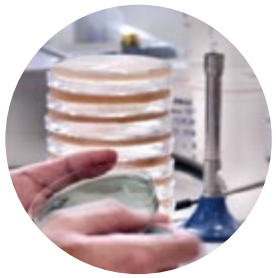
LAB

THE GATEWAY TO MICROBIOLOGY™

The Microbiology Manual



Spring 2012

About Lab M

Lab M has long enjoyed an international reputation for the high quality of its dehydrated culture media, particularly for the culture and isolation of anaerobes. The introduction of Harlequin™ chromogenic media, the company´s development of ISO compliant media formulations for food industry testing, and the provision of services such as the SMART QA Proficiency Testing scheme have all confirmed Lab M´s leading position.
Lab M also supplies bulk peptones, extracts and other raw materials to pharmaceutical and large-scale fermentation industries, in compliance with current EDQM certification. Custom-designed and custom-blended products are also a speciality.

Lab M is certified in accordance with ISO 9001:2000 and ISO 13485; products for the clinical market are supplied in compliance with the European IVD directive and carry the CE mark.

The Lab M team combines decades of knowledge and experience with their customer commitment ethic to ensure that you, the customer, receive the best possible attention and service.

From R&D through to sales and support, expertise and knowledge characterise the Lab M teams. Solving application challenges, providing technical assistance and ensuring that customers get the best from their products are all part of the service.

Our Heritage

Established in 1971, Lab M initially manufactured pre-poured media for clinical laboratories in London and the North West of England — and its name, London Analytical and Bacteriological Media, reflects its origins.

During the 1970s, the company developed its own dehydrated culture media, and by the early 1980s had added consumables and diagnostics to its product list. At that time Lab M was the only UK supplier offering such a range, and with a growing reputation for quality it soon became the ideal supplier for medium-sized industrial laboratories.

In recent years we have continued to flourish, with Lab M consolidating its position as an innovative producer of reliable, high quality microbiology products.

The Future

Lab M's Research and Development Group works in collaboration with key institutions and industry leaders to develop and trial new products that give faster results at lower cost. Conventional culture remains the most cost-effective and reliable way to enrich and enumerate micro-organisms in complex samples, but we are also developing DCM products designed for faster methods such as ELISA and PCR.

Our proven magnetic bead technology can isolate and concentrate target organisms and, through our emerging chromogenic and fluorogenic products, we can culture and isolate specific groups of organisms with the presumptive diagnostic power of the chromogenic/fluorogenic reaction.

CONTENTS

LRQA
Business Assurance

CERTIFICATE OF APPROVAL

This is to certify that the Quality Management System of:

Lab M Limited
Bury, Lancashire
United Kingdom

has been approved by Lloyd's Register Quality Assurance
to the following Quality Management System Standards:

BS EN ISO 9001:2008

The Quality Management System is applicable to:

Design, manufacture and supply of microbiological culture media, antibiotic supplements and diagnostic products. Supply of laboratory consumables.

This certificate forms part of the approval identified by certificate number LRQ 0924069

Approval
Certificate No: LRQ 0924069/A

Original Approval: 3 June 1993

Current Certificate: 1 June 2011

Certificate Expiry: 31 May 2014

J.K. Andrews

Issued by: Lloyd's Register Quality Assurance Limited

UKAS
MANAGEMENT
SYSTEMS
001

LRQA
Business Assurance

CERTIFICATE OF APPROVAL

This is to certify that the Quality Management System of:

Lab M Limited
Bury, Lancashire
United Kingdom

has been approved by Lloyd's Register Quality Assurance
to the following Quality Management System Standards:

BS EN ISO 13485:2003

The Quality Management System is applicable to:

Design, manufacture and supply of microbiological culture media, antibiotic supplements and diagnostic products.

This certificate forms part of the approval identified by certificate number LRQ 0924069

Approval
Certificate No: LRQ 0924069/B

Original Approval: 3 June 1993

Current Certificate: 1 June 2011

Certificate Expiry: 31 May 2014

J. K. Andrews

Issued by: Lloyd's Register Quality Assurance Limited

UKAS MANAGEMENT SYSTEMS
001

Customised Media Service

The Lab M Customised Media Service

Since its inception, Lab M has been committed to customer needs by offering a wide range of quality media products for global markets. Our continued programme of product innovation and development has ensured that the Company has been responsive to market trends and changes in custom and practice. The increasing importance of regulatory compliance has also been a significant factor in this process of innovation.

From time to time, however, Lab M has been asked to 'design' media to meet specific customer applications. This has evolved into a more proactive approach to resolving customer needs:

The Lab M Customised Media Service.

What do we offer?

- Individually designed media, engineered to meet specific customer requirements.
- Close client collaboration.
- Development programmes that are both cost and time efficient.
- A process approach to development, commencing with feasibility tests, through product trials and into routine production.
- Customised packaging and labelling.
- Assurance of compliance with regulatory requirements.
- Total client confidentiality.
- Intellectual Property options.
- Post-development consultancy.
- Commitment to on-going production.
- Quality products and service.

This is a unique service that differentiates us from our competitors and our Technical Support Group would be happy to discuss your needs on a confidential basis.

Lab M Culture Media: The Process Outline

RAW MATERIALS

Agars, Peptones, Extracts, Dyes, Chemicals etc.

Each component is individually tested for suitability

PRODUCTION

Weighing, Milling, Blending

Aproduction batch is made from raw materials of specified batch number which have been pre-tested for compatibility. The components are individually milled to ensure uniform particle size. Weighings are double checked before the components are blended.

QUALITY CONTROL

Physical, Biological parameters. Comparison with previous batch and competition

Quality control first checks that the batch is completely blended, then a series of physical and biological tests are performed to ensure the product meets the exacting standards required by our customers. Comparisons with previous and competitor's batches are made. Results are recorded and a reference sample stored.

BOTTLING

Into 500g sealed containers, or bulk containers at request

Automated equipment delivers pre-weighed amounts into containers which are hermetically sealed. Each container is immediately labelled with product details, code and batch number.

CUSTOMERS

Lab M products are dispatched all over the world to microbiologists in all types of laboratory. Strict batch traceability in accordance with ISO9001 ensures we can recall all products if necessary, safeguarding your products/process.

Lab M Culture Media – The quality criteria

Raw materials

Peptones and Extracts – Clarity, pH, moisture, growth promoting properties with Gram positive and Gram negative organisms aerobically and anaerobically, freedom from toxicity. Compatibility with other components, haemolysis patterns, antibiotic antagonists.

Agar – Clarity, pH, gel strength, melting point, setting point, heavy metal content (particularly Ca^{++}, Mg^{++}) compatibility with other components. Clarity on re-melt.

Bile Salts – Clarity, pH, thin layer chromatography, compatibility with other components.

Dyes & Chemicals – pH, chemical parameters, growth promotion inhibition, properties after incorporation into culture media.

Production

All components from specified batch numbers. All components weighed accurately and checked.

Components milled to uniform particle size. Components blended for specified time, multiple samples taken to ensure thorough blending.

Quality control

Physical – pH, clarity, gel strength, colour, heat stability, viscosity, redox.

Biological – Growth characteristics, productivity ratio, chemical reactions and colour changes, comparison with previous batch and competition.

Preparing Culture Media

Quality Assured

Before each batch of Lab M Culture media is passed for sale it undergoes a rigorous quality control procedure to ensure it gives maximum recovery and reproducibility. Reconstitution of media in the user's laboratory must be done with care to ensure the same high standards of performance.

The following section outlines the correct procedures which will ensure high quality reconstituted products, and suggests simple quality control techniques that can be used to check the performance of prepared media.

Dehydrated Culture Media

Storage

Dehydrated media stored unopened under optimal conditions have a shelf life of 2-5 years but once the container is opened the contents should be used within six months. In-house quality control by the user will help determine the condition of product in opened containers. The best conditions for storing dehydrated media are in a cool, even temperature away from any sources of moisture such as washing up areas or laboratory autoclaves and away from strong light. Storage in a refrigerator is generally not recommended (unless otherwise stated on the product label) as there is the risk of condensation on the container when it is brought out of the refrigerator. Storage instructions for Lab M media products are stated on the product label.

TABLE 1 – Deterioration of SS Agar stored in various conditions for 6 months.

Storage conditions	moisture gain %
Unopened bottle stored in cool, dark, dry conditions	0
Loose cap, stored in light on bench	1.1
Loose cap, stored in light in autoclave room	4.4

The effect of the moisture gain on the performance of the agar can be quite dramatic. A 1.1% gain in moisture on storage will lead to a 53% reduction in the numbers of *Salmonella* isolated. Similarly a 4.4% gain in moisture will result in a 78% reduction in isolation rate. This demonstrates the importance of ensuring the container lid is tightly closed and the pot stored in cool, dry, dark conditions. *Barry, A. L. and Fay, G. D. A review of some common sources of error in the Preparation of Agar Media. (1972). Am. J. Med. Tech. Vol. 38 No. 7.* When a container is opened for the first time the date should be noted on the container. Dehydrated media should not be used if it shows any sign of moisture gain i.e. becomes lumpy or discoloured. The lid on the container should be replaced quickly after media has been taken out and closed tightly.

Weighing Out

Using a top-pan balance with an accuracy of ±0.1 gram the powder should be spooned onto a weighing boat or clean beaker. Do not tip the media out of the container as this will cause excess dust which may be irritating and will certainly need cleaning up. The components of some formulations can be irritant so the wearing of a suitable face mask at this stage is advisable.

Water

Purification by distillation, deionisation or reverse osmosis is advisable. It is important that the equipment is properly maintained; the output of ion resins need to be electronically monitored and microbial colonisation of the resin and tubing must be avoided. Storage vessels for purified water must also be monitored for microbial colonisation. It is advisable to use only fresh purified water with a conductivity of less than 10 microsiemens. Stored water tends to become acidic because it absorbs atmospheric CO_2. Tap water is not recommended because of the potential presence of heavy metal ions which can cause inhibition and precipitation problems.

Quality Control of Culture Media

The routine quality control of culture media is an essential 'good laboratory practice' necessary to maintain the standards and performance of any bacteriological culture technique. Such practices are a key requirement for many laboratory accreditation schemes such as UKAS, and CLAS etc.

More recently, the International Standards Organisation (ISO) has issued guidelines on the preparation and production of culture media (ISO/TS 11133). Part 1 sets out general guidelines on the quality assurance for the preparation of culture media in the laboratory. Part 2 provides practical guidelines on performance testing of culture media. These guidelines currently take the form of a detailed technical specification, setting out the minimum acceptable performance criteria and the methodology and organisms required for quality control of specific media products.

Example of a typical quality control process

A typical dehydrated culture medium (DCM) product will be subjected to a battery of tests. The manufacturer tests the product in its final prepared form (as a plate or broth) for all criteria. An end user needs only to perform a minimum QC assessment.

A typical manufacturer's testing regime is as follows:

Determinants of physical quality.

- Final pH.
- Clarity and presence of optical artefacts.
- Gel stability and consistency.

Determinants of microbiological quality.

Tests to assess microbial contamination and microbiological growth characteristics are required for each batch of end product.

Microbial contamination.

The samples tested include at least one plate or tube from the beginning, and one plate or tube from the end of a pouring or dispensing process. Plates or tubes are incubated for at least 18 hours under the routine incubation conditions specified for a particular media type. Target limits for the percentage of contaminated plates or containers of liquid medium should be established for each medium or specified by the manufacturer.

Microbiological growth.

Each batch of complete culture medium, nutrient components or supplements, are assessed for microbiological growth in terms of productivity, selectivity and specificity. Assessment may be by the quantitative, semi-quantitative or qualitative methods described in the guidelines, or by another generally accepted technique. Results are interpreted by comparing the amount of growth on the test medium with that on a specified reference medium. The growth of target strains should be typical in appearance, size and morphology, while the growth of non-target strains should be partly or completely inhibited.

Test strains.

Micro-organism cultures from the ATCC reference collection are documented in this standard, however it is stated that 'the use of equivalent strains from other culture collections is permitted'. Lab M typically test media with QC organisms drawn from ATCC, NCTC and NCIMB approved sources.

Productivity.

Solid, semi-solid or liquid culture media are inoculated with an appropriate inoculum of the working culture of each of the defined test micro-organisms. Productivity should reach a minimum limit as defined in the corresponding standard or as detailed in the technical specification. Quantitative methods require determination of the productivity ratio: a score of growth based on a comparison between the test medium and the defined reference medium.

Selectivity and specificity.

Quantitative assessment of selectivity requires inoculation of both selective culture media and a reference medium with the specified micro-organism, at an appropriate inoculum for testing. Selectivity, as defined by the selectivity factor, has to reach the value given in the corresponding standard or as set out in the technical specification. For semi-quantitative and qualitative methods, the growth of the non-target strain(s) should be inhibited partly or completely.

Assessment of growth

Solid Culture Media

Productivity Ratio (P.R.)

Productivity ratio is determined by assessing performance related to a control medium, which should be a nutritious agar such as Tryptone Soy Agar (LAB011). A controlled inoculum of approximately 100 colony forming units (cfu) must be used for both media and the P.R. is calculated by counting the colonies on the test and control media:

$$\text{P.R.} = \frac{\text{No. of colonies on test x dilution factor}}{\text{No. of colonies on control x dilution factor}}$$

Or to express as a percentage:

$$\text{P.R. (\%)} = \frac{\text{No. of colonies on test x 100}}{\text{No. of colonies on control}}$$

There are many inoculation methods that may be used to determine the productivity ratio, including:

- Spiral plate
- Miles-Misra and Modified Miles-Misra technique
- Pour plate
- Surface inoculation e.g. serial dilution surface incoulation using 'L-shaped spreader'

Relative Growth Index (Ecometric Technique)

The ecometric technique of Mossel is simple and gives numerical readings that can form the basis of records suitable for trend analysis. Both absolute growth index (AGI) and relative growth index (RGI) can be obtained by this method.

This plating technique is less-frequently used, but still offers a robust method of determining productivity. The ecometric technique is based on streaking an inoculum to extinction. The results obtained can be compared with previous batches of the same medium or with batches of the same medium from different manufacturers. The results can also be compared with results obtained using the same organisms on non-selective media.

Liquid Culture Media

Liquid media are challenged with 10-100cfu of the target organism. Recovery of the organism is assessed qualitatively (visual) or semi-quantitatively (by sub-culture).

1. Prepare an overnight culture of the test organism in Tryptone Soy Broth (LAB004).
2. Prepare a tenfold serial dilution (to 10^{-12}) in Maximum Recovery Diluent (LAB103).
3. Add 1ml of each dilution to 9ml of test and control broths. Incubate at 37°C for 18 hours.
4. Examine the broths and note the highest dilution showing growth (turbidity of the broth).

This method can be used in conjunction with the Miles-Misra technique to demonstrate recovery of known levels of CFU's in broth media.

References:

ISO/TS 11133 Microbiology of food and animal feeding stuffs – Guidelines on preparation and production of culture media

- Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory ISO/TS 11133-1:2009.
- Part 2: Practical guidelines on performance testing of culture media ISO/TS 11133-2:2003.
- Part 2 AMENDMENT 1: Test microorganisms for commonly used culture media ISO/TS 11133-2:2003/Amd.1:2011(E).

Media for Bacteria, Moulds and Yeasts: An Attempt at Standardisation at the International Level. J. Appl. Bacteriol. 54 313-327

Mossel D.A.A. *et al* (1983) Quality Assurance of Selective Culture

Preservation of Stock Cultures

The following method has been used in our laboratory for several years for long-term storage of microorganisms. For most strains a freezer at -20°C will suffice, however it should be noted that storage temperature may affect growth characteristics and viability.

Preservation of Bacteria using Protect™ Microorganism Preservation System

Culture Preparation

1. Grow the organism on an appropriate non-selective solid medium using the appropriate incubation temperature and atmospheric conditions to achieve a heavy growth. Use several plates with organisms forming small colonies.
2. Remove growth from plate using a sterile cotton swab and emulsify in the Protect™ cryopreservation fluid, making a thick suspension.
3. Carefully cap the vial and invert it six times. Leave the vial to stand for at least 30 seconds. . The vial may be tapped to remove the bubbles from bead centres.
4. Withdraw as much liquid as possible using a sterile, fine-tip pipette.

Cap the vial, label and place in freezer.

Culture Recovery

1. Remove Protect™ vial from the freezer or liquid nitrogen container. Use a cryoblock, which has been stored in a freezer for at least thirty minutes to extend the time available for working with frozen vial.
2. Open vial. Remove one bead using sterile needle and recover the culture by rubbing bead over suitable agar medium or placing bead directly into broth. Removed beads must not be returned to the vial.

Duplicate vials can be prepared from the same original culture. This will assist should the vial become contaminated or should the pedigree of the organism be questioned.

The Protect™ Microorganism Preservation System is available to purchase from Lab M. Full details are available on the Lab M website, **www.labm.com**.

Details of recommended QC strains of organisms are contained in the individual entries for media.

TABLE – Culture collection strains for the quality control of culture media

Organism	WDCM	ATCC	NCIMB	NCTC
Actinomyces israelii	-	12102	-	12972
Aeromonas hydrophila	00063	7966	9240	8049
Aspergillus niger	-	16404	50097	-
Aspergillus restrictus	00183	42693	-	-
Bacillus cereus	-	14579	9373	2599
Bacillus cereus	00001	9634 / 11778	8012 / 50014	10320
Bacillus licheniformis	00068	14580	9375	10341
Bacillus subtilis	-	6633	8054 / 8566	10400
Bacillus subtilis	00070	-	13061	5398
Bacteroides fragilis	-	25285	-	9343
Burkholderia cepacia	-	25416	-	10743
Campylobacter coli	00004	43478	-	-
Campylobacter jejuni	-	33560	-	11351
Campylobacter jejuni	00005	33291	-	-
Candida albicans	-	10231	50010	-
Citrobacter freundii	00006	43864	-	-
Citrobacter freundii	-	8090 / 13316	11490	9750
Clostridium difficile	-	9689	10666	-
Clostridium perfringens	00007	13124	6125 / 50027	8237
Clostridium sporogenes	00008	19404	532	532
Corynebacterium diphtheriae var. *mitis*	-	-	-	10356
Corynebacterium flavescens	-	10340	8707	-
Cronobacter muytjensii	-	51329	-	-
Cronobacter sakazakii	-	12868	5920	5920
Cronobacter sakazakii	-	-	8272	8155
Enterobacter aerogenes	00175	13048	10102 / 50029	10006
Enterobacter cloacae	00083	13047	10101	10005
Enterococcus faecalis	00087	29212	-	12697
Enterococcus faecalis	00084	-	8260 / 50030	8213
Enterococcus faecalis	00009	19433	775	775
Escherichia coli	00013	25922	12210	12241
Escherichia coli	-	10536	8879	10418
Escherichia coli	00012	8739	-	-
Escherichia coli	-	11229	9517	-
Escherichia coli	-	-	-	9111
Escherichia coli O26	-	-	-	8783
Escherichia coli O157:H7	00014	700728	50139	12900
Eurotium rubrum	00184	42690	-	-
Fusobacterium necrophorum	-	25286	-	-
Haemophilus influenzae	-	-	-	11931
Klebsiella pneumoniae	00097	13883	-	9633
Lactobacillus brevis	-	367	947	947
Lactobacillus casei subsp. *rhamnosus*	00101	7469	6375 / 8010	6375
Lactobacillus delbrueckii subsp. *lactis*	-	4797	8117	7854
Lactobacillus fermentum	-	9338	6991 / 8028	6991
Lactobacillus plantarum	-	8014	-	6376
Lactococcus lactis subsp. lactis	-	-	8763	-
Legionella pneumophila	00107	33152	-	11192
Leuconostoc mesenteroides	-	10830a	-	10817
Listeria innocua	00017	33090	-	11288
Listeria ivanovii	00018	19119	50095	11846
Listeria monocytogenes	00020	19111	-	-
Listeria monocytogenes	00019	-	50002	11994
Listeria monocytogenes	00021	13932	-	10527

Organism	WDCM	ATCC	NCIMB	NCTC
Listeria seeligeri	-	35967	50000	11856
Mucor racemosus	00181	42647	-	-
Neisseria gonorrhoeae	-	19424	-	8375
Obesumbacterium proteus	-	12841	8771	-
Pediococcus acidilactici	-	8042	-	6990
Penicillium chrysogenum	-	9178	-	-
Penicillium cyclopium	-	16025	-	-
Peptostreptococcus anaerobius	-	27337	-	11460
Porphyromonas asaccharolytica	-	25260	-	-
Prevotella loescheii	-	15930	-	11321
Proteus mirabilis	00023	29906	-	11938
Proteus mirabilis	-	25933	13283	-
Proteus vulgaris	-	13315	4175	4175
Pseudomonas aeruginosa	00025	27853	12469	-
Pseudomonas aeruginosa	00026	9027	8626	-
Pseudomonas aeruginosa	00114	25668	13063 / 50067	10662
Pseudomonas fluorescens	00115	13525	9046	10038
Pseudomonas fluorescens	-	-	10559	-
Pseudomonas fragi	-	-	8987	-
Saccharomyces cerevisiae	-	9763	-	10716
Saccharomyces pastorianus	-	2700	-	903
Salmonella Enteritidis*	00030	13076	-	-
Salmonella Typhimurium*	00031	14028	13284	12023
Salmonella Typhimurium*	-	-	13034 / 50076	-
Serratia marcescens	-	274	-	1377
Shigella flexneri	00126	12022	50140	-
Shigella sonnei	00127	29930	-	12984
Shigella sonnei	-	-	-	8574
Staphylococcus aureus	00032	6538	9518	10788
Staphylococcus aureus	00034	25923	12702	-
Staphylococcus aureus	00033	6538P	8265 / 50080	7447
Staphylococcus aureus	00035	9144	-	6571
Staphylococcus aureus	-	-	-	11940
Staphylococcus aureus	-	-	-	12493
Staphylococcus epidermidis	00036	12228	8853	-
Staphylococcus epidermidis	00132	14990	12721 / 50082	11047
Streptococcus agalactiae	-	13813	-	8181
Streptococcus pyogenes	-	12344	11841	8198
Streptococcus salivarius	-	9222 / 9757	8883	-
Taylorella equigenitalis	-	35865	-	11184
Veillonella parvula	-	10790 / 17742	-	-
Vibrio cholerae	00136	-	-	11348
Vibrio furnissii	00186	-	-	11218
Vibrio parahaemolyticus	00037	17802	1902	10903
Wallemia sebi	00182	42694	-	-
Yersinia enterocolitica	00038	9610	-	12982

**Salmonella enterica* subsp. *enterica* serovar *Enteritidis/Typhimurium*

Obtaining stock cultures

The World Data Centre for Microorganisms (WDCM) catalogue is a unique system of identifiers for strains recommended for use in quality assurance. The WDCM catalogue was produced to enable broader and easier access to the reference strains listed by the ISO TC 34 SC 9 Joint Working Group 5 and by the Working Party on Culture Media of the International Committee on Food Microbiology and Hygiene (ICFMH-WPCM) in their publication Handbook of Culture Media for Food and Water Microbiology. The WDCM was pioneered by the World Federation for Culture Collections (WFCC) and is supported by ISO, AFNOR, FDA and ICFMH.

WDCM – World Data Centre for Microorganisms
http://refs.wdcm.org/home.htm

WFCC – World Federation for Culture Collections
http://www.wfcc.info/home/

As the WDCM reference list is a recent introduction, we have included some of the more commonly used culture collection references which may be more familiar to microbiologists.

ATCC – American Type Culture Collection
10801 University Boulevard, Manassas, Virginia 20110, USA
www.atcc.org/

NCIMB – National Collection of Industrial and Marine Bacteria
Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen, AB21 9YA, UK
http://www.ncimb.com/

NCTC – National Collection of Type Cultures
Health Protection Agency (HPA) Culture Collections also include:
ECACC - European Collection of Cell Cultures
NCPF - National Collection Pathogenic Fungi
NCPV - National Collection of Pathogenic Viruses
Health Protection Agency Culture Collections, Health Protection Agency, Centre for Emergency Preparedness and Response, Porton Down, Salisbury, SP4 0JG, UK
http://www.hpacultures.org.uk/

Microbiology Methods

There are numerous sources of information regarding microbiology methods, just some of which are listed below:

Bacteriological Analytical Manual.

Food and Drug Administration. Available online at **www.fda.gov**
(FDA's Bacteriological Analytical Manual (BAM) presents the agency's preferred laboratory procedures for microbiological analyses of foods and cosmetics. AOAC International published previous editions of this manual in a loose-leaf notebook format, and, more recently, on CD-ROM. This online BAM is now available to the public. Some changes have been made to methods since the previous version. A listing of chapters updated since the last hard-copy version (Edition 8, Revision A /1998) can be found in **About the Bacteriological Analytical Manual**)

British Standards Institute.
389 Chiswick High Road, London W4 4AL. www.bsigroup.co.uk

Compendium of Methods for the Microbiological Examination of Foods 4th edition.
2002. Edited by Downes, F.P. and Ito, K. American Public Health Association.
ISBN-10: 087553175X / ISBN-13: 978-0875531755

European Pharmacopeia, Supplement 6.3 to the 6th Edition.
2008. European Directorate for the Quality of Mediicnes and Healthcare (EDQM). www.edqm.eu ISBN: 978-92-871-6312-7

Manual of Microbiological Methods for the Food and Drink Industry, 5th edition
2007. Campden BRI, Station Road, Chipping Campden, Goucestershire, GL55 6LD. Guideline G43. ISBN 978-0-905942-93-3.

The Microbiology of Drinking Water
2002. Methods for the Examination of Water and Associated Materials. Environment Agency. Available online from www. http://www.environment-agency.gov.uk/

Practical Food Microbiology, 3rd edition
2002. Edited by Roberts, D. and Greenwood, M. Wiley-Blackwell. ISBN-10: 1405100753 / ISBN-13: 978-1405100755

Format and abbreviation guide

Product Name

(Alternative name or commonly used abbreviation)

Product Code

Description:
A brief outline which may include any of the following information on the medium:

- **History**
- **Mechanisms**
- **Applications**
- **Recognition By Regulatory/Advisory Bodies**
- **Advantages**

Formula: The product composition in grams per litre; minor adjustments to the published formula may be made to meet performance criteria.

Method for reconstitution
Distilled water can be substituted for deionised water. "Allow to soak times" are not critical. If agar media are to be dispensed prior to sterilising, first bring to the boil to dissolve the agar.

Appearance: – of the finished cooled medium.

pH: at 20˚C. For agars, pour a small quantity into a universal bottle, allow to set and plunge the probe into the medium.

Minimum Q.C. organisms – for use every time a new batch of prepared medium is reconstituted. This short form check should not be confused with a full Q.C. of the medium. Where an organism should show inhibition this could be complete or partial. Records should be kept of these results to help recognise changes in performance over a period of time.

Storage of Prepared Media – All prepared media should be stored in the dark. If a medium is to be used beyond the suggested shelf life, appropriate quality control should be performed to demonstrate that there has been no detectable fall off in performance.

Growth characteristics

Abbreviation key for colonial descriptions:

CV = convex	**CR** = crenated
F = flat	**Rz** = rhizoid
E = entire	**G** = glossy
P.P. = pinpoint	**D** = dull

( ) brackets are used to denote occasional variations.

References
A list of related publications and sources of information.

N.B. The formulae in this manual and on the product label are adhered to wherever possible. However it is occasionally necessary to make minor adjustments to meet performance criteria.

Dehydrated culture media selection guide

Anaerobes

LAB160 Brazier's CCEY Agar
LAB220 DRCM (ISO) - Differential Reinforced Clostridial Medium
LAB090 Fastidious Anaerobe Agar (F.A.A.)
LAB071 Fastidious Anaerobe Broth (F.A.B.)
LAB025 Fluid Thioglycollate Medium (U.S.P.)
LAB109 Perfringens Agar (O.P.S.P.)
LAB194 Perfringens Agar (TSC)
LAB023 Reinforced Clostridial Agar
LAB022 Reinforced Clostridial Medium (Broth)
LAB064 Thioglycollate Medium (Brewer)

Bacillus

LAB020 Dextrose Tryptone Agar
LAB193 PEMBA (Bacillus Cereus Medium)
LAB073 PREP Bacillus cereus Medium

Biomolecular

HAL004 Harlequin™ LB Agar
HAL005 Harlequin™ LB Top Agar
LAB168 LB Agar
LAB174 LB Agar (Lennox)
LAB169 LB Broth
LAB173 LB Broth (Lennox)
LAB191 Luria Bertani (Hi-Salt) Broth
LAB182 NZCYM Broth
LAB181 NZY Broth (NZYM)
LAB183 Terrific Broth
LAB175 YPD Broth
LAB176 YPD with Agar
LAB180 2xYT Agar
LAB179 2xYT Broth

Blood Agar Bases

LAB028 Blood Agar Base
LAB015 Blood Agar Base No. 2
LAB001 Columbia Agar Base
LAB525 Eugon Agar
LAB090 Fastidious Anaerobe Agar (F.A.A.)
LAB011 Tryptone Soy Agar (U.S.P.)

Blood Culture Media

LAB049 Brain Heart Infusion Broth
LAB071 Fastidious Anaerobe Broth (F.A.B.)
LAB004 Tryptone Soy Broth (USP)
LAB205 Tryptone Soy Broth (without dextrose)

Brewing

LAB201 Lysine Agar
LAB198 Raka-Ray No.3 Agar
LAB199 Raka-Ray No.3 (Increased gel strength)
LAB079 W.L. Nutrient Agar
LAB038 Wort Agar
LAB200 Yeast & Mould Agar

Clostridia

LAB160 Brazier's CCEY Agar
LAB220 DRCM (ISO) - Differential Reinforced Clostridial Medium
LAB109 Perfringens Agar (O.P.S.P)
LAB194 Perfringens Agar (TSC)
LAB022 Reinforced Clostridial Medium (R.C.M.)
LAB023 Reinforced Clostridial Medium Agar

Coliform/Enterobacteriaceae

LAB051 Brilliant Green Bile 2% Broth
LAB091 E. E. Broth (Enterobacteriacae Enrichment Broth)
LAB060 Endo Agar
LAB061 Eosin Methylene Blue Agar
HAL008 Harlequin™ E. coli/Coliform Medium
HAL009 Harlequin™ mLGA
HAL003 Harlequin™ Tryptone Bile Glucuronide Agar (TBGA)
LAB126 Lactose Broth
LAB196 Lauryl Tryptose Broth
LAB045 MacConkey Agar No. 3
LAB005 MacConkey Broth (Purple)
LAB077 MLSTB-MT (ISO) Modified Lauryl Sulphate Tryptose Broth with MUG & Tryptophan
LAB072 Tryptone Bile Agar
LAB031 Violet Red Bile Agar (VRBA)
LAB088 Violet Red Bile Glucose Agar (VRBGA)
LAB573 VRBA with MUG

Dairy

LAB081 CSEB (ISO) - Cronobacter sakazakii Enrichment Broth
LAB060 Endo Agar Base
HAL012 Harlequin™ CSIM (ISO)
HAL013 Harlequin™ CSA-DFI - Cronobacter sakazakii Agar DFI Formulation
HAL010 Harlequin™ Listeria Chromogenic Agar
LAB126 Lactose Broth
LAB196 Lauryl Tryptose Broth
LAB092 M17 Agar
LAB019 Milk Agar
LAB115 Milk Plate Count Agar
LAB077 MLSTB-MT (ISO) Modified Lauryl Sulphate Tryptose Broth with MUG & Tryptophan
LAB093 MRS Agar
LAB094 MRS Broth
LAB098 Potato Dextrose Agar
LAB087 Sugar Free Agar
LAB063 Tryptone Glucose Extract Agar
LAB031 Violet Red Bile Agar (VRBA)
LAB088 Violet Red Bile Glucose Agar (VRBGA)
LAB573 VRBA with MUG
LAB038 Wort Agar
LAB099 Wort Broth
LAB018 Yeast Extract Agar
LAB119 Yeast Extract Dextrose Chloramphenicol Agar

Diagnostic Medical Microbiology

LAB195 BCYE Legionella Isolation Medium
LAB121 Bromocresol Purple Lactose Agar
LAB006 CLED (Bevis)-double indicator
LAB041 CLED (Mackey & Sandys)-single indicator
LAB090 Fastidious Anaerobe Agar
LAB067 GC Agar Base
LAB027 Hoyle's Medium
LAB035 TYC Medium

Refer to other sections for our full range of Medical Products.

Diluents/ Isotonic solutions

LAB103 Maximum Recovery Diluent
LAB100Z Ringer's Solution (1/4 Strength) - Tablets

Enteric Pathogens

LAB167 Aeromonas Agar
LAB013 Bismuth Sulphite Agar
LAB034 Brilliant Green Agar
LAB046 Buffered Peptone Water - pre-enrichment broth
LAB204 Buffered Peptone Water (ISO)
LAB112 Campylobacter Agar (Blood Free - Improved)
LAB135 Campylobacter Enrinchment Broth
LAB161 Sorbitol MacConkey Agar (SMAC)
LAB003 DCLS
LAB029 Desoxycholate Citrate Agar (DCA)
LAB065 Desoxycholate Citrate Agar (Hynes)
LAB537 Diassalm
HAL001 Harlequin™ Salmonella ABC
HAL006 Harlequin™ Sorbitol MacConkey Agar (SMAC-BCIG)
LAB110 Hektoen Enteric Agar
LAB116 MLCB Agar
LAB150 MSRV
LAB030 MacConkey Agar (with salt)
LAB002 MacConkey Agar (without salt)
LAB216 MacConkey Agar No.2
LAB042 Mueller Kauffman Tetrathionate Broth
LAB202 Mueller Kaufmann Tetrathionate Novobiocin Broth (MKTTn)
LAB165 O157 Broth (MTSB)
LAB086 Rappapport Vassiliadis Medium
LAB209 Rhamnose MacConkey (VTEC O26) Agar

Enteric Pathogens continued

LAB052 S.S. Agar (Salmonella Shigella Agar)
LAB044 Selenite Broth
LAB055 Selenite Cystine Broth
LAB096 TCBS Cholera Medium
LAB097 Tetrathionate Broth Base
LAB032 XLD Agar
LAB120 Yersinia CIN Agar
LAB221 XLT4 Agar – Xylose Lysine Tergitol 4 Agar

Enterococci / Streptococci

LAB028 Blood Agar Base
LAB015 Blood Agar Base No. 2
LAB207 Bile Aesculin Agar
LAB001 Columbia Agar Base
LAB106 Kanamycin Aesculin Azide Agar
LAB107 Kanamycin Aesculin Azide Broth
LAB216 MacConkey Agar No.2
LAB092 M17 Agar
LAB166 Slanetz and Bartley (m Enterococcus Medium)
LAB035 TYC Medium
LAB075 Todd Hewitt Broth

Food Microbiology

LAB167 Aeromonas Agar
LAB085 Baird-Parker Medium
LAB285 Baird Parker Medium Base (ISO)
LAB207 Bile Aesculin Agar
LAB034 Brilliant Green Agar (Modified)
LAB582 Buffered Listeria Enrichment Broth^{PLUS}
LAB046 Buffered Peptone Water
LAB204 Buffered Peptone Water (ISO)
LAB112 Campylobacter (Blood Free - Improved)
LAB135 Campylobacter Enrichment Broth
LAB081 CSEB (ISO) - Cronobacter sakazakii Enrichment Broth
LAB218 DG18 Agar (ISO) - Dichloran (18%) Glycerol Agar
LAB537 Diassalm
LAB217 DRBC Agar (ISO) - Dichloran Rose Bengal Chloramphenicol Agar
LAB220 DRCM (ISO) - Differential Reinforced Clostridial Medium
LAB171 EC Medium (*E. coli* Medium)
LAB164 Fraser Broth
LAB212 Fraser Broth^{PLUS}
LAB211 Half Fraser Broth^{PLUS}
HAL013 Harlequin™ CSA-DFI - Cronobacter sakazakii Agar DFI Formulation
HAL012 Harlequin™ CSIM (ISO)
HAL008 Harlequin™ E.coli/Coliform Medium
HAL010 Harlequin™ Listeria Chromogenic Agar
HAL006 Harlequin™ Sorbitol MacConkey Agar (SMAC-BCIG)
HAL003 Harlequin™ Tryptone Bile Glucuronide Agar (TBGA)
LAB106 Kanamycin Aesculin Azide Agar
LAB107 Kanamycin Aesculin Azide Broth
LAB196 Lauryl Tryptose Broth
LAB138 Listeria Enrichment Broth
LAB139 Listeria Enrichment Broth (Buffered)
LAB579 Listeria Enrichment Broth^{PLUS}
LAB122 Listeria Isolation Medium (Oxford)
LAB206 Listeria Isolation Media, Oxford (ISO)
LAB172 Listeria Monocytogenes Blood Agar (LMBA)
LAB216 MacConkey Agar No.2
LAB077 MLSTB-MT (ISO) Modified Lauryl Sulphate Tryptose Broth with MUG & Tryptophan
LAB219 Modified Giolitti and Cantoni Broth (ISO)
LAB093 MRS Agar
LAB094 MRS Broth
LAB150 MSRV
LAB042 Mueller Kauffman Tetrathionate Broth
LAB202 Mueller Kaufmann Tetrathionate Novobiocin Broth (MKTTn)
LAB116 MLCB Agar
LAB165 O157 Broth (MTSB)
LAB147 Orange Serum Agar
LAB148 Palcam Agar
LAB144 Palcam Broth
LAB193 PEMBA - Bacillus Cereus Medium
LAB194 Perfringens Agar (TSC)
LAB109 Perfringens Agar (O.P.S.P.)
LAB149 Plate Count Agar
LAB010 Plate Count Agar A.P.H.A.
LAB098 Potato Dextrose Agar
LAB073 PREP Agar
LAB108 Pseudomonas Agar
LAB086 Rappaport Vassiliadis Medium (Broth)
LAB023 Reinforced Clostridial Agar
LAB209 Rhamnose MacConkey (VTEC O26) Agar
LAB055 Selenite Cystine Broth
LAB161 Sorbitol MacConkey Agar
LAB087 Sugar Free Agar
LAB097 Tetrathionate Broth Base A.P.H.A.
LAB155 UVM Broth
LAB031 VRBA
LAB573 VRBA with MUG
LAB088 VRBGA
LAB079 W.L. Agar
LAB038 Wort Agar
LAB099 Wort Broth
LAB032 XLD Agar
LAB221 XLT4 Agar – Xylose Lysine Tergitol 4 Agar

Identification Media

LAB059 Kligler Iron Agar
LAB126 Lactose Broth
LAB054 Lysine Iron Agar
LAB104 Peptone Water
LAB069 Simmons Citrate Agar
LAB053 Triple Sugar Iron Agar
LAB129 Tryptone Water
LAB130 Urea Agar
LAB131 Urea Broth

Lactic Acid Bacteria

LAB092 M17 Agar
LAB093 MRS Agar - de Man, Rogosa and Sharpe Agar
LAB094 MRS Broth
LAB147 Orange Serum Agar
LAB198 Raka-Ray No.3 Agar
LAB199 Raka-Ray No.3 (Increased gel strength)

Listeria

LAB139 Buffered Listeria Broth
LAB582 Buffered Listeria Enrichment Broth^{PLUS}
LAB212 Fraser Broth^{PLUS}
LAB164 Fraser Broth Base
LAB211 Half Fraser Broth^{PLUS}
HAL010 Harlequin™ Listeria Chromogenic Agar
LAB138 Listeria Enrichment Broth
LAB579 Listeria Enrichment Broth^{PLUS}
LAB122 Listeria Isolation Medium (Oxford formulation)
LAB206 Listeria Isolation Media, Oxford (ISO)
LAB172 Listeria Monocytogenes Blood Agar (LMBA)
LAB148 PALCAM Agar Base
LAB144 PALCAM Broth
LAB155 UVM Broth Base

Neutralising

LAB188 D/E Neutralising Agar
LAB187 D/E Neutralising Broth
LAB186 D/E Neutralising Broth Base
LAB185 Letheen Agar (AOAC)
LAB184 Letheen Broth (AOAC)
LAB189 Microbial Content Test Agar

Nutrient Media for general use

LAB048 Brain Heart Infusion Agar
LAB049 Brain Heart Infusion Broth
LAB525 Eugon Agar
LAB526 Eugon Broth
LAB008 Nutrient Agar
LAB214 Nutrient Agar (ISO)
LAB068 Nutrient Broth 'E'
LAB014 Nutrient Broth No. 2
LAB062 Tryptose Phosphate Broth
LAB018 Yeast Extract Agar

Sensitivity Testing

LAB039 Mueller Hinton Agar
LAB114 Mueller Hinton Broth
LAB012 Sensitivity Test Agar
LAB170 Susceptibility Test 'ISO' Agar

Staphylococci

LAB085 Baird-Parker Medium
LAB285 Baird Parker Medium Base (ISO)
LAB095 DN'ase Test Agar
LAB007 Mannitol Salt Agar
LAB219 Modified Giolitti and Cantoni Broth (ISO)
LAB192 ORSIM - Oxacillin Resistant Staphylococci Isolation Medium

Streptococci / Enterococci

LAB028 Blood Agar Base
LAB015 Blood Agar Base No. 2
LAB207 Bile Aesculin Agar
LAB001 Columbia Agar Base
LAB106 Kanamycin Aesculin Azide Agar
LAB107 Kanamycin Aesculin Azide Broth
LAB216 MacConkey Agar No.2
LAB092 M17 Agar
LAB166 Slanetz & Bartley Medium (Membrane Enterococcus Agar)
LAB035 TYC Medium
LAB075 Todd Hewitt Broth

Sterility Test Media

LAB025 Fluid Thioglycollate U.S.P.
LAB159 Malt Extract Broth
LAB014 Nutrient Broth No. 2 B.P.
LAB033 Sabouraud Liquid Medium U.S.P.
LAB011 Tryptone Soy Agar U.S.P.
LAB004 Tryptone Soy Broth U.S.P.
LAB205 Tryptone Soy Broth (without dextrose)

Total Viable Counts

LAB019 Milk Agar
LAB115 Milk Plate Count Agar
LAB010 Plate Count Agar A.P.H.A.
LAB149 Plate Count Agar
LAB163 R2A Medium
LAB063 Tryptone Glucose Extract Agar A.P.H.A.
LAB011 Tryptone Soy Agar
LAB197 Water Plate Count Agar (ISO)
LAB018 Yeast Extract Agar

Water Testing

LAB085 Baird Parker Medium Base
LAB207 Bile Aesculin Agar
LAB013 Bismuth Sulphite Agar
LAB048 Brain Heart Infusion Agar
LAB034 Brilliant Green Agar (Modified)
LAB051 Brilliant Green Bile 2% Broth
LAB195 BCYE Legionella Isolation Medium
LAB046 Buffered Peptone Water
LAB001 Columbia Agar Base
LAB537 Diagnostic Semi Solid Salmonella Agar (Diassalm)
LAB220 DRCM (ISO) - Differential Reinforced Clostridial Medium
LAB171 EC Medium
LAB060 Endo Agar Base
LAB061 Eosin Methylene Blue Agar (Levine)
HAL009 Harlequin™ mLGA
HAL006 Harlequin™ SMAC-BCIG
LAB110 Hektoen Enteric Medium
LAB106 Kanamycin Aesculin Azide Agar
LAB126 Lactose Broth
LAB196 Lauryl Tryptose Broth
LAB054 Lysine Iron Agar
LAB005 MacConkey Broth (Purple)
LAB002 MacConkey Agar (Without Salt)
LAB030 MacConkey Agar (With Salt)
LAB216 MacConkey Agar No.2
LAB045 MacConkey Agar No 3
LAB103 Maximal Recovery Diluent
LAB082 Membrane Lauryl Sulphate Broth
LAB080 Minerals Modified Glutamate Broth
LAB008 Nutrient Agar
LAB014 Nutrient Broth No. 2 BP
LAB165 O157 Broth (MTSB)
LAB109 Perfringens Agar (O.P.S.P)
LAB194 Perfringens Agar (TSC)
LAB010 Plate Count Agar A.P.H.A
LAB108 Pseudomonas Agar Base
LAB163 R2A Medium
LAB203 R2A Broth
LAB086 Rappaport-Vassiliadis Medium (RVS)
LAB100Z Ringer's Solution (1/4 Strength) Tablets
LAB044 Selenite Broth
LAB166 Slanetz & Bartley Medium (Membrane Enterococcus Agar)
LAB161 Sorbitol MacConkey Aga
LAB096 T.C.B.S. Cholera Medium
LAB097 Tetrathionate Broth Base APHA
LAB053 Triple Sugar Iron Agar
LAB129 Tryptone Water
LAB131 Urea Broth Base
LAB197 Water Plate Count Agar (ISO)
LAB032 XLD Agar
LAB221 XLT4 Agar – Xylose Lysine Tergitol 4 Agar
LAB018 Yeast Extract Agar

Refer to other sections for our full range of Water Test Media.

Yeasts and Moulds

LAB117 DTM Dermatophyte Test Medium
LAB218 DG18 Agar (ISO) - Dichloran (18%) Glycerol Agar
LAB217 DRBC Agar (ISO) - Dichloran Rose Bengal Chloramphenicol Agar
LAB201 Lysine Agar
LAB037 Malt Extract Agar
LAB159 Malt Extract Broth
LAB089 OGYE Agar
LAB098 Potato Dextrose Agar
LAB036 Rose Bengal Chloramphenicol Agar
LAB009 Sabouraud Dextrose Agar
LAB033 Sabouraud Liquid Medium
LAB111 Sabouraud Maltose Agar
LAB079 W.L. Nutrient Agar
LAB038 Wort Agar
LAB099 Wort Broth
LAB119 Yeast Extract Dextrose Chloramphenicol Agar
LAB200 Yeast & Mould Agar
LAB175 YPD Broth
LAB176 YPD Agar

1. Media Range

Aeromonas Agar

Bile Salt Irgasan Brilliant Green Agar

LAB167

Description

Aeromonas Agar is a highly selective medium for the isolation of *Aeromonas* spp. from food, clinical and environmental samples. Based on the selective agents brilliant green and irgasan, this medium will not inhibit those strains of *Aeromonas* spp. sensitive to ampicillin used in other media.

Formulation	g/litre
Beef Extract	5.0
Meat Peptone	5.0
Xylose	10.0
Bile Salts No.3	8.5
Sodium thiosulphate	5.44
Irgasan	0.005
Brilliant green	0.005
Neutral red	0.025
Agar	11.5

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, purple gel

pH: 7.0 ± 0.2

Method for reconstitution

Weigh 45.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by bringing to the boil. Cool to 47°C and mix well before dispensing into Petri dishes. Dry the agar surface prior to use..

Inoculation:

Faecal specimens: Inoculate surface of medium directly, spreading for single colonies.

Samples requiring enrichment: Inoculate alkaline peptone water and incubate at 37°C for 18-24 hr. Subculture onto Aeromonas Agar, surface spreading for single colonies.

Incubation: Incubate plates aerobically at 37°C for 18-24 hr. Examine for typical colonies and confirm as *Aeromonas* spp.

Storage:

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark (may be extended if moisture tight packaging used).

Minimum Q.C. organisms: *Aeromonas hydrophila* NCTC 8049
E. coli ATCC 25922 (inhibited)

Confirmation

Typical colonies (translucent, pale green colonies 0.5-3.0mm diameter) should be confirmed as presumptive Aeromonas spp. by performing an oxidase test and inoculating into Hugh & Leifsons O/F medium.

- *Aeromonas spp*. will give a positive oxidase reaction and demonstrate both oxidative and fermentative metabolism.
- *Pseudomonas spp*. will also be oxidase positive, but do not possess fermentative metabolism.

An alternative method is to inoculate triple sugar iron tubes.

- *Aeromonas* will typically produce an acid butt (yellow) and an alkaline or unchanged slant (red).
- *Pseudomonas spp*. will remain unchanged in both the butt and slant.

To fully identify colonies as *Aeromonas spp*. the above tests should be supported using a proprietary kit such as API 20NE or Microbact 24E (other products may be available).

Interpretation

Organism	Size	Shape	Colour
Aeromonas spp.*	0.5-3.0	CV.E.G	Translucent pale green
Pseudomonas spp.	0.5-1.0	CV.E.G	Translucent pale green
S.aureus	No growth		
E. coli	No growth		

* The selective nature of the medium may mean occasional strains do not grow, or grow poorly.

Bacillus Cereus Medium

Phenol Red Egg Yolk Polymyxin Agar (P.R.E.P.)
Mannitol Egg Yolk Polymyxin Agar

LAB073

Description

Introduced by Mossel and his co-workers in 1967 for the enumeration of *Bacillus cereus* in foods, this formula was shown to be the most effective for this purpose by Inal in 1972. Two reactions on this medium differentiate *B. cereus* from other members of the Bacillus group, these are mannitol fermentation and lecithinase production. Mannitol fermentation on this medium produces a yellow colour, *B. cereus* is mannitol negative and produces red colonies. The lecithinase production of *B. cereus* is indicated by a white precipitate around the colonies. Polymyxin is added to suppress coliforms but some *Proteus* spp and Gram positive cocci may grow through.

Formula	g/litre
Beef Extract	1.0
Balanced Peptone No. 1	10.0
D-Mannitol	10.0
Sodium chloride	10.0
Phenol red	0.025
Agar No. 1	15.0

Method for reconstitution

Weigh 46 grams of powder, disperse in 900ml of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C and aseptically add 100ml of X073 egg yolk emulsion and 2 vials of X074 Polymyxin.

Appearance: Pink, opaque gel.

pH: 7.2 ± 0.2

Minimum Q.C. organisms: *B. cereus* NCIMB 50014
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface, spreading or streaking for single colonies.

Incubation: 30˚C aerobically for 24-48 hours.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour
B. cereus	3.0-4.0	F.CR.D.	Pink, white halo
B. subtilis	2.0-3.0	F.CR.D.	Yellow
B. coagulans	2.0	F.CR.D.	Yellow
B. licheniformis	2.0	F.Rz.D.	Yellow
Proteus spp.	1.0	CV.E.G.	Pink (swarms)
E. faecalis	0.5	CV.E.G.	Yellow
E. coli	no growth		
S. aureus	1.0	CV.E.G.	Yellow (white halo)

References

Inal, T.: Vergleictiende Untersuchungen über die Selektivmedien zum qualitativen und quantitativen Nachweis von Vacillus cereus in Lebensmitteln.

Mitteilung I. : Fleischwritsch, 51: 1629-1632 (1971). IV. Mitteilung: Fleischwritsch, 52: 1160-1162 (1972).

Mossel, D.A.A., Koopman, M.J. and Jongerius, E. (1967). Enumeration of Bacillus cereus in foods. Appl. Microbiol. 15: 650-653. Thatcher, F.S., Clarke, D.S. (1978) Micro-organisms in foods. Volume 1 second edition. University of Toronto.

BS5763 Part 1L:1994. ISO7932:(1993) 3/100

Baird-Parker Medium Base

LAB085

Description

Originally introduced in 1962, this medium was developed by Baird-Parker to overcome the problems of recovering damaged *Staphylococcus aureus* from foodstuffs.

Baird-Parker medium is highly selective by nature, due to the presence of potassium tellurite and lithium chloride. Tellurite inhibits most coliforms and is also reduced to telluride by *S. aureus*, giving the typical black colonies. Glycine and sodium pyruvate are both used as growth factors by staphylococci while the pyruvate also neutralises any toxic peroxides that may be formed.

Unlike some commercially available preparations, Lab M Baird-Parker Medium can be used with either Egg Yolk Tellurite (X085) or Rabbit Plasma Fibrinogen (X086).

When Baird-Parker medium is used with Egg Yolk Tellurite X085, presumptive *S. aureus* appear as black colonies demonstrating lecithinase activity (an opaque zone around the colony) and lipase activity (a zone of clearing encircling the opaque zone). Suspected *S. aureus* colonies should be confirmed with RPF for coagulase or latex agglutination test.

Rabbit plasma fibrinogen (RPF X086) is a more specific alternative to egg yolk tellurite and allows the direct detection of coagulase-positive *S. aureus*. Typical *S. aureus* appear as black colonies surrounded by a zone of precipitation (demonstrating coagulase activity). This is recognised as the gold standard method for the identification of *S.aureus*. RPF overcomes any issues with atypical colony forms and its use means further confirmatory tests are not necessary.

Formula	g/litre
Tryptone	10.0
Beef Extract	7.5
Yeast Extract	1.0
Lithium chloride	5.0
Glycine	12.0
Sodium pyruvate	10.0
Agar No. 2	20.0

Method for reconstitution

For Baird-Parker Medium LAB085 with Egg Yolk Tellurite X085

Weigh 65.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise at 121°C for 15 minutes. Cool to 47°C and add 5% (50mL) X085. Mix well before aseptically pouring into sterile Petri dishes. Dry the agar surface prior to use.

For Baird-Parker Medium LAB085 with Rabbit Plasma Fibrinogen (RPF) Supplement X086

Weigh 6.55 grams of powder and disperse in 90mL of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise at 121°C for 15 minutes. Cool to 47°C and add 1 vial of reconstituted X086. Mix well before aseptically pouring into sterile Petri dishes. Dry the agar surface prior to use.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Final medium: opaque cream/pale fawn gel (with X085)

translucent, pale straw gel (with X086)

pH: 6.8 ± 0.2

Minimum Q.C. organisms:

Staphylococcus aureus ATCC 6538	>50% recovery, typical colonies
Staphylococcus aureus ATCC 25923	>50% recovery, typical colonies
Staphylococcus aureus ATCC 6538P	>50% recovery, typical colonies
Staphylococcus epidermidis ATCC 12228	Growth, typical colonies
Escherichia coli ATCC 25922	Inhibited

Storage of Prepared Medium: Dehydrated culture media: 10-25°C

Poured plates: LAB085+X085 upto 3 days at 2-8°C in the dark; LAB085+X086 use on day of preparation.

Inoculation: Surface inoculation.

Incubation:
LAB085+X085: 37°C aerobically for 48 hours.
LAB085+X086: 37°C aerobically for 24-48 hours.

Growth characteristics(with X085)

organism	colony size (mm)	shape & surface	colour	other
S. aureus	1.0-3.0	CV.E.G.	Black	Narrow opaque margin surrounded by a zone of clearing
S. saprophyticus	0.5-2.0	CV.E.G.	Black	(poor growth)
Other Coagulase negative staphylococci	0.5-1.0	CV.E.G.	Black	(no growth)
Proteus spp.	0.5-2.0	F.Rz.G	Brown Black	(no growth)
Bacillus spp.	0.5-1.0	F.Rz.D.	Brown	(no growth)
Entero-bacteriaceae	no growth			

Growth characteristics(with X086)

organism	colony size (mm)	shape & surface	colour	other
Coagulase positive *S. aureus*	1.0-3.0	CV.E.G. . .	White Grey Black	Narrow opaque zone of coagulase activity
Coagulase negative staphylococci	0.5-2.0	CV.E.G. .	White Grey Black	(poor growth)
Proteus spp.	0.5-2.0	F.Rz.G	Brown Black	(no growth)
Bacillus spp.	0.5-1.0	F.Rz.D.	Brown	(no growth)
Entero-bacteriaceae	no growth			

References

Baird-Parker, A.C. (1962). An improved diagnostic and selective medium for isolating coagulase positive staphylococci. J. Appl. Bact. 25(1): 12-19.

Baird-Parker, A.C. and Davenport, E. (1965). The effect of Recovery medium on the isolation of S. aureus after heat treatment and after storage of frozen or dried cells. J. Appl. Bact 28: 390-402.

Ten Broeke, R. (1976). The Staphylococcus medium of Baird-Parker in practical use. The occurrence of coagulase-positive, egg yolk nonclearing staphylococci. Antonie van Leeuwenhoek 33: 220-236.

Smith, B.A. and Baird-Parker, A.C. (1964). The use of sulphamethazine for inhibiting Proteus spp. on Baird-Parker's isolation medium for Staphylococcus aureus. J. Appl. Bact 27(1): 78-82.

Beckers N J. et al (1984). Canad. J. Microbiol. 30: 470-474.

Sawhney D. (1986) J. Appl Bact. 61:149-155.

Baird-Parker Medium Base (ISO)

LAB285

Description

For the isolation of coagulase-positive staphylococci. Formulated to ISO 6888-1 and compliant to ISO 6888-2 and ISO 6888-3.

Originally introduced in 1962, this medium was developed by Baird-Parker to overcome the problems of recovering damaged *Staphylococcus aureus* from foodstuffs. This version of the medium is formulated according to ISO 6888-1:1999+A1:2003 and is in compliance with ISO 6888-2:2003+A1:2003 and ISO 6888-3:2003.

Baird-Parker medium is highly selective by nature, due to the presence of potassium tellurite and lithium chloride. Tellurite inhibits most coliforms and is also reduced to telluride by *S. aureus*, giving the typical black colonies. Glycine and sodium pyruvate are both used as growth factors by staphylococci while the pyruvate also neutralises any toxic peroxides that may be formed.

As with Lab M's traditional Baird-Parker Medium, LAB085, and unlike some commercially available preparations, the new ISO formulated Baird-Parker medium can be used with either Egg Yolk Tellurite (X085) or Rabbit Plasma Fibrinogen (X086).

When Baird-Parker medium is used with Egg Yolk Tellurite X085, presumptive *S. aureus* appear as black colonies demonstrating lecithinase activity (an opaque zone around the colony) and lipase activity (a zone of clearing encircling the opaque zone). Suspected *S. aureus* colonies should be confirmed with RPF for coagulase or latex agglutination test.

Rabbit plasma fibrinogen (RPF X086) is a more specific alternative to egg yolk tellurite and allows the direct detection of coagulase-positive *S. aureus*. Typical *S. aureus* appear as black colonies surrounded by a zone of precipitation (demonstrating coagulase activity). This is recognised as the gold standard method for the identification of *S.aureus*. RPF overcomes any issues with atypical colony forms and its use means further confirmatory tests are not necessary.

Formula	g/litre
Pancreatic digest of casein	10.0
Yeast extract	1.0
Meat extract	5.0
Sodium pyruvate	10.0
L-Glycine	12.0
Lithium chloride	5.0
Agar	20.5

Method for reconstitution

For Baird-Parker Medium LAB285 with Egg Yolk Tellurite X085

Weigh 63.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise at 121°C for 15 minutes. Cool to 48°C and add 5% (50mL) X085. Mix well before aseptically pouring into sterile Petri dishes. Dry the agar surface prior to use. Sulphamezathine may be added at 0.05g/L to suppress the swarming of *Proteus* spp.

For Baird-Parker Medium LAB285 with Rabbit Plasma Fibrinogen (RPF) Supplement X086

Weigh 6.35 grams of powder and disperse in 90mL of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise at 121°C for 15 minutes. Cool to 48°C and add 1 vial of reconstituted X086. Mix well before aseptically pouring into sterile Petri dishes. Dry the agar surface prior to use.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Final medium: opaque cream yellow gel (with X085) clear, straw gel (with X086)

pH: 7.2 ± 0.2

Minimum Q.C. organisms:	
Staphylococcus aureus ATCC 6538	>50% recovery, typical colonies
Staphylococcus aureus ATCC 25923	>50% recovery, typical colonies
Staphylococcus aureus ATCC 6538P	>50% recovery, typical colonies
Staphylococcus epidermidis ATCC 12228	Growth, typical colonies
Escherichia coli ATCC 25922	Inhibited
Escherichia coli ATCC 8739	Inhibited

Storage:

Dehydrated culture media: 10-25°C

Poured plates: LAB285+X085 upto 3 days at 2-8°C in the dark; LAB285+X086 use on day of preparation.

Inoculation:

LAB285+X085: surface inoculation as per user's validated methods.
LAB285+X086: surface inoculation or pour plate as per user's validated methods.

Incubation:

LAB285+X085: 37°C aerobically for 48 hours.
LAB285+X086: 37°C aerobically for 24-48 hours.

Interpretation:

LAB285+X085: Presumptive *S. aureus* colonies appear as black colonies demonstrating lecithinase activity and lipase activity. All black colonies (suspected *S. aureus*) should be confirmed with a coagulase test (RPF) or a latex agglutination kit.

LAB285+X086: Typical *S. aureus* appear as black colonies surrounded by a zone of coagulase activity.

References

ISO 6888-1:1999+A1:2003 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird-Parker agar medium (includes amendment A1:2003).

ISO 6888-2:1999+A1:2003 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using rabbit plasma fibrinogen agar medium (includes amendment A1:2003).

ISO 6888-3:2003 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 3: Detection & MPN technique for low numbers.

ISO/TS 11133-2:2003 Microbiology of food and animal feeding stuffs - Guidelines on preparation and production of culture media - Part 2: Practical guidelines on performance testing of culture media (ISO/TS 11133-2:2003).

Baird-Parker, A.C. (1962). An improved diagnostic and selective medium for isolating coagulase-positive staphylococci. *J. Appl.* Bact. 25(1):12-19.

Smith, B.A. and Baird-Parker, A.C. (1964). The use of sulphamezathine for inhibiting *Proteus* spp. on Baird-Parker's isolation medium for *Staphylococcus aureus*. J. Appl. Bact. 27(1):78-82

BCYE Legionella Isolation Medium

LAB195

Description

BCYE (Buffered Charcoal Yeast Extract) Legionella Isolation Medium (LAB195) is a base medium used for the isolation of Legionella from clinical and environmental samples. This medium is based on the charcoal yeast extract formulation of Feeley *et al*.[1&2] The performance of this medium is further enhanced by the additions of ACES (N-2-acetamido-2-aminoethane - sulphonic acid) buffer and α-ketoglutarate as defined by Edelstein[3]. This medium is also detailed in internationally recognized methodology[4] for the isolation of *Legionella* spp. from water.

Specimens or samples are often heavily contaminated with other bacteria and consequentially a range of selective supplements have been developed to aid isolation. Lab M provide the GVPC supplement (X195) which is most effective for the isolation of *L. pneumophila*. It is recommended that this supplement is used in conjunction with heat and acid sample treatments, to further reduce the growth of non-*Legionella* bacteria.

This product contains the ACES buffer and ferric pyrophosphate in the base medium. This negates the need for complex freeze dried supplements. A complementary growth supplement is provided (X196) which contains the L-cysteine and α-ketoglutarate. In addition, an α-ketoglutarate supplement (X197) is also available for the preparation of confirmatory media for suspected *Legionella* colonies.

Principle of isolation

Water samples are concentrated either by membrane filtration or centrifugation (turbid samples may also be centrifuged). To reduce the growth of unwanted bacteria, separate portions of the concentrated sample may be subjected to heat and acid treatments. Treated and untreated portions are then inoculated onto *Legionella* selective media.

Formula	g/litre
Yeast Extract	10.0
Charcoal	2.0
Ferric	0.25
Pyrophosphate ACES Buffer	10.0
Potassium Carbonate	2.28
Agar	14.0

Supplements

GVPC Selective Supplement (X195)	
Formula	
Glycine	3000mg
Vancomycin	1mg
Polymyxin B	79200IU
Cycloheximide	80mg

BCYE Growth Supplement (X196)	
Formula	
L-Cysteine	400mg
α-ketoglutarate	1000mg

Presumptive ID (X197)	
Formula	
α-ketoglutarate	1000mg

Add one vial per 500mL of sterilised medium as appropriate.

Method for reconstitution

Selective Isolation (GVPC BCYE)

Weigh 38.5 grams of powder and disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix and sterilise by autoclaving at 110ºC for 10 minutes. Cool to 47ºC and aseptically add 2 vials of reconstituted growth supplement X196 and 2 vials of reconstituted selective supplement X195. Mix well and pour into sterile Petri dishes.

Maintenance (BCYE)

Weigh 38.5 grams of powder and disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix and sterilize by autoclaving at 110ºC for 10 minutes. Cool to 47ºC and aseptically add 2 vials of reconstituted growth supplement X196. Mix well and pour into Petri dishes.

Presumptive Identification (BCYE no L-Cysteine)

Weigh 38.5 grams of powder and disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix and sterilize by autoclaving at 110ºC for 10 minutes. Cool to 47ºC and aseptically add 2 vials of reconstituted growth supplement X197. Mix well and pour into Petri dishes.

pH: 6.9 ± 0.1

Inoculation:

The concentrated sample should be split into 3 portions. One portion is used without any further treatment, the other 2 portions should be treated, one with heat and the other with acid.

Heat Treatment

Take 1ml of the concentrated sample and place in a water bath at 50ºC for 30 minutes.

Acid Treatment

Take 1-10ml of the concentrated sample and centrifuge at 6000g for 10 minutes. Decant the supernatant to leave half the original volume. Vortex to re-suspend the pellet and make up to the original volume using an HCl-KCl buffer. Leave to stand for 5 minutes.

Inoculate the first plate of GVPC supplemented media with 0.1mL of the untreated portion and spread over the entire surface of the plate. Inoculate the second plate of GVPC supplemented media in the same way with 0.1ml of the heat treated portion as soon as possible after removal from the water bath. Inoculate the third plate of GVPC supplemented media in the same way with 0.1mL of the acid treated portion immediately after acid treatment.

Incubation:

Incubate at 36 + 1ºC in a humid atmosphere under aerobic conditions for up to 10 days.

Interpretation:

The plates should be examined for growth on days 3, 5, 7 and 10. Suspect colonies should be sub-cultured on to "maintenance" supplemented BCYE medium and "presumptive ID" supplemented BCYE medium, incubate as before. Isolates that fail to grow on the "presumptive ID" medium but grow on the maintenance medium and have typical morphology should be regarded as presumptive *Legionella*.

Presumptive isolates should be confirmed using a serological method, e.g. Microgen M45 Latex.

Minimum Q.C. organisms: *Legionella* spp. - Growth
Staphylococcus epidermidis - Growth
Escherichia coli - Inhibited

References

Feeley, J.C., Gibson, R.J. *et al.* (1979). *Journal of Clinical Microbiology* **10**: 437-441

Pesculle, A.H., Feeley, J.C. *et al.* (1980). *Journal of Infectious Disease* **141**: 727-732

Edelstein, P.H. (1982). *Journal of Clinical Microbiology* **14**: 298-303

International Standard. ISO 11731:1998(E). Water Quality – Detection & Enumeration of *Legionella*.

Bile Aesculin Agar

LAB207

Description

For the isolation and presumptive identification of Enterococci / Group D Streptococci. The aesculin produced by organisms positive for aesculin hydrolysis reacts with ferric citrate to form a dark brown or black complex. Bile salts inhibit Gram-positive organisms other than Enterococci or Group D Streptococci. This medium can also be used for presumptive differentiation of the Klebsiella-Enterobacter-Serratia group from other *Enterobacteriaceae*.

Formula	g/litre
Peptone	8.0
Bile salts	20.0
Ferric citrate	0.5
Aesculin	1.0
Agar	15.0

Method for reconstitution

Weigh 44.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121ºC. Cool to 47°C, mix well and dispense into Petri dishes..

Appearance:

Powder: fine, free-flowing, homogeneous, buff.

Finished medium: Buff/pale brown gel.

pH: 7.1 ± 0.2

Hazard classification

Xi – Irritant

Minimum Q.C. organisms:
Enterococcus faecalis ATCC 29212
Enterobacter aerogenes ATCC 13048
Streptococcus pyogenes NCTC 8198

Storage:

Dehydrated culture media: 10-25ºC.

Poured plates: 7 days at 2-8°C in the dark.

Inoculation: Surface inoculation as per user's validated methods.

Incubation: Incubate at 37°C for 18-24 hours.

Interpretation

organism	colony size (mm)	observations
Enterococcus spp.	0.1 - 0.3	Blackening of media around colony
Enterobacter spp.	1 - 2	Blackening of media around colony
Pseudomonas aeruginosa	0.3 - 0.6	
Escherichia coli	1.5 - 2.5	
Stahpylococcus aureus	0.5	
Streptococcus pyogenes	Inhibited	

Bismuth Sulphite Agar

(Wilson and Blair Medium)

LAB013A + LAB013B

Description

A modification of Wilson and Blair's original medium for the isolation of *Salmonella typhi* and other *Salmonella* from clinical samples, sewage and other materials. The presence of bismuth sulphite and brilliant green make this medium highly selective. As the medium contains neither lactose nor sucrose it can be used to detect lactose and sucrose fermenting *Salmonella.*

Formula	g/litre
Bismuth Sulphite Agar Base 'A' LAB013A	
Beef Extract	6.0
Balanced Peptone No. 1	10.0
Ferric citrate BPC	0.4
Brilliant Green	0.01
Agar No. 2	20.0
Bismuth Chemical Mixture 'B' LAB13B	
Bismuth ammonium citrate	3.0
Sodium sulphite	5.0
Disodium phosphate	5.0
Glucose	5.0

Method for reconstitution

Agar Base 'A': Weigh 36.4 grams of powder and mix with 1 litre of deionised water. Sterilise for 15 minutes at 121˚C. Cool to 50˚C approx. and add 100ml of Chemical Mixture 'B'. Mix well and pour thin plates. Store at 4˚C for 3 days to mature, before use.

Chemical Mixture 'B': Suspend 18 grams of powder in 100ml of deionised water. Bring to boil over a tripod and gauze, and cool quickly in cold water. Add to 1 litre of Agar Base 'A' prepared as above.

Appearance: Pale green, opaque gel.

pH: 7.6 ± 0.2

Minimum Q.C. organisms: *Salmonella* sp. NCIMB 50076
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Plates – store 3 days before use. Use within 7 days. Store at 2-8˚C in the dark.

Inoculation: Surface, streak out to single colonies.

Incubation: 37˚C for 24 hours aerobically.

Growth characteristics

organism	colony size (mm)	shape & surface	colour	other
S. typhi	1.5-2.0	CV.E.G.	Black	Metallic sheen black deposit in medium. (H_2S-ve strains green)
Other *Salmonella* spp.	1.0-2.5	CV.E.G.	Black/ Green	Metallic sheen especially in heavy growth, single colonies may give rabbit eye appearance
E. coli	P.P.-1.0	CV.E.G.	Green	
Klebsiella spp.	P.P-2.0	CV.E.G.	Green	
Citrobacter spp.	1.0-2.5	CV.E.G.	Green	(black centre)
Proteus spp.	1.0-2.5	CV.E.G.	Green / Brown	(black centre)

References

Wilson, W.J. and Blair, E.M. M'V (1926). A combination of bismuth and sodium sulphites affording an enrichment and selective medium for the typhoid-paratyphoid groups of bacteria. J. Pathol. Bacteriol., 29: 310-311.
International Journal of Food Microbiology (1987) 5:3:200-202.
I.C.M.S.F. (1978) Micro organisms in Foods I. Their significance and enumeration. 2nd edition Univ of Toronto Press. Speck M.L. Compendium of methods for microbiological examination of foods. (1984) 2nd edition. American Public Health Association, Washington. 3/102

Blood Agar Base

LAB028

Description

An inexpensive general purpose agar base which, with the addition of 5% sterile blood, can be used to cultivate a wide range of micro organisms of clinical significance. Typical haemolysis patterns are obtained with this medium.

Formula	g/litre
Beef Extract	10.0
Balanced Peptone No. 1	10.0
Sodium chloride	5.0
Agar No. 2	12.0

Method for reconstitution

Weigh 37 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving for 15 minutes at 121˚C. Cool to 47˚C and add 5-7% sterile defibrinated blood. Mix by swirling the flask and pour into Petri dishes.

Appearance: Dependent upon blood additive.

pH: 7.4 ± 0.2

Minimum Q.C. organisms: S. *aureus*. NCIMB 50080
S. pyogenes ATCC 19615

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface, streaking to single colonies.

Incubation: 37˚C aerobically, anaerobically or microaerobically for 24 hours.

Growth characteristics

organism	colony size (mm)	shape & surface	colour	other
S. aureus	0.5-1.5	CV.E.G.	White-Golden	haemolytic
S. pyogenes	P.P.-1.0	CV.E.G.	Grey	beta haemolytic alpha haemolytic non-haemolytic
S. pneumoniae	P.P.-1.0	F.E.G.	Grey	alpha haemolytic draughtsman
N. meningitidis	P.P.-1.5	CV.E.G.	Grey	mucoid
E. coli	1.5-2.5	CV.E.G.	Grey	haemolytic
Ps. aeruginosa	0.5-3.0	F.CR.D.	Grey	many colonial forms green pigment
C. perfringens	0.5-1.5	CV.CR.G.	Grey	Target haemolysis non-haemolytic
B. fragilis	0.5-1.5	CV.E.G.	Grey	mucoid
P. anaerobius	P.P.-0.5	CV.E.G.	Grey-White	
F. necrophorum	P.P.	CV.E.G.	Trans-parent	haemolytic

References

Cruikshank, R. (1972). Medical Microbiology. 11th edn. Livingstone, London

Blood Agar Base No. 2

LAB015

Description

A very rich agar base which, with the addition of blood, is capable of growing delicate clinical pathogens. The medium gives colonial appearances, haemolysis patterns and pigment production of diagnostic value. When the blood is 'chocolated' the medium gives good recovery of *Haemophilus* spp. The medium can be made selective for various groups by the addition of appropriate antibiotic mixtures eg:

Streptococci –Colistin/Oxolinic acid (X013)
Gardnerella spp. – Colistin/Oxolinic acid (X011)
C. perfringens – Neomycin (X015) (X016)
Staphylococci/streptococci – Colistin/Naladixic acid (X012)

Formula	g/litre
Tryptose	15.0
Soy Peptone	2.5
Yeast Extract	5.0
Sodium chloride	5.0
Agar No. 2	12.0

Method for reconstitution

Weigh 39.5 grams of powder, disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix then sterilise for 15 minutes at 121˚C. Cool to 47˚C then aseptically add 5-7% sterile, defibrinated horse or sheep blood. Mix well before pouring.

Appearance: Dependent upon blood additive.

pH: 7.4 ± 0.2

Minimum Q.C. organisms: *S. aureus* NCIMB 50080
S. pyogenes ATCC 19615

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface, streaking out to single colonies.

Incubation: 37˚C aerobically or microaerobically for 24 hours, anaerobically for 24 and 48 hours.

Growth characteristics

organism	colony size (mm)	shape & surface	colour	other
S. aureus	1.5-2.0	CV.E.G.	White/ Golden	(haemolytic)
S. pyogenes	1.0-1.5	CV.E.G.	Grey	beta haemolytic) (alpha or non haemolytic)
S. pneumoniae	0.5-1.0	F.E.G.	Grey	(draughtsman (alpha haemolytic) (mucoid) (require CO_2)
N. meningitidis	0.5-1.0	CV.E.G.	Grey	(May require CO_2)
E. coli	2.0-3.0	CV.E.G.	Grey	(haemolytic)
Ps. aeruginosa	1.0-3.0	F.CR.D.	Grey	(green pigment (haemolytic)
C. perfringens	1.0-2.5	CV.CR.-(E)G	Grey	"Target"-haemolysis (non-haemolytic)
B. fragilis	1.0-1.5	CV.E.G.	Grey	non haemolytic
P. anaerobius	0.5-1.0	CV.E.G.	White	non haemolytic

Brain Heart Infusion Agar

LAB048

Description

A general purpose nutritious agar base. This medium was first used for the isolation of dental pathogens. The mixture of brain and heart infusions is particularly useful in the isolation of *Actinomyces israeli* and *Histoplasma capsulatum.* With the addition of 7% defibrinated blood the medium will support the growth of a wide range of fastidious organisms, the phosphate buffer will help neutralise the acids produced from the utilisation of glucose and thus maintain viability. The medium is not recommended for the determination of haemolytic reactions because of the glucose content.

The use of porcine material in this product ensures there are no Specified Risk Materials (SRM'S) with respect to Transmissible Spongeform Encephalopathies (TSE'S).

Formula	g/litre
Brain-Heart Infusion Solids (porcine)	17.5
Tryptose	10.0
Glucose	2.0
Sodium chloride	5.0
Disodium phosphate	2.5
Agar No. 2	12.0

Method for reconstitution

Weigh 49 grams of powder, disperse in 1 litre of deionised water. Allow to stand for 10 minutes then swirl to mix. Sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C then pour into Petri dishes.

Appearance: Pale Straw colour, clear gel.

pH: 7.4 ± 0.2

Minimum Q.C. organisms: *S. aureus* NCIMB 50080
E. coli NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark. Capped container – up to 3 months at 15-20˚C in the dark.

Inoculation: Surface, streaking out to single colonies.

Incubation: Time and temperature to suit specimen/organisms.

Growth characteristics (with horse blood)

organism	colony size (mm)	shape & surface	colour
S. aureus	1.0-1.5	CV.E.G.	White/ Golden
other Staphylococci	0.5-1.5	CV.E.G.	White/ Yellow
S. pyogenes	0.5-1.0	CV.E.G.	White
S. milleri	P.P.-0.1	CV.E.G.	Transp. (White)
E. faecalis	1.0-1.25	CV.E.G.	Grey/ Green
S. pneumoniae	0.5-1.0	F.E.G.	Grey/ Green
E. coli	2.0-3.0	CV.E.G.	Grey
Pseudomonas aeruginosa	2.0-4.0	F.CR.D.	Grey

References

Roseburg. T., Epps, L.J. and Clarke, A.R. (1944). A study of the isolation, cultivation and pathogenicity of *Actinomyces israeli* recovered from the human mouth and from actinomycosis in man. J. inf. Dis., 74: 131-149.

Howell, E. (1948) Efficiency of methods of isolation of *Histoplasma capsulatum.* Pbl. Hlth. Rep. 63: 173-178. 3/108

Brain Heart Infusion Broth

LAB049

Description

A rich isotonic infusion medium with tryptose (a mixture of meat and milk peptones) providing a wide range of substrates. A low concentration of glucose is used to stimulate early growth. The medium is lightly buffered to prevent the early death of some species due to acid production. Organisms which produce significant amounts of acid may well overwhelm the buffering system and auto-sterilise. The medium is suitable for use as a blood culture medium or as an enrichment broth for fastidious organisms.

The use of porcine material in this product ensures there are no Specified Risk Materials (SRM'S) with respect to Transmissible Spongeform Encephalopathies (TSE'S).

Formula	g/litre
Brain-Heart Infusion solids (porcine)	17.5
Tryptose	10.0
Glucose	2.0
Sodium chloride	5.0
Disodium hydrogen phosphate	2.5

Method for reconstitution

Weigh 37 grams of powder then disperse in 1 litre of deionised water. Allow to stand for 10 minutes then dissolve with gentle heat before dispensing into tubes or bottles. Sterilise at 121˚C for 15 minutes. Overheating will cause caramelisation and darkening of the medium.

Appearance: Straw colour, clear liquid.

pH: 7.4 ± 0.2

Minimum Q.C. organisms: *S. aureus* NCIMB 50080
E. coli NCIMB 50034

Storage of Prepared Medium: Capped container – up to 3 months at 15-20˚C in the dark.

Inoculation: (as a blood culture medium). Using a minimum volume of 50ml of medium add the blood to a dilution of from 1:10 to 1:20. Use in conjunction with an anaerobic culture medium e.g. Fastidious Anaerobe Broth LAB071.

Incubation: 37˚C aerobically for 7 to 15 days.

Interpretation: Observe daily, subculture after 1, 2, 3, 7 and 15 days or immediately on showing signs of growth.

References

Rosenow. E.C. (1919). Studies on selective localisation; focal infection with special reference to oral sepsis. J. Dent. Res. 1:205-267.

Brazier's CCEY Agar

LAB160

Description

Brazier's CCEY agar is the formulation currently used by the Anaerobe Reference Unit for the isolation of *C.difficile,* resulting from work initiated by Ken Phillips and Paul Levett, and completed by Jon Brazier.

Based upon the market leading anaerobe medium, Fastidious Anaerobe Agar, it incorporates additional ingredients to improve the isolation and differentiation of *C.difficile* from clinical specimens.

Cholic acid is present to promote spore germination following alcohol shock treatment, and p-hydroxyphenylacetic acid to enhance the production of p-cresol, a distinctive metabolite of *C.difficile.*

Selectivity is achieved by addition of supplement X093 (cefoxitin cycloserine), whilst egg yolk emulsion X073 is added to help differentiate *C.difficile* from lecithinase positive clostridia. Finally, the addition of lysed horse blood optimises the recognition of colony fluorescence when cultures are examined using UV light.

Formula	g/litre
Peptone Mix	23.0
Sodium chloride	5.0
Soluble Starch	1.0
Agar No. 2	12.0
Sodium bicarbonate	0.4
Glucose	1.0
Sodium pyruvate	1.0
Cysteine HCl	0.5
Haemin	0.01
Vitamin K	0.001
L-arginine	1.0
Soluble pyrophosphate	0.25
Sodium succinate	0.5
Cholic acid	1.0
p-Hydroxyphenylacetic acid	1.0

Method for reconstitution

Weigh 48 grams of powder and add to 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix, and sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C and aseptically add the following: 2 vials of X093, 40ml of Egg Yolk Emulsion X073 and 10ml lysed horse blood. Mix well and pour into Petri dishes.

Appearance: Tan opaque gel.

pH: 7.0 ± 0.2

Minimum Q.C. organisms: *C. difficile*
E. coli (inhibition) NCIMB 50034

Storage of prepared medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface streak untreated or alcohol shocked specimens for single colonies.

Incubation: 37˚C for 24-48hrs under anaerobic conditions

Characteristics of *C.difficile:* Gray opaque flat colonies, raised elevation, 2-3mm diameter, generally circular but tending to elongate in the direction of spreading, ground glass appearance and a rough, fimbriate edge. Lecithinase negative. Incubation longer than 48hrs may result in a lighter gray or white centre to the colony. Phenolic odour due to the production of p-cresol. Colonies fluoresce yellow-green under UV light. Confirm by latex agglutination.

References

Brazier J.S. (1993) Rôle of the Laboratory in Investigations of Clostridium difficile Diarrhoea. Clinical Infectious Diseases 16 (4) 228-33.

Brilliant Green Agar (modified)

(Phenol Red Brilliant Green Agar, BPLS)

LAB034

Description

First introduced by Kristensen *et al* in 1925 as a selective medium for the isolation of salmonellae (except *S. typhi*). The medium was modified by the Netherlands Institute for Public Health, Utrecht. The modification was to increase the selectivity of the medium by increasing the dye concentration. This formulation is quoted by the International Standards Organisation, standard European Community Methods, the American Public Health Association and the Association of Official Analytical Chemists. The medium is suitable for subcultures from selective enrichment media. However because this medium is highly selective, small numbers of salmonellae may be missed. This medium is definitely not recommended for *S. typhi* and *Shigella* spp. Less inhibitory media such as X.L.D. and Hektoen Enteric Agar will be useful in detecting salmonellae and shigellae inhibited by Brilliant Green Agar.

Formula	g/litre
Beef Extract	5.0
Balanced Peptone No. 1	10.0
Yeast Extract	3.0
Disodium hydrogen phosphate	1.0
Sodium dihydrogen phosphate	0.6
Lactose	10.0
Sucrose	10.0
Phenol red	0.09
Brilliant green	0.0047
Agar No. 2	12.0

Method for reconstitution

Weigh 52 grams of powder and disperse in 1 litre of deionised water. Allow to soak for ten minutes and then bring to the boil with frequent swirling to dissolve the solids and cool to 47˚C in a water bath. Pour plates and dry the surface before inoculation. DO NOT remelt or autoclave: overheating causes precipitation of the medium. Store plates away from light.

Appearance: Tan, clear gel.

pH: 6.9 ± 0.2

Minimum Q.C. organisms: *Salmonella* sp. NCIMB 50076
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface streaking for single colonies, a heavy inoculum can be used.

Incubation: 37˚C for 18-24 hours aerobically.

Growth characteristics

organism	colony size (mm)	shape & surface	colour	other
Salmonella spp.	1-1.5	CV.E.G.	Pink colonies	(red zone in medium)
S. typhi	1.0	CV.E.G.	Pink/Red	(may not grow)
E. coli	no growth			(0.5-1.0 yellow colony)
Proteus spp	no growth			
Ps aeruginosa	no growth			(crenated small red colonies)
Klebsiella spp.	1-1.5	CV.E.G.	Green colonies	(yellow) (NG)
Enterococcus spp.	no growth			
S. sonnei	no growth			(0.5 mm red)

References

Edel, W. and Kamplemacher, E.H. (1968). Comparative studies on Salmonella isolation in eight European laboratories. Bull. Wld. Hlth. Org. 39: 487-491.

Edel, W. and Kamplemacher, E.H. (1969). *Salmonella* infections in nine European laboratories using a standard technique. Bull Wld. Hlth. Org. 41: 297-306.

American Public Health Association (1966). Recommended Methods for the Microbiological Examination of Foods, 2nd end. (ed. J.M. Sharf) A.P.H.A. Washington.

Association of Official Analytical Chemists (AOAC) (1978) Bacteriological Analytical Manual, 5th edn., Washington D.C.

Pharmacopoeia of culture media for food microbiology. (1987). Int. J. Food Microbiol. 513: 245-247. 3/112

Brilliant Green Bile 2% Broth

LAB051

Description

A modification of MacConkey's medium, formulated in 1926 by Dunham and Schoenlein, for the recovery of coliform bacteria in foodstuffs and water. The brilliant green and bile inhibit most Gram positive organisms thus overcoming the problem of some *Clostridium* spp. fermenting lactose and giving false positive results.

Formula	g/litre
Balanced Peptone No. 1	10.0
Lactose	10.0
Ox Bile	20.0
Brilliant green	0.0133

Method for reconstitution

Weigh 40 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then warm to dissolve. Dispense into tubes or bottles with inverted Durham tubes. Sterilise by autoclaving at 115˚C for 15 minutes.

Appearance: Green, clear.

pH: 7.4 ± 0.2

Minimum Q.C. organisms: *Salmonella* sp. NCIMB 50076
E. coli NCIMB 50034

Storage of Prepared Medium: Capped containers – up to 1 month at 2-8˚C in the dark.

Inoculation: Serial 1:10 dilutions of homogenised sample are inoculated into the broth in the proportion of 1ml sample to 9ml broth. Ensure the Durham tube is free from gas bubbles before commencing inoculation. B.G.B. broth can be used at double strength if required but cannot be sterilised by autoclaving, pasteurisation must be used instead.

Incubation: *E. coli* and thermotrophs 44˚C for 18 hours aerobically. Mesopholic coliforms 32˚C for 24-48 hours aerobically. Psychrotrophic coliforms 4˚C for 10 days aerobically.

Interpretation: Turbidity, colour changes (to yellow or yellowish green) and production of gas are all presumptive evidence of the growth of organisms of the coli-aerogenes group. Confirmation by indole production in Tryptone Water LAB129 (44˚C for *E. coli*).

References

Pharmacopoeia of Culture Media for Food Microbiology (1987). Int. J. Food Microbiol. 5:3:206-207.

American Public Health Association, American Water Works Association and Water Pollution Control Federation, (1975), Standard Methods for the Examination of Water and Wastewater, 14th ed., Washington D.C.

Association of Official Analytical Chemists (AOAC). Bacteriological Analytical Manual, 5th ed., Washington, D.C. Association of Official Analytical Chemists. 1978.

Hausler, W. J. (ED) (1972). Standard Methods for the Examination of Dairy Products. 13th ed., Washington. D.C. American Public Health Association.

Shane, M.S. (1947). Studies on false confirmed test using B.G.B. and comparison studies on Lauryl Sulfate Tryptose Broth as presumptive medium. J. Am. Water Works Assoc., 39: (4), 337.

Bromocresol Purple Lactose Agar

(Drigalski agar)

LAB121

Description

A non-selective differential medium for the isolation and enumeration of *Enterobacteriaceae* from urine, water and food products. Lactose fermenting organisms produce yellow colonies, non lactose fermenters produce purple colonies.

Formula	g/litre
Peptone mixture	7.4
Lactose	8.5
Bromocresol purple	0.025
Agar No. 1	12.0

Method for reconstitution

Weigh 28 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Allow to cool to 47˚C then pour into Petri dishes.

Appearance: Purple, clear agar.

pH: 6.8 ± 0.2

Minimum Q.C. organisms: *E. coli.* NCIMB 50034
S. aureus NCIMB 50080

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface, plating either over entire surface for colony count or streak out to single colonies.

Incubation: 37˚C aerobically for 18-24 hours.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
E. coli	1.5-2.0	CV.E.G.	Yellow	(N.L.F. – purple)
Klebsiella spp.	4.0-6.0	CV.E.G.	Yellow	(mucoid)
Citrobacter spp.	2.0-2.5	CV.E.G.	Yellow	
Proteus spp.	1.0-1.5	CV.E.G.	Purple	
Salmonella spp.	1.0-2.0	CV.E.G.	Purple	
S. aureus	0.5	CV.E.G.	Cream	(purple if N.L.F.)
E. faecalis	0.5	CV.E.G.	Yellow	

References

Drigalski, C. (1902). Uber ein Verfahren zum Nachweis der Typhusbacillen. Z. Hyg. Infekt. 39:283-300.

Buffered Listeria Enrichment Broth

LAB139

Description

A medium for the selective enrichment of food and environmental samples for *Listeria* spp, LAB139 is a buffered version of the 'FDA' broth LAB138. The extra buffering capacity maintains the pH of the enrichment culture during incubation, ensuring optimum conditions for the recovery of *Listeria* spp.

Formula	g/litre
Tryptone	17.0
Soy peptone	3.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	2.5
Glucose	2.5
Yeast Extract	6.0
Potassium dihydrogen phosphate	1.35
Disodium hydrogen phosphate	9.6

Method for reconstitution

Weigh 47 grams of powder and add to 1 litre of deionised water. Allow to soak for 10 minutes then swirl to mix and sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C and add 2 vials of X138 (X139 can be used as an alternative) reconstituted in 50% alcohol. Aseptically dispense into sterile tubes or bottles.

Appearance: Yellow, clear.

pH: 7.2 ± 0.2

Minimum Q.C. organisms: *L. monocytogenes* NCIMB 50007
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Capped containers – up to 14 days at 2-8˚C in the dark.

Inoculation: Add 25 grams of sample to 225mls of Buffered Listeria Enrichment Broth and homogenise.

Incubation: 30˚C aerobically for up to 48 hours.

Subculture: After 24 and 48 hours onto Listeria Isolation Medium – LAB122.

Buffered Peptone Water

LAB046

Description

A pre-enrichment medium designed to help sublethally damaged salmonellae recover before introducing them into a selective medium. This nutrient medium is free from inhibitors and is well buffered to maintain the pH at 7.2 for the incubation period. Sublethal injury to salmonellae occurs in many food processes and this pre-enrichment step greatly increases recovery of these organisms.

Formula	g/litre
Peptone	10.0
Sodium chloride	5.0
Disodium hydrogen phosphate	3.7
Potassium dihydrogen phosphate	1.5

Method for reconstitution

Weigh 20 grams of powder and disperse in 1 litre of deionised water. Mix to dissolve then distribute into tubes or bottles. Sterilise by autoclaving at 121˚C for 15 minutes.

Appearance: Pale straw, clear liquid.

Minimum Q.C. organisms: *E. coli* NCIMB 50034

pH: 7.2 ± 0.2

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation: Add 25 grams of sample to 225ml of Buffered Peptone Water and homogenise.

Incubation: Aerobically at 37˚C for 18-24 hours.

Subculture: 10ml aliquots in 100ml of Selenite Cystine Broth LAB055 and 0.1ml into 10ml Rappaport Vassiliadis Medium LAB086.

References

Edel W. and Kampelmacher E.H. (1973). Bull. Wld Hlth Org. 48: 167-174.

Poemla P.K. and Silliker J.H. (1976) *Salmonella* in Compendium of Methods for microbiological examination of foods. Am. Pub. Health Ass., Washington.

Buffered Peptone Water (ISO)

LAB204

Description

Formulated to ISO 6579, this pre-enrichment medium is designed to help sublethally damaged salmonellae recover before introducing them into a selective medium. This nutrient medium is free from inhibitors and is well buffered to maintain pH 7.0 for the incubation period. Sublethal injury to salmonellae occurs in many food processes and this pre-enrichment step greatly increases recovery of these organisms.

Formula	g/litre
Enzymatic digest of casein	10.0
Sodium chloride	5.0
Disodium hydrogen phosphate (anhydrous)	3.6
Potassium dihydrogen phosphate	1.5

Method for reconstitution

Weigh 20.1 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then distribute into tubes or bottles. Sterilise by autoclaving for 15 minutes at 121ºC.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: pale straw, clear liquid

pH: 7.0 ± 0.2

Hazard classification

NR – Not regulated

Minimum Q.C. organisms:
Salmonella typhimurium ATCC 14028
Escherichia coli ATCC 25922

Storage:

Dehydrated culture media: 10-25°C away from direct sunlight

Prepared media: capped containers – up to 3 months at 15-20°C in the dark.

Inoculation: Add 25 grams of sample to 225ml of Buffered Peptone Water and homogenise.

Incubation: Aerobically at 37°C for 18-24 hours.

References

BS EN ISO 6579:2002 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. (Incorporating Corrigendum No. 1)

Campylobacter Blood Free Selective Medium

(Modified CCDA-Improved)

LAB112

Description

A blood free medium which will support the growth of most enteric campylobacters. The selective cocktail X112 (or X212) makes the medium selective for *C. jejuni. C. coli* and *C. laridis* when incubated at 37˚C. With this product incubation at 42˚C is no longer necessary and higher recovery rates have been reported at 37˚C than at 42˚C.

The supplement X112 (or X212) consists of cefoperazone and amphotericin and is superior to the selective cocktails of Skirrow, Butzler and Blazer-Wang all of which contain antibiotics shown to be inhibitors to *C. coli.* The colonial morphologies of *Campylobacter* spp. on this medium are distinctive.

Formula	g/litre
Peptone blend	25.0
Bacteriological Charcoal	4.0
Sodium chloride	3.0
Sodium desoxycholate	1.0
Ferrous sulphate	0.25
Sodium pyruvate	0.25
Agar No. 2	12.0

Method for reconstitution

Weigh 45.5 grams of powder, disperse in 1 litre of deionised water and allow to soak for 10 minutes. Swirl to mix, then sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C then add 2 vials of X112 supplement, mix well and pour into Petri dishes. Continuously mix whilst pouring to prevent the charcoal settling.

Appearance: Black agar.

pH: 7.4 ± 0.2

Minimum Q.C. organisms: *C. jejuni*
E. coli (inhibition) NCIMB 50034
Candida albicans (inhibition) NCIMB 50010

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: *C. jejuni, C. coli. C. laridis* surface streaking to single colonies.

Incubation: 37˚C for 48 hours in an atmosphere of 5% oxygen, 10% carbon dioxide and 85% nitrogen. *C. cinaedi* and *C. fennelliae* require up to 7 days.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
C. jejuni	2.0-3.0	F.E.G.	Grey/ White	Efflorescent (spreading moist)
C. coli	1.0-2.5	CV.E.G.	Creamy Grey	Moist
C. laridis	1.5-3.0	F.E.G.	Grey	(C.V. moist)
C. cinaedi	2.0-3.0	F.E.G.	Pale Grey	requires 7 days in absence of X112
C. fenneliae	2.0-3.0	F.E.G.	Pale Grey	requires 7 days in absence of X112

Other *Enterobacteriacae* – No growth if sensitive to cefoperazone.

References

Bolton F.J. Hutchinson D.N., Parker G. Reassessment of Selective Agars and Filtration Techniques for Isolation of Campylobacter Species from Feces. Eur.J. Clin. Microbiol. Infects. Dis. (1988) 7 p 155-160.

Bolton F. J. (1988) Personal Communication.

Bolton F.J. Hutchinson D.N., Parker G. Isolation of Campylobacter: What are we missing? J.Clin.Path. (1987) 40 p 702-703.

Goosens H., De. Boeck M., Coignau H., Vlaes L., Van Den Borre C., Butzler J.P. Modified Selective Medium for Isolation of Campylobacter spp from Feces: Comparison with Preston Medium, a Blood Free Medium, and a Filtration System. J.Clin. Micro. (1986) 24 p 840-843.

Gun-Munro J., Rennie R.P., Thornley J.H. Richardson H.L., Hodge D., Lynch J. Laboratory and Clinical Evaluation of Isolation Media for Campylobacter jejuni J. Clin Micro. (1987). 25 p 2274-2277.

Herbert G.A., Hollis D.G., Weaver R.E., Karmali M.A., Simor A.E., Roscoe M., Fleming P.C., Smith, S.S. Lane J. Evaluation of a Blood-Free, Charcoal-Based, Selective Medium for the Isolation of Campylobacter organisms from Faeces. J. Clin. Micro. (1986) 23 p 456-459.

Campylobacter Enrichment Broth

(Bolton Formulation)

LAB135

Description

A selective enrichment broth for the isolation of *Campylobacter* spp. from food, environmental samples and faeces. The use of a selective enrichment broth enhances the recovery of sub-lethally damaged organisms due to processing of foods, or if small numbers of campylobacters are present in heavily contaminated specimens. This broth has been shown to give appreciably better results than Preston Broth.

Formula	g/litre
Meat Peptone	10.0
Lactalbumin Hydrolsates	5.0
Yeast Extract	5.0
Sodium chloride	5.0
Haemin	10.0mg
Sodium pyruvate	0.5
α – ketoglutaric acid	1.0
Sodium metabisulphite	0.5
Sodium carbonate	0.6

Method for reconstitution

Weigh 27.6 grams of powder, disperse in 1 litre of deionised water and allow to soak for 10 minutes. Swirl to mix and autoclave at 121˚C for 15 minutes. Cool to 47˚C, add 2 vials of selective supplement X131 (X132 can be used as an alternative) reconstituted with 5ml of 50% alcohol and 50ml of saponin lysed horse blood, mix well and dispense into sterile containers.

Appearance: Translucent, wine-red with a fine black suspension.

pH: 7.4 ± 0.2

Minimum Q.C. organisms: *Campylobacter jejuni*
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Capped containers: 7 days at 2-8˚C in the dark.

Inoculation: Food homogenate is added to broth in a ratio of 1:4 (w/v) in screw cap containers leaving a head space of 1.5 cm. For faeces 1ml of a 10% suspension in Buffered Peptone Water LAB046 is added to 5ml of broth.

Incubation: Aerobically at 37˚C for 2-4 hours, followed by a further 16-44 hours at 42˚C.

Subculture: Onto Campylobacter Blood Free Selective Medium LAB112.

References

Bolton, F.J. Personal Communication.

Hunt J.M., Abeyta C., and Tran T. (1998) Chapter 7 Campylobacter in FDA Bacteriological Analytical Manual 8th Edition.

Cary-Blair Medium

LAB505

Description

Cary-Blair medium is a transport medium for the collection and shipment of clinical specimens based on the formulation of Cary and Blair. The low nutrient content of the medium and the inclusion of phosphate buffer prevents bacterial overgrowth by *E. coli*, *Citrobacter freundii* and *Klebsiella aerogenes*, which can occur in other transport medium containing sodium glycerophosphate. The low oxidation-reduction potential of the medium ensures bacterial survival over long periods.

Cary and Blair reported recovery of cholera vibrios up to 22 days, *Salmonella* and *Shigella* after 49 days and *Yersinia pestis* up to 75 days storage at 28°C. Survival of *Vibrio parahaemolyticus* has been reported after a 35-day period at 70-80°F.

The medium may be prepared as a pre-reduced anaerobic sterilised medium (PRAS) by the Holdeman and Moore method.

Formula	g/litre
Disodium hydrogen phosphate	1.1
Sodium thioglycollate	1.5
Sodium chloride	5.0
Calcium chloride	0.09
Agar	5.6

Method for reconstitution

Weigh 13.3 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix then bring to the boil, with mixing, to dissolve the agar. Distribute into bijou bottles and sterilise by immersing in free-steam for 15 minutes. Allow the medium to cool and tighten the screw caps to prevent water loss.

For transport of fastidious anaerobic bacteria prepare the medium as directed and fill into long narrow screw capped tubes, or to the neck of the Bijou bottle.

Appearance: Colourless soft gel.

pH: 8.4 ± 0.2

Minimum Q.C. organisms: *Shigella sonnei* ATCC® 25931
Vibrio furnissi NCTC 11218

Storage of Prepared Medium: Store away from light at 2-8°C or at room temperature (22-25°C) for up to 19 months.

Inoculation: Use sterile, cotton-tipped swab on wooden sticks to collect the specimen. Push the swab down one third of the depth of the medium and cut the stick. Screw the cap firmly on the bottle. Label the bottle and send to the testing laboratory without delay.

References

Cary, S.G. and Blair, E.B. (1964). J. Bact. 88. 96-98.

Cary, S.G., Matthew, M.S., Fusillo, M.H., and Harkins, C. (1965). Survival of *Shigella* and *Salmonella* in a new transport medium for shipment of clinical samples. Am. J. Clin, Path. 43. 294-296.

Crookes, E.M. and Stuart, R.D. (1959) J. Pathol. Bacteriol. 78. 283-288.

Stuart, R.D. (1959) Public Health Reports 74. 431-438.

Neumann D.A., Benenson, M.W., Hubster, E. and Tuan, N.T.N. (1971). Am. J. Clin. Path. 57.

Wren, M.W.D. J. Med. Microbiol. 10. 195-201.

Holdeman, L.V. and Moore, W.E.C (1975) Anaerobe Laboratory Manual, Virginia Polytechnic Institute Anaerobe Laboratory, 3rd Ed.

C.E.M.O. Agar Base

(Contagious Equine Metritis Organism)

LAB078

Description

This medium is a selective isolation medium for *Taylorella equigenitalis* the causative organism of contagious equine metritis. The medium is a sugar free base with a mixture of high grade casein and soy peptones as nutrients and with L-cystine and sodium sulphite as supplements and reducing agents. The medium is made selective with the addition of amphotericin (5 mg/L) and trimethoprim (10 mg/L). Streptomycin (200 mg/L) can also be used but sensitive variants of *T. equigenitalis* have been described.

Formula	g/litre
Tryptone	15.0
Soy Peptone	5.0
Sodium chloride	5.0
Agar No. 2	12.0
L-Cystine	0.3
Sodium sulphite	0.2

Method for reconstitution

Weigh 37.5 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Allow to cool to 80˚C, add 50ml of sterile horse blood and allow to 'chocolate'. Further cool to 47˚C before adding antibiotic selective agents. Mix well and pour into Petri dishes.

Appearance: Chocolated Blood Agar.

pH: 7.3 ± 0.2

Minimum Q.C. organisms: *T. equigenitalis*
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface, streaking out for single colonies.

Incubation: 37˚C in 10% CO_2 for 2-3 days.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
T. equigenitalis	0.1-1.0	CV.E.G.	Cream	colony size variation is common

References

Atherton, J.G. (1978). Inhibition of the C.E.M. organism in mixed cultures. Vet. Rec. 432.

Mackintosh, M.E. (1981). Bacteriological techniques in the diagnosis of equine genital infections. Vet. Rec. 108, 52-55. Atherton, J.G. Personal Communication.

Fleming, M.P. Tribe. G. W. (1977). Vet. Rec. 101, 1470.

Cetrimide Agar

U.S.P.

LAB133

Description

A medium recommended by the United States Pharmacopoeia for the isolation of *Pseudomonas aeruginosa* from pharmacological preparations. Subculture is carried out onto the medium after enrichment in LAB004 Tryptone Soy Broth. Cetrimide inhibits the growth of many micro organisms whilst allowing *P. aeruginosa* to develop typical colonies which will fluoresce in ultraviolet light and produce green pigment.

Formula	g/litre
Pancreatic Digest of Gelatin	20.0
Magnesium chloride	1.4
Potassium sulphate	10.0
Cetyl trimethylammonium bromide (cetrimide)	0.3
Agar	13.6

Method for reconstitution

Weigh 45.3 grams of powder, disperse in 1 litre of deionised water. Add 10ml of glycerol, allow to soak for 10 minutes then swirl to mix. Sterilise at 121˚C for 10 minutes.

Appearance: Opalescent, pale yellow agar.

pH: 7.2 ± 0.2

Minimum Q.C. organisms: *P. aeruginosa* NCIMB 50067
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Subculture from enrichment broth, streak out for single colonies.

Incubation: 30-35˚C aerobically for 24-48 hours.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour
P. aeruginosa	0.5-1.0	F.CR.D.	green pigment (non pigment) green/yellow fluorescence
P. fluorescens	0.5	CV.R.E.G.	green/yellow fluorescence
E. coli	N.G.		
S. aureus	N.G.		
Proteus spp.	N.G.		

References

United States Pharmacopoeia XXI. 1985.

Brown V.I., Lowbury E.J.L. (1965). Use of an improved Cetrimide Agar Medium and other culture methods for *Pseudomonas aeruginosa.* J. Clin. Pathol. 18, 752-756.

C.L.E.D. Medium

(Mackey and Sandys)
(Cystine Lactose Electrolyte Deficient-Single Indicator)

LAB041

Description

A medium for urine culture first described by Mackey and Sandys in 1960. The absence of electrolytes inhibits the swarming of *Proteus* spp. Cystine is added for the benefit of those organisms which have a specific cystine requirement. Differentiation of lactose and non lactose fermenters is achieved using bromothymol blue as pH indicator. This medium supports the growth of *Streptococcus pyogenes* and most other fastidious organisms that do not require blood.

Formula	g/litre
Balanced Peptone No. 1	4.0
Beef Extract	3.0
Tryptone	4.0
Lactose	10.0
L-Cystine	0.128
Bromothymol blue indicator	0.02
Agar No. 1	15.0

Method for reconstitution

Weigh 36 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving for 15 minutes at 121˚C. Cool to 47˚C mix and distribute into Petri dishes.

Appearance: Green/blue clear gel.

pH: 7.3 ± 0.2

Minimum Q.C. organisms: *E. coli*
NCIMB 50034
S. aureus
NCIMB 50080

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface inoculation either spreading for single colonies or spread evenly over entire surface for colony counts.

Incubation: 37˚C aerobically for 18-24 hours.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
E. coli	2.0-3.0	CV.E.G.	Yellow	(blue if non lactose fermenters)
K. aerogenes	3.0-4.0	CV.E.G.	Yellow	(mucoid)
Proteus spp.	2.0-3.0	CV.E.G.	Blue	
Ps. aeruginosa	1.0-4.0	F.CR.D.	Blue	(green pigment & odour)
Shigella spp.	1.5-2.5	CV.E.G.	Blue	
Salmonella spp.	2.0-3.0	CV.E.G.	Blue	(yellow if lactose +ve)
Staph. aureus	1.0-1.5	CV.E.G.	Yellow	(blue if non-lactose fermenting)
other *Staphylococcus* spp.	0.5-1.5	CV.E.G.	Blue-white	(yellow if lactose fermenting)
Enterococcus spp.	0.5	CV.E.G.	Yellow	

References

Mackey, J.P. and Sandys, G.H. (1966). Diagnosis of urinary infections. Brit.Med.J. 1: 1173.

Guttman, D and Naylor, G.R.E. (1967). Dip-slide: an aid to quantitative urine culture in general practice. Brit.Med. J. 3: 343-345.

C.L.E.D. Medium (Bevis modification)

(Cystine Lactose Electrolyte Deficient – Double Indicator)

LAB006

Description

Bevis modified Mackey and Sandys original medium by introducing a double indicator to improve the differentiation of lactose and non lactose fermenting coliforms, staphylococci and streptococci. The swarming of *Proteus* spp. is inhibited. Lab M C.L.E.D. will grow many of the more demanding streptococci of Lancefield groups A, B, C, G and F. This medium may not grow *Pasteurella* spp. or halophilic organisms.

Formula	g/litre
Balanced Peptone No. 1	4.0
Beef Extract	3.0
Tryptone	4.0
Lactose	10.0
L-Cystine	0.128
Bromothymol blue indicator	0.02
Andrade's indicator	0.08
Agar No. 1	15.0

Method for reconstitution

Weigh 36 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving for 15 minutes at 121˚C. Cool to 47˚C and mix before pouring.

Appearance: Green/blue, clear gel.

pH: 7.5 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034
S. aureus NCIMB 50080

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation method: Surface inoculation, either streaking for single colonies or spread evenly over entire surface for colony counts.

Incubation: 37˚C for 24 hours aerobically.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
E. coli	2.0-3.0	CV.E.G.	Yellow/ Orange	(Blue if non-lactose fermenter)
K. aerogenes	3.0-4.0	CV.E.G.	Yellow/ Orange	mucoid
Proteus spp.	2.0-3.0	CV.E.G.	Blue	
Ps. aeruginosa	1.0-4.0	F.CR.D.	Blue	(Green pigment & odour)
Shigella spp.	1.5-2.5	CV.E.G.	Blue	
Salmonella spp.	2.0-3.0	CV.E.G.	Blue	(Yellow-orange if lactose +ve)
S. aureus	1.0-1.5	CV.E.G.	Yellow/ Orange	(Blue if non-lactose fermenting)
Other staphylococci	0.5-1.5	CV.E.G.	Blue-White	(Yellow if lactose fermenting)
Enterococcus spp.	0.5	CV.E.G.	Yellow-Orange	

References

Bevis, T.D. (1968). A modified electrolyte-deficient culture medium. J. Med. Lab. Tech., 25: 38-41.

Mackey, J.P. and Sandys, G.H. (1966). Diagnosis of urinary infections, Brit.Med. J., 1: 1173.

Sandys, G.H. (1960). A new medium for preventing swarming of *Proteus* spp. with a description of a new medium suitable for use in routine laboratory practice. J. Med.Lab. Tech., 17: 224-233.

Columbia Agar Base

LAB001

Description

A general purpose nutritious agar base formulated by Ellner *et al*. When further enriched by the addition of sterile blood, Columbia agar can be used for the isolation of most clinically significant pathogens. The blood can be 'chocolated' if required. The medium can be made selective for various groups by the addition of appropriate antibiotic mixtures eg:

Streptococci – Colistin/Oxolinic acid (X013)
Gardnerella spp. – Colistin/Nalidixic acid (X011)
C. perfringens – Neomycin (X015) (X016)
Campylobacters - (X214)
Staphylococci/streptococci – Colistin/Naladixic acid (X012)

Formula	g/litre
Columbia Peptone Mixture	23.0
Corn Starch	1.0
Sodium chloride	5.0
Agar No. 2	12.0

Method for reconstitution

Weigh 41 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving for 15 minutes at 121˚C. Cool to 48˚C and add 5-7% sterile, defibrinated horse or sheep blood. Mix well before pouring.

Appearance: Cherry red if blood is fresh and well oxygenated.

Minimum Q.C. organisms: *S. aureus* NCIMB 50080
S. pyogenes ATCC 19615

pH: 7.3 ± 0.2

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface plating, streaking out for single colonies.

Incubation: 37˚C aerobically or microaerobically for 24 hours. Anaerobically for 24 and 48 hours.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
S. aureus	1.5-2.0	CV.E.G.	White-Yellow	Haemolytic
S. pyogenes	0.5-1.0	CV.E.G.(D)	White	α, β-haemolytic dependent on strain
S. pneumoniae	0.5-1.5	F.E.G.	Grey	greenish discolouration in medium, mucoid in $H_2/C0_2$
Neisseria meningitidis	1.0-2.0	CV.E.G.	trans/ Grey	(mucoid)
E. coli	2.0-3.0	CV.E.G.	Opaque/ Grey	(haemolytic)
Ps. aeruginosa	0.5-4.0	F.CR.D.	Opaque Grey	many colonial forms (green pigment) (haemolytic) (mucoid)
C. perfringens	1.5-2.0	CV.CR.G.	Grey	usually target haemolysis (non haemolytic)
B. fragilis	1.0-1.5	CV.E.G.	Grey	(mucoid)
P. anaerobius	P.P.-0.5	CV.E.G.	White/ Grey	

References

Ellner, P.D., Stoessel, C.J., Drakeford, E and Vasi, F. (1966). A new culture medium for medical bacteriology. Amer. J. Clin Pathol., 45:502-504.

Goldberg, R.L., and Washington, J.A., (1976). Comparison of isolation of *Haemophilus vaginalis (Corynebacterium vaginale)* from Peptone-Starch-Dextrose Agar and Columbia Colistin-Nalidixic Acid Agar. J. Clin. Microbiol., 4:245-247.

Thayer, D.D. and Martin, H. E. (1966). An improved medium for the cultivation of *N. gonorrhoeae* and *N. meningitidis*. Publ. Hlth. Report, 81:559-562.

CSEB (ISO) - Cronobacter sakazakii Enrichment Broth (ISO)

Modified Lauryl Sulphate Tryptose Broth Vancomycin Medium

NEW **LAB081**

Description

Cronobacter sakazakii (formerly *Enterobacter sakazakii*) is a member of the *Enterobacteriaceae* family and has been associated with serious outbreak infections in neonates (premature infants) which have been fed on infant formula milk. Although rarely causing infections in immunocompetent adults, *C. sakazakii* has been implicated in sepsis, meningitis and necrotising enterocolitis with a high death rate in neonates. This opportunistic pathogen is common in the environment and its ability to survive desiccation presents a significant risk for post pasteurisation contamination and survival in spray dried milk products.

Based on lauryl sulphate tryptose broth, Cronobacter sakazakii Enrichment Broth (CSEB) has added sodium chloride for extra selectivity against competing organisms. The antibiotic vancomycin is also added to inhibit Gram-positive organisms such as *Staphylococccus aureus* which may be able to grow in this medium.

This media formulation is the secondary enrichment broth as currently recommended in the isolation protocol under ISO/TS 22964:2006(E) for the isolation of *Enterobacter sakazakii* from milk and milk products.

Formula	g/litre
Enzymatic digest of animal and plant tissue	20.0
Lactose	5.0
Sodium chloride	34.0
Dipotassium hydrogen phosphate	2.75
Potassium dihydrogen orthophosphate	2.75
Sodium lauryl sulphate	0.1

Method for reconstitution

Weigh 64.6 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and if required, heat gently to dissolve. Dispense in 10ml volumes and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C.

Prepare a solution of vancomycin in distilled water at a concentration of 1mg/ml. Add 0.1ml of the vancomycin solution to the sterile broth to obtain a final concentration of 0.1mg per 10ml (10mg/L) of CSEB.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, straw liquid

pH: 6.8 ± 0.2

Hazard classification

NR – Not regulated

Minimum Q.C. organisms:
Cronobacter sakazakii ATCC 12868
Cronobacter muytjensii ATCC 51329
Escherichia coli ATCC 25922 (inhibition)

Storage:

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media (with vancomycin): 1 day at 2-8°C in the dark..

Inoculation: Following pre-enrichment in Buffered Peptone Water, transfer 0.1mL of the obtained culture into 10ml LAB081 CSEB.

Incubation: Incubate at 44°C + 0.5°C for 24 hours + 2 hours.

Sub-culture & Interpretation: After incubation, tubes showing turbidity should be streaked onto HAL012 CSIM (ISO).

References

Bowen AB, Braden CR (2006). "Invasive Enterobacter sakazakii disease in infants". *Emerging Infect Dis* **12** (8): 1185–9.

Caubilla-Barron J & Forsythe S (2007). "Dry stress and survival time of *Enterobacter sakazakii* and other *Enterobacteriaceae* in dehydrated infant formula". *Journal Food Protection* **13**: 467-472.

"Enterobacter sakazakii infections associated with the use of powdered infant formula--Tennessee, 2001" (2002). *MMWR Morb Mortal Wkly Rep* **51** (14): 297–300.

Farmer JJ III, Asbury MA, Hickman FW, Brenner DJ, the Enterobacteriaceae Study Group (USA) (1980). "*Enterobacter sakazakii*: a new species of "Enterobacteriaceae" isolated from clinical specimens". *Int J Syst Bacteriol* **30**: 569–84.

ISO/TS 22964:2006(E) Milk and milk products – Detection of *Enterobacter sakazakii*.

Iversen C, Lehner A, Mullane N, *et al* (2007). "The taxonomy of *Enterobacter sakazakii*: proposal of a new genus *Cronobacter* gen. nov. and descriptions of *Cronobacter sakazakii* comb. nov. *Cronobacter sakazakii* subsp. *sakazakii*, comb. nov., *Cronobacter sakazakii* subsp. *malonaticus* subsp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov. and *Cronobacter genomospecies* 1". *BMC Evol Biol* **7**: 64.

Iversen C, Mullane N, Barbara McCardell, *et al* (2008). "*Cronobacter* gen. nov., a new genus to accommodate the biogroups of Enterobacter sakazakii, and proposal of Cronobacter sakazakii gen. nov. comb. nov., C. malonaticus sp. nov., C. turicensis sp. nov., C. muytjensii sp. nov., C. dublinensis sp. nov., Cronobacter genomospecies 1, and of three subspecies, C. dublinensis sp. nov. subsp. dublinensis subsp. nov., C. dublinensis sp. nov. subsp. lausannensis subsp. nov., and C. dublinensis sp. nov. subsp. lactaridi subsp. nov.". IJSEM.

Lai KK (2001). "*Enterobacter sakazakii* infections among neonates, infants, children, and adults. Case reports and a review of the literature". *Medicine (Baltimore)* **80** (2): 113–22.

D.C.A.

(Desoxycholate Citrate Agar)

LAB029

Description

This is Leifson's original formulation of this selective medium for the isolation of *Salmonella* spp. and *Shigella* spp. from faeces and environmental samples. It has approximately half the quantity of inhibitors used in the Hynes modification. The medium uses sodium citrate and sodium desoxycholate as inhibitors. Sodium thiosulphate is the substrate for the enzyme thiosulphate reductase being broken down to form sulphite and hydrogen sulphide. The hydrogen sulphide reacts with the ferric ions to produce a black precipitate of ferrous sulphide. This gives a typical black centre to the colonies of most species of *Salmonella.*

Formula	g/litre
Beef Extract	5.0
Balanced Peptone No. 1	5.0
Lactose	10.0
Sodium citrate	5.0
Sodium thiosulphate	5.0
Ferric citrate	1.0
Sodium desoxycholate	2.5
Neutral Red	0.025
Agar No. 2	12.0

Method for reconstitution

Weigh 45.5 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix, then bring to the boil with frequent stirring. When the medium boils up into the neck of the flask, quickly remove from the source of heat and allow the froth to subside. Return to the heat and allow the foam to boil up into the neck of the flask once more. Remove at once and cool to 47˚C approx. before pouring plates. Dry the surface before inoculation. DO NOT REMELT OR AUTOCLAVE THIS MEDIUM.

Appearance: Pale pink, translucent, a fine precipitate of desoxycholate may be present which may clear if the pH is increased by the growth of organisms.

pH: 7.0 ± 0.2

Minimum Q.C. organisms: *Salmonella* sp. NCIMB 50076
E. coli NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface, streaking for single colonies.

Incubation: 37˚C for 18-24 hours aerobically.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
S. typhi	0.5. 1.5	CV.E.G.	transp Yellow	(black centre)
Other *Salmonella* spp.	1.5-2.0	CV.E.G	transp (Opaque) Yellow	(black centre) (clearing around colony)
S. sonnei	1.5-2.0	CV.E.G.	transp (pinkish)	(more opaque centre)
S. flexneri group	1.0-1.5	CV.E.G.	transp-(pinkish)	
S. dysenteriae	0.5-1.0	CV.E.G.	transp	
E. coli	P.P.-1.5 (uninhibited)	CV.E.D. (G)	Red/Pink	ppt in medium
Citrobacter spp.	P.P.-2.0	CV.E.D. (G)	Red/Pink	ppt in medium (black centre)
Proteus spp.	1.0-2.0	CV.E.G.	Yellow	(black/grey centre) fishy odour (clearing around colony)
Pseudomonas spp.	0.5-1.5	CV.E.D. (G)	Yellow/ Pink	(green pigment)

References

Hynes. M. (1942). The isolation of intestinal pathogens by selective media. J. Path. Bact. 54. 193-207.

Liefson. E. (1935). New culture media based on Sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Path. Bact. 40: 581-589.

D.C.A. Hynes

(Desoxycholate Citrate Agar -Hyne's modification)

LAB065

Description

This modification of Leifson's D.C.A. medium was introduced in 1942. The medium was designed to be more inhibitory to commensal flora whilst allowing for adequate growth of *Salmonella* spp and *Shigella* spp. The citrate and desoxycholate levels are significantly increased. To keep the desoxycholate in solution the pH also had to be increased. The medium still uses lactose fermentation and hydrogen sulphide production as differential indicators.

Formula	g/litre
Beef Extract	5.0
Balanced Peptone No. 1	5.0
Lactose	10.0
Sodium thiosulphate	5.4
Sodium citrate	8.5
Ferric citrate	1.0
Sodium desoxycholate	5.0
Neutral red	0.02
Agar No. 2	12.0

Method for reconstitution

Weigh 52 grams of powder, disperse in 1 litre of deionised water in a two litre flask. Bring to the boil over a gauze, swirling frequently to prevent burning. Simmer for 30 seconds to dissolve. Cool to 47˚C before pouring plates. Dry the surface before inoculation. DO NOT REMELT OR AUTOCLAVE THIS MEDIUM.

Appearance: Pink, clear, bile aggregates may appear on the surface on refrigeration.

pH: 7.4 ± 0.2

Minimum Q.C. organisms: *Salmonella* sp. NCIMB 50076
E. coli NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface, streaking out for single colonies.

Incubation: 37˚C aerobically for 24 hours.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
S. sonnei	1.0-2.0	CV.E.G.(D)	Colourless - pale pink	
S. flexneri	1.0-2.0	CV.E.G.	Colourless	
Salmonella spp.	1.0-4.0	CV.E.G.	Colourless	(black centre)
S. typhi	0.5-1.5	CV.E.G.	Colourless	(Black/grey centre)
E. coli	P.P.-1.5	CV.CR.D.	Red	(No growth)
K. aerogenes	1.0-2.5	CV.E.G.	Pink	(mucoid)
Proteus spp	0.5-2.0	CV.E.G.	Colourless	(Yellow) fishy odour
P. aeruginosa	0.5-1.0	CV.CR.D.	Colourless	(green)

References

Hynes, M. (1942). The isolation of intestinal pathogens by selective media. J. Path. Bact, 54: 193-207

D.C.L.S. Agar

(Desoxycholate Citrate Lactose Sucrose Agar)

LAB003

Description

A modification of Leifson's D.C.A. medium which incorporates sucrose as an additional fermentable substrate to differentiate lactose negative sucrose positive coliforms from *Salmonella* spp. This medium is unsuitable for the isolation of *Yersinia* spp. which are sucrose positive.

Formula	g/litre
Balanced Peptone No. 1	7.0
Beef Extract	3.0
Lactose	5.0
Sucrose	5.0
Sodium citrate	10.5
Sodium thiosulphate	5.0
Sodium desoxycholate	2.5
Agar No. 2	12.0
Neutral Red	0.03

Method for reconstitution

Weigh 50 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes then heat gently with frequent mixing and bring to the boil. Simmer for 1 minute to complete dissolution of the solids. Cool to 47˚C then distribute 20ml into 90mm Petri dishes. Dry the surface by partial exposure, before use. DO NOT REMELT OR AUTOCLAVE THIS MEDIUM.

Appearance: Pale Pink, clear.

pH: 7.2 ± 0.2

Minimum Q.C. organisms: *Salmonella* sp. NCIMB 50076
E. coli NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface plating, streaking out to single colonies.

Incubation: 37˚C aerobically for 24 hours

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
S. typhi	0.5-1.0	CV.E.G.	Trans. colourless	
Other *Salmonella* spp.	1.5-2.0	CV.E.G.	Slight cloudy colourless	
S. sonnei	1.5-2.0	CV.E.G	Trans. Pinkish	(More opaque centre)
S. flexneri	0.5-1.0	CV.E.G	Trans. Pinkish	
S. dysenteriae	0.5	CV.E.G	Trans. colourless	
E. coli	P.P.-1.5 (inhibited)	CV.E.G.(D)	Red	(ppt around colonies)
Citrobacter spp	P.P.-2.0 (inhibited)	CV.E.D.(G)	Red	(ppt around colonies)
Proteus spp.	1.0-2.0	CV.E.G.	Yellow	(Fishy odour)
Pseudomonas spp.	0.5-1.0	CV.E.D.	Yellow Pink	(Green pigment)

References

Hynes, M. (1942). The isolation of intestinal pathogens by selective media. J. Path. Bact. 54: 193-207.

Leifson, E. (1935). New culture media based on sodium desoxycholate for the isolation of colon bacilli in milk and water. J. Path. Bact. 40: 581-589.

D/E Neutralising Agar

(Dey & Engley)

LAB188

Description

D/E Neutralising Agar is used to neutralise and determine the bactericidal activity of antiseptics and disinfectants. Developed by Dey and Engley, this agar neutralises a broad spectrum of antimicrobial chemicals, producing better results than those obtained using alternatives such as Letheen Agar. Complete neutralisation is required to prevent false results arising from disinfectant carryover. D/E Neutralising Agar is used as the plating medium when testing disinfectants using D/E Neutralising Broth and D/E Neutralising Broth Base. It can also be used to test disinfectants by a disc diffusion method. D/E Neutralising Agar contains thioglycollate to neutralise mercurial compounds, sodium thiosulphate to neutralise iodine and chlorine and sodium bisulphite to neutralise formaldehyde and gluteraldehyde. Lecithin is included to neutralise quaternary ammonium compounds and Tween®80 neutralises phenols, hexachlorophene, formalin, and combined with lecithin, ethanol. Bromocresol purple allows detection of growth via a colour change from purple to yellow when organisms ferment the glucose contained in the medium.

Formula	g/litre
Glucose	10.0
Lecithin	7.0
Sodium thiosulphate	6.0
Tween®80	5.0
Tryptone	5.0
Sodium bisulphite	2.5
Yeast extract	2.5
Sodium thioglycollate	1.0
Bromocresol purple	0.02
Agar	15.0

Method for reconstitution

Weigh 54.0 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and pour into sterile Petri dishes and allow to set.

Appearance: Purple opaque gel.

pH: 7.6 ± 0.2

Minimum Q.C. organisms:
Bacillus subtilis NCIMB 8054
Escherichia coli NCIMB 12210
Pseudomonas aeruginosa NCIMB 12469
Salmonella typhimurium NCIMB 13284
Staphylococcus aureus NCIMB 12702

Storage of Powder: Store at 2-8°C in the dark. Formulation is very hygroscopic, keep container tightly closed after use.

Storage of Prepared Medium: Plates – up to 7 days at 2-8°C in the dark.

Inoculation: Consult appropriate references as this product is used in several procedures.

Incubation: 37°C aerobically for 24-48 hours.

Interpretation: Count all colonies for total counts, count yellow colonies for differential acid producer count. Non-acid producing colonies are grey to colourless.

References

Roberts, D., Hooper, W. and Greenwood, M., (1995). Methods for the examination of food for micro-organisms of public health significance, 2nd edition, section 5.10, Practical Food Microbiology. Butler & Tanner. ISBN 0 901144 36 3.

Engley Jr., F.B. and Dey, B.P. (1970). A universal neutralising medium for antimicrobial chemicals. Presented at the Chemical Specialities Manufacturing Association (CSMA) Proceedings, 56th Mid Year Meeting.

Dey, B.P. and Engley Jr., F.B. (1995). Comparison of Dey and Engley
(D/E) Neutralising medium to Lethhen medium and Standards
Methods Medium for recovery of *Staphylococcus aureus* from sanitised surfaces. J. Ind. Microbiol. 14:21-25.

Curry, A.S., Graf, J.G. and McEwen Jr., G.N. (ed.) (1993) CFTA Microbiology Guidelines. The Cosmetic, Toiletry and Fragrance Association, Washington, D.C.

D/E Neutralising Broth

(Dey & Engley)

LAB187

Description

D/E Neutralising Broth is used to neutralise and determine the bacteriocidal activity of antiseptics and disinfectants. Developed by Dey and Engley, D/E Neutralising Broth neutralises a broad spectrum of antimicrobial chemicals, producing better results than those obtained using alternatives such as Letheen Broth, Thioglycollate Medium and Neutralising Buffer. Complete neutralisation is required to prevent false results arising from disinfectant carryover. When used with D/E Neutralising Broth Base the action of the antimicrobial agent can be assessed, i.e. whether it is bacteriostatic or has bactericidal properties. The procedure is based upon D/E Neutralising Broth Base being deficient of all neutralising agents, therefore the potency of the disinfectant is not diminished after addition to the medium. Whereas, when disinfectant is added to the D/E Neutralising Broth, its activity is neutralised allowing for the detection of any bacteria presence. D/E Neutralising Broth contains thioglycollate to neutralise mercurial compounds, sodium thiosulphate to neutralise iodine and chlorine and sodium bisulphite to neutralise formaldehyde and gluteraldehyde. Lecithin is included to neutralise quaternary ammonium compounds and Tween®80 neutralises phenols, hexachlorophene, formalin, and combined with lecithin, ethanol. Bromocresol purple allows detection of growth via a colour change from purple to yellow when organisms ferment the glucose contained in the medium.

Formula	g/litre
Glucose	10.0
Lecithin	7.0
Sodium thiosulphate	6.0
Tween®80	5.0
Tryptone	5.0
Sodium bisulphite	2.5
Yeast extract	2.5
Sodium thioglycollate	1.0
Bromocresol purple	0.02

Method for reconstitution

Weigh 39.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and dispense into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Appearance: Purple opaque liquid.

pH: 7.6 ± 0.2

Minimum Q.C. organisms:
Bacillus subtilis NCIMB 8054
Escherichia coli NCIMB 12210
Pseudomonas aeruginosa NCIMB 12469
Salmonella typhimurium NCIMB 13284
Staphylococcus aureus NCIMB 12702

Storage of Powder: Store at 2-8°C in the dark. The formulation is very hygroscopic therefore keep the container tightly closed after use.

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20°C in the dark.

Inoculation: Consult appropriate references as this product is used in several procedures.

Incubation: 37°C aerobically for 24-48 hours.

Interpretation: Examine all tubes for increased turbidity, formation of a pellicle or a colour change from purple to yellow, indicating bacterial growth.

References

Roberts D., Hooper, W. and Greenwood, M., (1995). Methods for the examination of food for micro-organisms of public health significance, 2nd edition, section 5.10, Practical Food Microbiology. Butler & Tanner. ISBN 0 901144 36 3.

Engley Jr., F.B. and Dey, B.P. (1970). A universal neutralising medium for antimicrobial chemicals. Presented at the Chemical Specialities Manufacturing Association (CSMA) Proceedings, 56th Mid Year Meeting.

Dey, B.P. and Engley Jr., F.B. (1995). Comparison of Dey and Engley
(D/E) Neutralising medium to Lethhen medium and Standards
Methods Medium for recovery of *Staphylococcus aureus* from sanitised surfaces. J. Ind. Microbiol. 14:21-25.

Curry, A.S., Graf, J.G. and McEwen Jr., G.N. (ed.) (1993) CFTA Microbiology Guidelines. The Cosmetic, Toiletry and Fragrance Association, Washington, D.C.

D/E Neutralising Broth Base

(Dey & Engley)

LAB186

Description

D.E. Neutralising Broth Base is a nutritious medium deficient of all neutralising agents. Therefore when a test disinfectant is added to the broth, the potency is undiminished. Developed for use with Dey and Engley's Neutralising Broth (LAB187), incorporating D.E. Neutralising Broth Base into the test procedure allows the user to differentiate between bacteriostatic and bactericidal activity, and to detect viable organisms that remain after treatment. Its use is recommended in disinfectant evaluation, environmental sampling and water-miscible cosmetics in accordance with Cosmetic, Toiletry and Fragrance Association (CTFA) guidelines..

Formula	g/litre
Glucose	10.0
Tryptone	5.0
Yeast extract	2.5
Bromocresol purple	0.02

Method for reconstitution

Weigh 17.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and dispense into final containers. Sterilise by autoclaving for 15 minutes at 121°C.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, purple liquid

pH: 7.6 ± 0.2

Minimum Q.C. organisms:
Bacillus subtilis ATCC 6633
Escherichia coli ATCC 25922
Pseudomonas aeruginosa ATCC 25953
Salmonella typhimurium ATCC 14028
Staphylococcus aureus ATCC 25923

Storage of Powder: 10-25°C away from direct sunlight.

Storage of Prepared Medium: in capped containers for up to 3 months at 15-20°C in the dark.

Inoculation: Consult appropriate references as this product is used in several procedures.

Incubation: 37°C aerobically for 24-48 hours.

Interpretation: Examine all tubes for turbidity, indicating growth.

References

Roberts, D., Hooper, W. and Greenwood, M., (1995). Methods for the examination of food for micro-organisms of public health significance, 2nd edition, section 5.10, Practical Food Microbiology. Butler & Tanner. ISBN 0 901144 36 3.

Engley Jr., F.B. and Dey, B.P. (1970). A universal neutralising medium for antimicrobial chemicals. Presented at the Chemical Specialities Manufacturing Association (CSMA) Proceedings, 56th Mid Year Meeting.

Dey, B.P. and Engley Jr., F.B. (1995). Comparison of Dey and Engley (D/E) Neutralising medium to Lethhen medium and Standards Methods Medium for recovery of *Staphylococcus aureus* from sanitised surfaces. J. Ind. Microbiol. 14:21-25.

Curry, A.S., Graf, J.G. and McEwen Jr., G.N. (ed.) (1993) CFTA Microbiology Guidelines. The Cosmetic, Toiletry and Fragrance Association, Washington, D.C..

Dermatophyte Test Medium (D.T.M.)

LAB117

Description

A modification of the formulation of Taplin, Zaias, Rebell and Blank for the detection of dermatophytic fungi. This medium helps in the differentiation between saprophytic and environmental fungi.

Formula	g/litre
Balanced Peptone No. 1	10.0
Glucose	40.0
Agar No. 2	12.0
Phenol Red	0.2

Method for reconstitution

Weigh 62 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes then bring to the boil with frequent stirring. Dissolve 2 vials of Chloramphenicol X009 (or 1 vial X209) in ethanol and add these to the agar, mix well and distribute into tubes or universal containers. Sterilise at 121˚C for 15 minutes, allow to cool in the sloped position.

Note: Do not exceed the times stated for sterilisation, overheated acidified agar loses gel strength and the sugars are caramelised.

Appearance: Orange, clear gel.

pH: 5.5 ± 0.2

Minimum Q.C. organisms: *Aspergillus* spp. NCIMB 50097
Trichophyton spp.

Storage of Prepared Medium: Slopes – up to 1 month at 2-8˚C in the dark.

Inoculation: Surface plating or stab inoculation.

Incubation: 22-25˚C aerobically for 10-14 days.

Interpretation: Dermatophytes appear as fluffy colonies, colour varies with species, the medium is reddened. Fungi other than dermatophytes cause the medium to become yellow due to acid production. If incubation is prolonged the medium may become reddened. Yeasts appear as white creamy colonies. Blastomyces, Histoplasma and Coccidiodes may also turn the medium red, though these are rarely encountered in lesions associated with ring worm.

References

Taplin, D., Zaias, N., Rebell, G., Blank, H. (1969). Isolation and recognition of dermatophytes on a new medium. (DTM) Arch. Dermatol. 99: 203-209.

Dextrose Tryptone Agar

LAB020

Description

A medium for the enumeration of thermophilic spore bearers in foods. The medium was designed to detect the thermophilic bacteria causing 'flat sour'spoilage of canned foods. The medium also detects the 'flat sour' organism *Bacillus stearothermophilus* in sugar and other sweetening agents used in the preparation of frozen dairy foods, cereals and other food products.

Formula	g/litre
Tryptone	10.0
Glucose	5.0
Bromocresol purple	0.04
Agar No. 2	12.0

Method for reconstitution
Weigh 27 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then bring to the boil to dissolve agar before dispensing in 20ml amounts for poured plate technique. Sterilise by autoclaving at 121˚C for 15 minutes.

Appearance: Purple clear agar.

pH: 6.9 ± 0.2

Minimum Q.C. organisms: *B. stearothermophilus*

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark. Capped container – up to 3 months at 15-20˚C in the dark.

Inoculation: Pour plate technique, pre-heat sample by steaming for 20 minutes if a spore count is required.

Incubation: For Thermophiles – Aerobically for 48 hours at 55˚C. For Mesophiles – Aerobically for 48-72 hours at 30-32˚C.

Interpretation: Count all colonies for total counts, count yellow colonies for differential acid producer count. Non acid producing colonies are grey to colourless.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour
B. stearothermophilus	2.0	Rz.D	Yellow zone mauve centre
Bacillus spp.	1.5-3.0	Rz.D	Mauve (Yellow halo)
S. aureus	0.5-1.5	CV.E.G.	Yellow
E. coli	1.0-1.5	CV.E.G.	Yellow
Klebsiella spp.	1.5-2.5	CV.E.G.	Yellow (mucoid)
Enterococci	0.5	CV.E.G.	Yellow
Proteus spp.	2.0-3.0	RzD	Yellow (spreads)

References

Williams, O.B. (1963). Tryptone Medium for the Detection of Flat Sour Spores. Food Research 1, (3): 217-221.

American Public Health Association. (1972). Standard Methods for the Examination of Dairy Products. 13th Edn. Ed. W.J. Hausler A.P.H.A. Washington.

Tanner, F.W. (1946). The Microbiology of Food 2nd edn., Garrard Press, Champners.

Baumgartner, J.G. and Hersom, A.C. (1956). Canned Foods. 4th Edn. Churchill, London.

DG18 Agar (ISO)

Dichloran (18%) Glycerol Agar (ISO)

NEW

LAB218

Description

Lab M's Dichloran 18% Glycerol Agar (DG18 Agar) is a medium for the enumeration of osmophilic yeasts and xerophilic moulds in food and animal products.

DG18 Agar is used for the enumeration of viable osmophilic yeasts and xerophilic moulds in food or animal feed products with a water activity of less than or equal to 0.95 by a colony count technique. This includes such foods as dry fruits, jams, cakes, dried meat, salted fish, grains, cereals, flours, nuts, spices, condiments and some animal feeds. This medium is not suitable for the examination of dehydrated products with a water activity of less than or equal to 0.60 and does not allow the enumeration of mould spores or the detection of halophilic xerophilic fungi found in dried fish.

The reduction in water activity in this medium is achieved by the addition of glycerol at approximately 18% and this is very important as many yeast and moulds actually require a low water activity to enhance growth and colony development. The medium also contains the antifungal agent dichloran, which restricts the spreading of mucoraceous fungi and restricts the colony size of other genera making colony counting an easier task.

Additional selectivity against bacterial growth is achieved by the incorporation of the heat-stable antibiotic Chloramphenicol. Glucose is incorporated as the fermentable carbohydrate source, with casein enzymatic digest providing the essential vitamins, minerals, amino acids, nitrogen and carbon.

Prepared according to ISO 21527-2:2008, this medium is based on the original formulation described by Hocking and Pitt (1980), and is also compliant with methods stated in the Bacteriological Analytical Manual (BAM).

Formula	g/litre
Casein enzymatic digest	5.0
D-Glucose	10.0
Potassium dihydrogen phosphate	1.0
Magnesium sulphate	0.5
Dichloran	0.002
Chloramphenicol	0.1
Agar	15.0

Method for reconstitution
Disperse 31.6g of powder in 1 litre of distilled water. Allow to soak for 10 minutes and swirl to mix. Add 220g Glycerol and if necessary, heat gently to dissolve. Sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C and mix well before dispensing into Petri dishes. Dry the agar surface prior to use.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, straw gel

pH: 5.6 ± 0.2

Hazard classification
T – Toxic

Minimum Q.C. organisms:
Saccharomyces cerevisiae ATCC 9763
Escherichia coli ATCC 25922 (inhibited)

Storage:

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

Inoculation (as per ISO 21527-2:2008): Inoculate plates in duplicate with 0.1ml of test sample. Spread the liquid over the agar surface using a sterile spreader until the liquid is completely absorbed.

Incubation (as per ISO 21527-2:2008): Incubate aerobically with lids uppermost at 25°C ± 1°C for 5-7 days.

Interpretation (as per ISO 21527-2:2008): Read plates between 2 – 5 days. Select dishes containing less than 150 colonies/propagules and count these colonies/propagules.

If fast-growing moulds are a problem, count colonies/propagules after 2 days and again after 5-7 days of incubation.

References

Bell, C., Neaves, P., Williams, A.P. (2005). Food microbiology and laboratory practice. Blackwell, Oxford. p324.

Beuchat, L.R. Media for detecting and enumerating yeasts and moulds. In Corry, J.E.L., Curtis, GDW., Baird, R.M., Editors. Handbook of Culture Media for Food Microbiology, p369-386.

Beuchat LR, Frandberg E, Deak T, Alzamora SM, Chen J, Guerrero AS, López-Malo A, Ohlsson I, Olsen M, Peinado JM, Schnurer J, de Siloniz MI, Tornai-Lehoczki J. (2001). Performance of mycological media in enumerating desiccated food spoilage yeasts: an interlaboratory study. *Int. J. Food Microbiol*. Oct 22;**70**(1-2):89-96.

BS ISO 21257-2:2008 Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeasts and moulds. Part 2: Colony count technique in products with water activity less than or equal to 0,95.

Deak, T., Chen. J., Golden D.A., Tapia, M.S., Tornai-Lehoczki, J., Viljoen, B.C., Wyder, M.T. Beuchat, L.R. (2001). Comparison of dichloran 18% glycerol (DG18) agar with general purpose mycological media for enumerating food spoilage yeasts. *Int. J. Food Microbiol.* **67**, 49-53.

Hocking, A.D., Pitt, J.I. (1980). Dichloran-glycerol based medium for the enumeration of xerophilic fungi form low moisture foods. *Appl. Environ. Microbiol.* **39**, 488-492.

Diagnostic Semi-Solid Salmonella Agar (Diassalm)

According to Van Netten and Van der Zee et al

LAB537

Description

Diassalm, as developed by Van Netten *et al* (1991), is a semi-solid differential medium for the isolation of *Salmonella* spp. from food and water. It is an improved modification of MSRV (De Smedt and Bolderdijk 1988) and SR (Perales and Audicana 1989) with regard to the composition of the basal medium, selective system and the introduction of a differential system.

The original basal medium was a commercially available sulphide mobility-indole medium (SIM BBL) (Blazevic 1968). Lab M have substituted their raw materials into Blazevic's formula to create a richer base for Diassalm. Selectivity is achieved by the use of malachite green oxalate, magnesium chloride and novobiocin. The diagnostic properties of Diassalm are based on the use of two indicator systems; saccharose combined with bromocresol purple; and ferro-iron in combination with thiosulphate.

The efficiency of Diassalm is due to the ability of salmonellae to move through the highly selective mobility medium in a Petri dish, whilst the double diagnostic system allows visualisation of motile and non-motile suspected salmonellae due to blacking zones against the turquoise background. Diassalm can be seeded after pre-enrichment or after 8hr enrichment in selective broth (De Smedt and Bolderdijk 1987).

Formula	g/litre
Tryptone	20.0
Meat Peptone	6.1
Ferrous ammonium sulphate	0.2
Sodium thiosulphate	5.0
Sucrose	7.5
Lactose	0.5
Bromocresol purple	0.08
Malachite green oxalate	0.037
Magnesium chloride anhyd.	11.0
Agar No.1	2.8

Method for reconstitution

Weigh 53.0 grams of powder, disperse in 1 litre of deionised water. Mix well, bring quickly to the boil. Allow to cool to 47˚C and add 1 vial of Novobiocin supplement – X150 (10mg/vial). Mix well and pour plates. Nitrofurantoin may be used instead of Novobiocin to improve the isolation of *S. enteritidis*.

Appearance:- Green transparent, soft gel

pH: 5.5 ± 0.2

Minimum QC organisms:
Salmonella typhimurium NCIMB 50076
Proteus mirabilis (Inhibition)

Storage of Prepared Medium: Plates – up to 7 days: at 2-8˚C in the dark.

Inoculation: 3 drops (0.1ml) of 8 to 20hr. incubated pre-enrichment broth are inoculated in one spot in the centre of one plate of Diassalm.

Incubation: At 42 ± 0.5˚C or 37˚C for 18-24 hours. Keep the lid uppermost at all times.

Interpretation

After incubation the plates are examined for a mobility zone with a purple/black colour change. When the mobility zone is absent, but the centre is blackened, non-motile salmonellae may be present. A loopfull of the motile zone which is the farthest from the sample inoculum (or the blackened centre if non-motile) is sub-cultured onto brilliant green agar and XLD agar. Futher biochemical and serological identification are performed according to recognised procedure.

Direct latex agglutination may also be carried out from the edge of the mobility zone.

References

Blazevics, D.J. (1968) Appl. Microbiol. 16, 688

De Smedt J.M. *et al* 1987 J. Food Protection 50, 658

Perales, I and Audicana. Evaluation of semi-solid Rappaport medium for detection of Salmonellae in meat products. J. Food Protection 52,

Van Netten, P., Van de Moosdijk, A., Perales, I. and Mossel, D.A.A. Letters in Applied Microbiology

Van Netten, P., Van der Zee, H., and Van der Moosdijk, A., (1991). The use of diagnostic selective semi-solid medium for the isolation of *Salmonella* enteritidis from poultry. Proceedings of the 10th Symposium on the quality of poultry meat, Spelderholt Beckbergen, pp. 59-67.

Van der Zee, H., and Van Netten, P., (1992). Diagnostic semi-solid media based on Rappaport-Vassiliadis Broth for the detection of *Salmonella* spp. and *S. enteritidis* in foods. Proceedings of the International Symposium of *Salmonella* and Salmonellosis.

Van der Zee, H., (1992). Detection of *Salmonella* spp. with the use of a standard method, diagnostic semi-solid agars and immunocapture kit. Proceedings Third World Congress Foodborne infections and intoxications, Berlin.

DN'ase AGAR

LAB095

Description

DN'ase agar provides a convenient means of identifying potentially pathogenic staphylococci, based on the ability of coagulase-positive species to split DNA. DN'ases produced by the organisms hydrolyse the DNA molecule to a mixture of smaller mono and poly nucleotides. DiSalvo observed perfect correlation between coagulase activity and DN'ase production using *S. aureus* strains from clinical specimens. Other publications have also reported a close correlation.

Formula	g/litre
Tryptone	20.0
Deoxyribonucleic acid (DNA)	2.0
Sodium chloride	5.0
Agar No. 2	12.0

Method for reconstitution

Weigh 39 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Allow to cool to 47˚C then pour into Petri dishes.

Appearance: Pale cream, clear.

pH: 7.3 ± 0.2

Minimum Q.C. organisms: *S. aureus* NCIMB 50080
S. epidermidis NCIMB 50082

Storage of Prepared Medium: Plates – up to 7 days: at 2-8˚C in the dark. Capped container – up to 1 month at 4˚C in the dark.

Inoculation: Use a heavy inoculum on a small area. Four or more organisms can be tested on one 90mm Petri dish.

Incubation: 37˚C aerobically for 18-24 hours.

Interpretation:

Having obtained good growth flood the plate with 1N hydrochloric acid. This will precipitate the DNA in the medium. DN'ase producing organisms will be surrounded by a clear area where the DNA has been broken down into fractions which are not precipitated by the Hydrochloric acid. Gram positive, catalase positive cocci that produce DN'ase can be provisionally classified as *S. aureus,* and confirmed by tube coagluase or thermostable DN'ase tests. DN.'ase is also produced by some Gram negative bacilli such as *Serratia marcescens, Pseudomonas aeruginosa.* Some corynebacteria and streptococci may also produce DN'ase.

References

Baird-Parker, A. C. 1965. The classification of staphylococci and micrococci from world-wide sources. J. Gen. Microbiol. 38, 363-387. Black, W. A., Hodgson, R. and McKechnie, A. 1971.

DiSalvo, J. W. 1958 Deoxyribunuclease and coagulase activity of micrococci. Med. Tech. Bull. U.S. Armed Forces Med. J. 9, 191.

Martin, W. J and Ewing, W. H. 1967. The deoxryibonuclease test as applied to certain gram-negative bacteria. Can. J. Microbiol. 13, 616-618.

Messinova, O. V., Yusupova, D. V. and Shamsutdinov, N. S. 1963. Deoxyribonuclease activity of Corynebacterium and its relation to virulence. Fed. Proc. 22, T1033.

Streitfeld, M. M., Hoffmann, E. M. and Janklow, H. M. 1962. Evaluation of extracellular deoxyribonuclease activity in Pseudomonas. J. Bacteriol. 84, 77. Wannamaker, L. W. 1964. Streptococcal deoxryribonuclease, pp. 140-165. J. W. Uhr (ed.). The Streptococcus, Rheumatic Fever, Glomerulophritis. Baltimore: Williams & Williams.

Weckman, B. G. and Catlin, B. W. 1957 Deoxryribonuclease activity of micrococci from clinical sources. J. Bacteriol. 73, 747-753.

Zierdt, C. H. and Golde, D. W. 1970. Deoxryribonuclease-positive Staphylococcus epidermidis strains. Appl. Microbiol. 20(1), 54-57.

DRBC Agar (ISO)

Dichloran Rose Bengal Chloramphenicol Agar (ISO)

NEW

LAB217

Description

Lab M's Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar) is a medium for the enumeration of yeasts and moulds in food and animal products.

Prepared according to ISO 21527-1:2008, this medium is based on the original formulation described by King, Hocking and Pitt in 1979, and is also compliant with methods stated in the Bacteriological Analytical Manual (BAM) and American Public Health Association (APHA).

Used for the enumeration of viable yeasts and moulds in products with a water activity of greater than 0.95 such as eggs, meat, some dairy products, fresh pastes, fruit and vegetables, DRBC Agar (ISO) is designed to suppress the colonial growth of 'spreader' moulds and in doing so allow easier performance of the colony count technique on yeasts and moulds.

The use of the anti-fungal agent, dichloran, restricts spreading of mucoraceous fungi and restricts the colony size of other genera. Rose bengal also assists in the reduction of colony sizes and is selective against bacteria.

Additional selectivity against bacterial growth is achieved by the incorporation of the heat-stable antibiotic Chloramphenicol. Glucose is incorporated as the fermentable carbohydrate source, with an enzymatic digest of animal & plant tissues providing the essential vitamins, minerals, amino acids, nitrogen and carbon.

Formula	g/litre
Enzymatic digest of animal & plant tissues	5.0
D-Glucose	10.0
Potassium dihydrogen phosphate	1.0
Magnesium sulphate	0.5
Dichloran	0.002
Chloramphenicol	0.1
Rose bengal	0.025
Agar	15.0

Method for reconstitution

Disperse 31.7g of powder in 1 litre of distilled water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C and mix well before dispensing into Petri dishes. Dry the agar surface prior to use.

Appearance:

Powder: fine, free-flowing, homogeneous, pink

Finished medium: clear, pink gel

pH: 5.6 ± 0.2

Hazard classification

T – Toxic

Storage:

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

Inoculation (as per ISO 21527-1:2008): Inoculate plates in duplicate with 0.1ml of test sample. Spread the liquid over the agar surface using a sterile spreader until the liquid is completely absorbed.

Incubation (as per ISO 21527-1:2008): Incubate aerobically with lids uppermost at 25°C ± 1°C for 5 days.

Interpretation (as per ISO 21527-1:2008): Read plates between 2 – 5 days. Select dishes containing less than 150 colonies/propagules and count these colonies/propagules.

If necessary, use a magnifier to distinguish between cells of yeasts or moulds and bacteria from colonies.

References

Bacteriological Analytical Manual, 8th edition, Revision A, 1998. Chapter 18 Yeasts, Molds and Mycotoxins. Authors: Valerie Tournas, Michael E. Stack, Philip B. Mislivec, Herbert A. Koch and Ruth Bandler. Revised: 2000-APR-17

Beuchat and Cousin (2001). In Downes and Ito (ed.). Compendium of Methods for the Microbiological Examination of Foods, 4th edition. American Public Health Association.

BS ISO 21257-1:2008 Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeasts and moulds. Part 1: Colony count technique in products with water activity greater than 0.95.

King Jr, A.D., Hocking, A.D. and Pitt, J.I. (1979). Dichloran-Rose Bengal Medium for Enumeration and Isolation of Molds from Foods. *J. Appl. Environ. Microbiol.* 1979, **37**, 959-964.

DRCM (ISO)

Differential Reinforced Clostridial Medium (ISO)

NEW **LAB220**

Description

Differential Reinforced Clostridial Medium ISO (DRCM) is a medium for the detection and enumeration of the spores of sulphite-reducing anaerobes as described in BS EN 26461-1.

Sulphite reducing anaerobes, in particular clostridia, can be indicators of remote and intermittent pollution. Widespread in the environment, being found in human and animal faeces, soil and waste water, the spores are more resistant to physical and chemical factors than vegetative cells and able to survive for long periods in water. The spores may also be resistant to chlorination at the levels commonly used in water treatment.

DRCM has been developed for use with the Most Probable Number (MPN) method to determine the MPN of anaerobes (Clostridia) per volume of sample. The formulation includes peptone, yeast extract, meat extract, starch & L-cysteine for nutrition with glucose providing the energy source. Sodium acetate provides partial selectivity.

Clostridia are able to reduce sulphite to sulphide – forming iron sulphide. Iron (III) citrate is included in the formulation as an indicator of sulphite reduction. Blackening in the medium indicates that iron sulphide has been formed and therefore that sulphite reduction has occurred.

Other bacteria are able to form sulphide, so vegetative cells must be first be removed from the test sample by an appropriate process e.g. heat treatment.

Formula	g/litre
Peptone tryptic digest of meat	10.0
Meat extract	10.0
Yeast extract	1.5
Starch	1.0
Hydrated sodium acetate	5.0
Glucose	1.0
L-Cysteine hydrochloride	0.5
Sodium sulphite	0.4
Iron (III) citrate	0.7

Method for reconstitution

Disperse 30.1g of powder in 1 litre of distilled water. Allow to soak for 10 minutes, swirl to mix and dispense into final containers. Sterilise by autoclaving for 15 minutes at 121°C.

Medium should be used on day of preparation. If medium is stored, tubes should be reheated to deoxygenate the medium. Tubes should not be reheated more than once.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, straw liquid

pH: 7.1 ± 0.2

Hazard classification: NR – Not regulated

Storage:

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

Inoculation (as per BS EN 26461-1:1993): Before the test, the sample of water should be heated in a water bath at 75 ± 5°C for 15 minutes from the time it reaches that temperature.

Add 50ml of sample to 50ml double strength medium (x5).
Add 10ml of sample to 10ml double-strength medium (x5).
Add 1ml of sample to 25ml single-strength medium (x5).

If required add 1ml of a 1 in 10 dilution of the sample to 25ml single-strength medium (x5).

To qualitatively examine 100ml drinking/bottled water without performing MPN, add 100ml sample to 100ml double-strength medium.

If required, top up all bottles with single-strength medium to bring the volume of liquid level with the neck of the bottle, and to ensure that only a very small volume of air remains. Seal the bottles hermetically, or incubate under anaerobic conditions.

Incubation (as per BS EN 26461-1:1993): Incubate aerobically with lids uppermost at 37°C ± 1°C for 44 ± 4 hours.

Large volumes of culture in hermetically sealed glass bottles may explode due to gas production. The addition of iron wire, heated to redness and placed into the medium before inoculation, may aid anaerobiosis

Interpretation (as per BS EN 26461-1:1993): Bottles in which blackening is observed, as a result of the reduction of sulphite and the precipitation of iron (II) sulphide, shall be regarded as positive.

References

BS EN 26461-1:1993 / BS 6068-4.8:1993 / ISO 6461-1:1986. Water quality – Detection and enumeration of he spores of sulfite-reducing anaerobes (clostridia) – Part 1: Method by enrichment in a liquid medium.

Freame, B. & Fitzpatrick, B.W.F. (1967). The use of Differential Reinforced Clostridial Medium for the isolation and enumeration of Clostridia from foods. *The Society for Applied Microbiology Technical Series n. 5: Isolation of Anaerobes,* ed. Shapton, D.A. & Board, R.G. **Vol. 5.** London Academic Press. 49-55.

Gibbs, M.B. (1973). The detection of Clostridium welchii in the Differential Reinforced Clostridial Medium technique. *J. Appl. Bact.* **36**. 23-33.

EC Medium

(Escherichia coli Medium)

LAB171

Description

EC Medium (*Escherichia coli* Medium) is a selective enrichment broth designed for the isolation of coliforms, including *E. coli*, from water and food samples. It is the recommended medium of the American Public Health Association (APHA) and the AOAC.

EC Medium is made selective for coliforms by the inclusion of Bile Salts No.3 in the dehydrated medium. The selective nature of this medium ensures that the growth of non-coliform bacteria is minimised. The medium is buffered by the addition of potassium phosphates and osmotically balanced by sodium chloride. The medium is used at 37°C for coliform organisms and 45.5°C is recommended for the isolation *E. coli.*

Formula	g/litre
Tryptone	20.0
Lactose	5.0
K_2HPO_4	4.0
KH_2PO_4	1.5
Sodium chloride	5.0
Bile Salts No. 3	1.5

Method for reconstitution

Weigh 37.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix. Dispense into tubes of appropriate volume and, where applicable, add Durham tubes. Sterilise by autoclaving at 121°C for 15 minutes.

Appearance: Clear straw broth.

pH: 6.9 ± 0.2

Minimum Q.C. organisms:
Escherichia coli NCIMB 50034
Enterococcus faecalis NCIMB 50030 (inhibition)
Bacillus subtilis NCIMB 13061 (inhibition)

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20°C in the dark.

Inoculation: Coliforms: Follow the methods and procedures as stated in Standard Methods for the Examination of Water and Wastewater and Compendium of Methods for the Microbiological Examination of Foods.

Incubation: 45.5°C for 18-24 hours aerobically for *E. coli* and 37°C for 18-24 hours, aerobically for coliforms.

Interpretation: Turbidity of broth and gas collection in the Durham tube indicates the presumptive growth of organisms from the coli-aerogenes group. All broths should be sub-cultured onto selective media whether turbid or not.

References

American Public health Association, (1980). Standards Methods for the Examination of Water and Wastewater, 15th Edition, American Public Health Association, Inc., Washington, D.C.

American Public health Association, (1976). Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Inc., Washington, D.C.

Association of Official Analytical chemists. (1995). Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Perry and Hajna, (1943). American Journal of Public Health, 33:550.

Perry and Hajna, (1944). American Journal of Public Health, 34:735.

E.E. Broth

(Enterobacteriaceae Enrichment Broth)

LAB091

Description

E.E. Broth is recommended as an enrichment medium when examining food and feedstuffs for *Enterobacteriaceae.* It is a modification of LAB051 Brilliant Green Bile Broth, with an improved buffering capacity to encourage early growth and prevent autosterilization. E.E. Broth uses glucose instead of lactose to make the medium a test for all enterobacteria including non lactose fermenting organisms.

Formula	g/litre
Balanced Peptone No. 1	10.0
Dextrose	5.0
Disodium hydrogen phosphate	6.45
Potassium dihydrogen phosphate	2.0
Bile Salts	20.0
Brilliant green	0.0135

Method for reconstitution

Weigh 43.5 grams of powder and add to 1 litre of deionised water. Swirl to dissolve, warm gently if necessary, then distribute into bottles or tubes and heat at 100˚C for 30 minutes only. Cool rapidly. OVERHEATING THIS MEDIUM WILL ADVERSELY AFFECT ITS PERFORMANCE.

Appearance: Green, clear.

pH: 7.2 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034
B. subtilis (inhibition)

Storage of Prepared Medium: capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation: Add 1 part of sample suspension or dilution to 10 parts of medium.

Incubation: 44˚C for 18 hours for thermotrophs. 32˚C for 24-48 hours for mesotrophs. 4˚C for 10 days for psychrotrophs.

Interpretation: Turbidity and a colour change to yellow-green is presumptive evidence of *Enterobacteriaceae.* Subculture onto confirmatory media e.g. LAB088 V.R.B.G.A. must be carried out.

References

Mossel, D. A. A., Visser, M. and Cornelissen, A. M. R. 1963. The examination of foods for *Enterobacteriaceae* using a test of the type generally adopted for the detection of salmonellae. J.Appl. Bacteriol. 26, 444-452.

Mossel, D. A. E., Harrewijn, G. A. and Nesselrooy-van Zadelhoff, C. F. M. 1974. Standardisation of the selective inhibitory effect of surface active compounds used in media for the detection of *Enterobacteriaceae* in food and water. Health Lab. Sci. 11, 260-267. Richard, N. 1982. Monitoring the quality of selective liquid media by the official French dilution technique used for the bacteriological examination of foods. In: Quality assurance and quality control of microbiological culture media, edited by J. E. L. Corry, G.I.T.-Verlag Darmstadt, pp. 51-57.

Endo Agar

LAB060

Description

This medium was developed in 1914 for the isolation of *Salmonella typhi;* other media have since proved superior for this purpose, but Endo agar has a role as a coliform medium. It is recommended by the American Public Health Association as a standard medium for the enumeration of coliforms in water and dairy products. In this medium acetaldehyde is produced by coliforms and then fixed by the sulphite to produce a metallic sheen with the basic fuchsin dye. Most enteric Gram negative organisms will grow well, whilst Gram positive organisms are mostly inhibited.

Formula	g/litre
Balanced Peptone No. 1	10.0
Lactose	10.0
Dipotassium phosphate	3.5
Sodium sulphite	2.5
Agar No. 1	15.0

Method for reconstitution

Weigh 41 grams of powder, disperse in 1 litre of deionised water. Add 4ml of a 10% w/v alcoholic solution of basic fuchsins (95% ethyl alcohol). Bring to the boil with frequent swirling to dissolve the solids. Sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C in a water bath before pouring. The precipitate typically associated with this medium should be dispersed by gentle swirling prior to pouring the plates.

This medium is light sensitive and should therefore be stored in the dark, preferably under refrigeration. The medium will become dark red in colour if exposed to light.

Basic Fuchsin is a potential Carcinogen and care should be taken when handling it to avoid inhalation of the powdered dye and contamination of the skin.

Appearance: Pale pink/orange

pH: 7.5 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface, streaking out for single colonies.

Incubation: 37˚C for 18-48 hours aerobically.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
E. coli	1.0-2.0	CV.E.G.	Deep Red	(Metallic sheen)
K. aerogenes	1.0-2.5	CV.E.G.	Red	(mucoid)
Proteus spp	2.0-3.0	CV.E.G.	Pale Pink colourless	
Ps. aeruginosa	0.5-1.0	F.CR.D.	Pale Pink	
Shigella spp	0.5-1.0	CV.E.G.	Pale Pink	
Salmonella spp	1.0-1.5			
Gram positive organisms	no growth.			

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface, streaking out for single colonies.

Incubation: 37˚C for 18-48 hours aerobically.

References

Endo, 1914, Centr. Bakt., Abt 1, Orig., 35: 109.

American Public Health Association, 1975. Standard Methods for the Examination of Water and Wastewater, 14th Edn. American Public Health Association, Inc. Washington D.C.

American Public Health Association, 1972. Standard Methods for the Examination of Dairy Products, 13th End., American Public Health Association, Inc., Washington, D.C.

Eosin Methylene Blue Agar (Levine)

LAB061

Description

This medium was introduced in 1916 by Holt-Harris and Teague to differentiate *Escherichia* spp. and *Aerobacter* spp. It was modified by Levine in 1918 who removed sucrose from the formula and increased the lactose content. The distinctive metallic sheen produced by *E. coli* on this medium is due to acid production resulting in an amide bonding between the eosin and methylene blue, other coliforms do not produce enough acid to cause this reaction. Eosin inhibits most Gram positive organisms. The prepared medium is sensitive to light.

Formula	g/litre
Balanced Peptone No. 1	10.0
Lactose	10.0
Dipotassium phosphate	0.7
Monopotassium phosphate	1.3
Eosin Y	0.4
Methylene Blue	0.065
Agar No. 2	15.0

Method for reconstitution

Weigh 37.5 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Cool to 50˚C and agitate gently to ensure uniform distribution of the flocculant precipitate (which is a feature of this medium) before pouring into Petri dishes.
STORE IN THE DARK.

Appearance: Blue/purple with a light precipitate.

pH: 6.8 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface, streaking for single colonies.

Incubation: 37˚C aerobically for 24 hours.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
E. coli	2.0-3.0	CV.E.G.	Blue Black	(Metallic sheen)
Klebsiella spp.	3.0-4.0	CV.E.G.	Brown Blue	(mucoid)
Salmonella spp.	2.0-3.0	CV.E.G.	Colourless	
Shigella spp.	1.0-2.0	CV.E.G.	Colourless	
Candida spp.	0.5-1.5	CV.Rz.G. (D)	White	
S. aureus	P.P.	CV.E.G.	Colourless	
E. faecalis	P.P.	CV.E.G.	Colourless	

References

American Public Health Association, American Water Works Association and Water Pollution Control Federation, (1975). Standard Methods for the Examination of Water and Wastewater, 14th Edn., Washington, D.C. American Public Health Association.

Girolami, R.L. and Stamm, J.M. (1976). Inhibitory effect of light on growth supporting properties of Eosin Methylene Blue Agar. Appl. Environ. Microbiol., 31:1 141-142.

Haesler, W. J. (ed) (1972). Standard Methods for the Examination of Dairy Products, 13th edn., Washington, D.C., American Public Health Association.

Levine, M. (1918). Differentiation of *E. coli* and *B. aerogenes* on a simplified Eosin-Methylene Blue agar. J. Infect. Dis., 23: 43-47.

Eugon Agar

(Eugonic Agar)

LAB525

Description

Eugon Agar is used for the cultivation of a wide variety of microorganisms, particularly in mass cultivation procedures. The medium is prepared according to the formulation of Vera and was developed to obtain eugonic (luxuriant) growth of fastidious microorganisms. The medium can be used with additions to enhance its performance with certain microorganisms, e.g. Eugon Agar supplemented with 5% sterile defibrinated blood will enable the growth of pathogenic fungi such as *Nocardia, Histoplasma* and *Blastomyces*.

Niven reported Eugon Agar for the detection of lactic acid bacteria in cured meats and recommended it for investigating spoilage in meats. Harrison and Hansen employed the medium for plate counts of the intestinal flora of turkeys and Frank showed its use for the germination of anaerobic spores pasteurised at 104°C. Eugon Agar is also specified in the APHA Compendium of Methods for the Microbiological Examination of Food.

The high sugar content of this medium dictates that it not suitable as a base for haemolytic reactions.

Formula	g/litre
Tryptose	15.0
Soy Peptone	5.0
Dextrose	5.5
L-Cystine	0.7
Sodium chloride	4.0
Sodium sulphite	0.2
Agar	15.0

Method for reconstitution

Weigh 45.4 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and then sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C before the addition of supplements or pouring into sterile Petri dishes.

Appearance: Light amber clear gel, may contain a slight precipitate.

pH: 7.0 ± 0.2

Minimum QC organisms:
Aspergillus niger NCIMB 50097
Candida albicans NCIMB 50010
Lactobacillus fermentum ATCC 9388
Streptococcus pyogenes NCIMB 13285

Storage of Prepared Medium: Plates can be stored up to 7 days at 2-8°C in the dark.

Inoculation: For the examination of clinical specimens for bacteria and fungi refer to the appropriate published references. For the examination of food for the examination of bacteria and fungi refer to standard methods.

Incubation: 35°C ± 2°C for up 72 ± 4 hours for bacteria. 30°C ± 2°C for up 72 ± 4 hours for fungi.

Interpretation: Refer to appropriate references and procedures.

References

Vera, H.D. (1947). The ability of peptones to support surface growth of lactobacilli. J. Bacteriol. 54:14.

MacFaddin, J.D. (1985). Media for the isolation-cultivation-identification-maintenance of medical bacteria. 301-303. vol. 1. Williams & Wilkens, MD.

Niven (1949). J. Bacteriol. 58:633.

Harrison, A.P.Jr. and Hansen, P.A. (1950). The bacterial flora of the cecal feces of healthy turkeys. J. Bacteriol. 59. 197.

Frank, H.A. (1955). The influence of various media on spore count determinations of a putrefactive anaerobe. J. Bacteriol. 70:269.

Vanderzant, C. and Splittstoesser, D.F. (ed.). (1992). Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Isenberg, H.D. (ed.) (1992). Clinical microbiological procedures handbook, American Society for Microbiology, Washington, D.C.

Murray, P.R. *et al* (ed) (1995). Manual of Clinical Microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Association of Official Analytical Chemists. (1995). Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Eugon Broth

(Eugonic Broth)

LAB526

Description

This is the broth version of Eugon Agar (LAB525) for the cultivation of a wide variety of microorganisms, particularly in mass cultivation procedures. The medium is prepared according to the formulation of Vera and was developed to obtain eugonic (luxuriant) growth of fastidious microorganisms. The medium can be used with additions to enhance its performance with certain microorganisms e.g. Eugon Broth supplemented with 5% sterile defibrinated blood the medium will support the growth of pathogenic fungi such as *Nocardia, Histoplasma* and *Blastomyces*.

Formula	g/litre
Tryptose	15.0
Soy Peptone	5.0
Dextrose	5.5
L-Cystine	0.7
Sodium chloride	4.0
Sodium sulphite	0.2

Method for reconstitution

Weigh 30.4 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool before the addition of enrichments and aseptically dispense into appropriate containers.

Appearance: Light amber solution, may contain a slight precipitate.

pH: 7.0 ± 0.2

Minimum QC organisms:
Aspergillus niger NCIMB 50097
Candida albicans NCIMB 50010
Lactobacillus fermentum ATCC 9388
Streptococcus pyogenes NCIMB 13285

Storage of Prepared Medium: Store the prepared medium at 2-8°C.

Inoculation: For the examination of clinical specimens for bacteria and fungi refer to the appropriate published references.

Incubation: 35°C ± 2°C for up 72 ± 4 hours for bacteria. 30°C ± 2°C for up 72 ± 4 hours for fungi.

Interpretation: Refer to appropriate references and procedures.

References

Vera, H.D. (1947). The ability of peptones to support surface growth of lactobacilli. J. Bacteriol. 54:14.

MacFaddin, J.D. (1985). Media for the isolation-cultivation-identification-maintenance of medical bacteria. 301-303. vol. 1. Williams & Wilkens, MD.

Isenberg, H.D. (ed.) (1992). Clinical microbiological procedures handbook, American Society for Microbiology, Washington, D.C.

Murray, P.R. *et al* (ed) (1995). Manual of Clinical Microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Fastidious Anaerobe Agar (F.A.A.)

LAB090

Description

A primary isolation medium capable of growing most clinically significant anaerobes. Developed by Lab M, comparisons have shown this medium to be superior to other formulations as a primary isolation medium for fastidious organisms. The peptones included have been chosen for maximum growth stimulation. Starch and sodium bicarbonate act as de-toxification agents whilst haemin encourages pigment production in *Porphyromonas melaninogenicus*. Specific growth promoting agents are Cysteine for *Fusobacterium necrophorum, Propionibacterium acne* and *Bacteroides fragilis,* arginine for *Eubacterium* spp. soluble pyrophosphate for *Porph. gingivalis* and *Porph. asaccharolytica.* Pyruvate helps neutralise hydrogen peroxide and is also utilised by *Veillionella* spp. as an energy source. Vitamin K and sodium succinate provide essential growth factors for some anaerobes as does the 0.1% glucose. The low level of glucose prevents the production of high levels of acids and alcohols which would inhibit colonial development.

Formula	g/litre
Peptone mix	23.0
Sodium chloride	5.0
Soluble starch	1.0
Agar No. 2	12.0
Sodium bicarbonate	0.4
Glucose	1.0
Sodium pyruvate	1.0
Cysteine HCl monohydrate	0.5
Haemin	0.01
Vitamin K	0.001
L-Arginine	1.0
Soluble pyrophosphate	0.25
Sodium succinate	0.5

Method for reconstitution

Weigh 46 grams of powder and add to 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C then aseptically add 5-10% of sterile defibrinated horse blood, mix well and pour into Petri dishes. This medium can be made selective for various species of anaerobes by the addition of appropriate selective cocktails e.g.

Gram negative anaerobes	X090
Non-sporing anaerobes	X291
Clostridium difficile	X093

Appearance: Red due to addition of blood. The blood will darken (reduce) because of the presence of reducing agents.

pH: 7.2 ± 0.2

Minimum Q.C. organisms: *B. fragilis*
P. anaerobious

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface plating, streaking out to single colonies.

Incubation: 37˚C anaerobically with 10% CO_2 for 48 hours to 5 days.

Growth Characteristics (48 hours)

organism	colony size (mm)	shape & surface	colour	other
Bacteroides fragilis	1.0 - 2.0	CV.E.G.	Grey	
Clostridium Perfringens	1.0 - 2.0	CV.E.G	Grey	'Target' haemolysis (non haemolytic)
Fusobacterium necrophorum	1.0 - 2.0	CV.E.G (D)	trans-parent	(grey) (haemolytic)
Porphyromonas asaccharolyticus	1.0 - 2.0	CV.E.G	Grey / Brown	(clearing)
Bacteroides ureolyticus	0.5	F.E.D.	translucent	pitting
Propionibacterium acne	0.5	CV.E.G	White	
Peptostreptococcus anaerobius	0.5 - 2.0	CV.E.G	White / Grey	
Actinomyces israeli	0.5 - 1.0	CV.E.G	White	('molar tooth') (smooth)

References

Brazier, J.S. (1986). Yellow fluorescence of Fusobacteria Letters in Applied Microbiol. 2: 124-126.

Brazier, J.S. (1986). A note on ultra violet red fluorescence of anaerobic bacteria in vitro. J. Appl. Bact. 60: 121-126.

Eley, A., Clarry, T., Bennett, K.W. (1989). Selective and differential medium for isolation of *Bacteriodes ureolyticus* from clinical specimens. European Journal of Clinical Microbiology, Infectious Diseases. 8: 83-85.

Wade W. Griffiths, M. (1987). Comparison of Media for cultivation of subgingival bacteria. J. Dent. Res. 66: no. 4 abstract 334.

Heginbotham M., Fitzgerald T.C., and Wade W.G. (1990). Comparison of solid media for the culture of anaerobes. J. Clin. Path. 43: 253-256.

Fastidious Anaerobe Broth (F.A.B.)

LAB071

Description

F.A.B. was developed by Lab M working in conjunction with the microbiology department of a University of Manchester teaching hospital. The medium was designed to give optimum growth of fastidious anaerobes and has found applications as a blood culture medium and an enrichment broth for the isolation of anaerobes. The medium is very rich in nutrients from the specially selected peptone mixture. Vitamin K. haemin and L-cysteine are all growth factors required by some anaerobes. L-cysteine together with sodium thioglycollate reduce the Eh of the medium and the agar content inhibits absorption of oxygen and convection currents. Resazurin is a redox indicator. Several published evaluations show F.A.B. to be the liquid medium of choice for fastidious anaerobes.

Formula	g/litre
Peptone mixture	15.0
Yeast Extract	10.0
Sodium thioglycollate	0.5
Sodium chloride	2.5
Agar No. 1	0.75
L-Cysteine HCl	0.5
Resazurin	0.001
Sodium bicarbonate	0.4
Haemin	0.005
Vitamin K	0.0005

Method for reconstitution

Weigh 29.7 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix. Boil to dissolve the agar then dispense into screw cap containers. Sterilise by autoclaving at 121˚C for 15 minutes. Tighten the caps as soon as possible after autoclaving.

Appearance: Pale straw, clear, viscous. May have a narrow band of red/purple at the surface due to action of oxygen on the resazurin. If the medium is reddish this indicates too much oxygen has been absorbed, the medium should be reheated to deoxygenate. Do not reheat more than once.

pH: 7.2 ± 0.2

Minimum Q.C. organisms: *Bacteroides fragilis*

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation: If used as a blood culture medium a minimum dilution of 1:10 should be used.

Incubation: 37˚C for 24-72 hours. Keep the container airtight.

Growth indicators: The broth may become turbid or individual colonies may form suspended in the medium.

References

Gould, J.H., Duerden, B.I. (1983). Blood culture – current state and future prospects. J. Clin. Pathol. 36: 963-977.

Ganguli, G.A., O'Hare, W., Hyde, W.A. (1984). Rapid Detection of Bacteraemia by early subculture. J. Med. Microbiol. 17: 311-315.

Ganguli, L.A., Keaney, M.G.L., Hyde, W.A., Fraser, B.J. (1985). More Rapid identification of bacteraemia by manual rather than radiometric methods. J. Clin. Pathol. 38: 1146-1149.

Junt, G.H., Price, E.H. (1982). Comparison of a home made blood culture broth containing a papain digest of liver, with four commercially available media, for the isolation of anaerobes from simulated paediatoic blood cultures. J. Clin. Pathol. 35: 1142-1149.

Ganguli, L.A., Turton, L.J., Tillotson, G.S. (1982). Evaluation of Fastidious Anaerobe Broth as a blood culture medium. J. Clin. Pathol. 35: 458-461.

Tillotson, G.S. (1981). Evaluation of ten commercial blood culture systems to isolate a pryridoxal dependent streptococcus. J. Clin. Pathol. 34: 930-934.

Fluid Thioglycollate Medium (U.S.P.)

LAB025

Description

A medium for sterility tests, prepared according to the specification of the United States Pharmacopeia. Aerobic and anaerobic organisms grow well in this medium even from small inocula. In appropriate tubes or bottles the thioglycollate ensures adequate anaerobic conditions. The low level of agar reduces oxygen diffusion into the medium. The thioglycollate will also serve to inactivate any mercurial compounds used as preservatives.

Formula	g/litre
Tryptone	15.0
L-Cystine	0.5
Glucose	5.5
Yeast Extract	5.0
Sodium chloride	2.5
Sodium thioglycollate	0.5
Resazurin	0.001
Agar	0.75

Method for reconstitution

Weigh 29.75 grams, disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix, then bring to the boil to dissolve and dispense 15ml into 6mm x 150mm tubes. Sterilise by autoclaving for 15 minutes at 121˚C. Store at ambient temperature in the dark, but not in the refrigerator. If more than 30% of the medium turns pink (oxidised) the Eh may be restored (once only) by heating in a boiling water bath or by free-steaming.

Appearance: Pale straw colour, clear. Surface may be pink/blue due to oxidation of Resazurin.

pH: 7.1 ± 0.2

Minimum Q.C. organisms: *C. sporogenes*
S. aureus NCIMB 50080

Storage of Prepared Medium: Capped container – up to 3 months at 15-20˚C in the dark.

Incubation: 30-35˚C aerobically for 14 days.

Growth indicators: Turbidity, colonies in medium.

References

The Pharmacopeia of the United States of America. 21st End. (1985).

Fraser Broth

LAB164

Description

Developed as a modification of UVM II medium, Fraser broth is a secondary enrichment broth for the isolation of *Listeria* spp., and is similar to Palcam broth in that it contains aesculin to indicate the presence of a potential *Listeria* isolate. It also contains lithium chloride in an attempt to suppress the growth of enterococci in the medium (as does Palcam). Fraser broth may also be used as a primary enrichment medium by incorporating 1/2 strength supplement into the broth base (X164 or X564).

Formula	g/litre
Peptone mixture	15.0
Yeast extract	5.0
Aesculin	1.0
Disodium hydrogen phosphate	9.6
Potassium dihydrogen phosphate	1.35
Sodium chloride	20.0
Lithium chloride	3.0

Method for reconstitution

Weigh 55 grams of power and add to 1 litre of deionised water (add to 900ml if preparing 1/2 Fraser). Allow to soak for 10 minutes, swirl to mix and sterilise at 121˚C for 15 minutes. Cool to 47˚C and add 2 vials of Fraser supplement X165 (or 2 vials of 1/2 Fraser supplement X164), mix well and aseptically dispense into sterile tubes or bottles.

Appearance: Straw opalescent broth with precipitate (clears on storage).

pH 7.2 ± 0.2

Minimum Q.C. organisms: *Listeria* sp NCIMB 50007
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface spreading.

Incubation: 30-37˚C for 24 and 48 hours aerobically.
Storage of Prepared Medium: Bottles – up to 14 days at 2-8˚C.

Inoculation: 1/2 Fraser – Add 25g sample to 225ml of 1/2 Fraser broth and homogenise Fraser – Subculture 0.1ml of primary enrichment broth (UVM I or 1/2 Fraser) into 10ml of Fraser broth.

Incubation: 1/2 Fraser – 30˚C aerobically for 24hrs.

Fraser – 35˚C aerobically for 24hrs and 48hrs. Subculture onto selective agars at 24 and 48hrs.

Interpretation

Blackening of the broth indicates the presence of a potential Listeria and should be subcultured onto Listeria isolation medium (Oxford) LAB122 or Palcam agar LAB148. All broths should be subcultured before discarding, irrespective of colour change.

References

Fraser J.A., and Sperber W.H., (1988) Rapid detection of Listeria spp in food and environmental samples by esculin hydrolysis. J.Food Protection 51 (10) 762-765.

McClain D., and Lee W.H. (1989) FSIS method for isolation of L.monocytogenes from processed meat and poultry products. Lab.Comm.No.57, Revised May 24, (1989). US Dept of Agric.FSIS, Microbiol. Div.

Fraser Broth^{PLUS} (ISO)

LAB212

Description

A secondary enrichment broth for the isolation of *Listeria* spp. formulated according to ISO 11290. The selective components acriflavine and nalidixic acid are blended into the base powder and the ferric ammonium citrate (X211) is added to the tempered broth after sterilisation.

Note: Acriflavine and Nalidixic Acid in LAB211 are half-strength of Fraser Broth^{PLUS} (ISO) LAB212.

Formula	g/litre
Peptone mixture	15.00
Yeast extract	5.00
Aesculin	1.00
Disodium hydrogen phosphate	9.60
Potassium dihydrogen phosphate	1.35
Sodium chloride	20.00
Lithium chloride	3.00
Acriflavine	0.025
Nalidixic acid	0.02

Method for reconstitution

Weigh 55 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C and add 2 vials X211. Mix well and dispense into sterile containers..

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: straw opalescent broth with yellow fluorescence (in final medium).

pH: 7.2 ± 0.2

Hazard classification

NR – Not regulated

Minimum Q.C. organisms (According to ISO/TS 11133-2):
Listeria monocytogenes ATCC 13932
Escherichia coli ATCC 25922
Enterococcus faecalis ATCC 29212

Storage:

Dehydrated culture media: 10-25°C

Final medium: 14 days at 2-8°C in the dark

Inoculation: Sub-culture 0.1mL of LAB211 into 10mL of LAB212 Fraser Broth^{PLUS} (ISO).

Incubation: 35-37°C aerobically for 24hrs and 48hrs. Sub-culture onto selective agar at 24 and 48 hours.

Interpretation: Blackening of the broth indicates the presence of a potential *Listeria* spp. and should be sub-cultured onto a selective *Listeria* isolation medium, e.g. Harlequin™ Listeria Chromogenic Agar (HAL010). All broths should be sub-cultured before discarding irrespective of colour change.

References

Fraser J.A. and Sperber W.H. (1988). Rapid detection of Listeria spp in food and environmental samples by esculin hydrolysis. *J. Food Protect.* 51, No.10, 762-765.

McClain D. and Lee W.H. (1989). FSIS method for isolation of L. monocytogenes from processed meat and poultry products. Lab. Comm.No.57, Revised May 24, (1989). US Dept of Agric.FSIS, Microbiol. Div.

ISO 11290-1:1997 (Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of *Listeria monocytogenes* - part 1, Incorporating Amendment 1.)

ISO/TS 11133-2:2003. Microbiology of food and animal feed stuffs-Guidelines on preparation and production of culture media – Part 2: Practical guidelines on performance testing of culture media.

G.C. Agar Base

LAB067

Description

A nutritious agar base described by Thayer and Martin for the isolation of Neisseria gonorrhoeae. The rich peptone mixture is enhanced by the use of corn starch to absorb toxic metabolites and a buffering system is used to maintain neutral pH. The medium is made selective by the use of various antibiotic cocktails. Thayer and Martin originally recommended the use of vancomycin, colistin and nystatin V.C.N. but the addition of trimethoprim (X068) is useful in preventing the swarming of proteus. More recently the emergence of vancomycin sensitive gonococci has made the New York City selective agents (lincomycin, colistin, amphotericin, trimethoprim X070, LCAT) the combination of choice. Enrichment of the base is usually by the addition of lysed blood. Alternatively chocolated blood or haemoglobin powder and Thayer and Martin's mixture of vitamins, amino acids and coenzymes can be used. The supplement X069, can be added as this is without Amphotericin and this permits the growth of yeasts. The growth supplement X271 can be added to this medium to aid in the isolation of *Neisseria* spp.

Formula	g/litre
Special Peptone	15.0
Corn Starch	1.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	4.0
Potassium dihydrogen phosphate	1.0
Agar No. 2	10.0

Method for reconstitution

Weigh 36 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Cool to 48˚C and add 50-70ml of lysed blood and 2 vials of X070 selective agent. Mix well and pour into Petri dishes.

Appearance: Dependent on blood supplement used.

pH: 7.2 ± 0.2

Minimum Q.C. organisms: *N. gonorrhoeae* ATCC CDC98
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface, streaking out for single colonies.

Incubation: 37˚C microaerobically for 24-48 hours.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
N. gonorrhoeae	1.0-2.0	CV.E.G.	Transparent	variations in colony size
N. lactamica	1.0-2.0	CV.E.G.	Grey	
N. meningitidis	2.0-3.0	CV.E.G.	Grey	
B. catarrhalis	2.0-3.0	CV.E.G.	Cream	

Other organisms should not grow with the exception of antibiotic resistant variants.

References

Young, H. 1978. Cultural diagnoses of gonorrhoea with modified New York City (MNYC) medium. Brit. Journ. Ven. Dis. 54: 36-40:

Thayer, J. D. and Martin, J. E. 1966. Improved medium selective for the cultivation of *N. gonorrhoeae* and *N. Meningitidis:* Public Health rep. 81: 559-562.

Half Fraser Broth^{PLUS} (ISO)

LAB211

Description

Primary enrichment broth for the isolation of *Listeria* spp. formulated according to ISO 11290. The selective components acriflavine and nalidixic acid are blended into the base powder and the ferric ammonium citrate (X211) is added to the tempered broth after sterilisation.

Note: Acriflavine and Nalidixic Acid in LAB211 are half-strength of Fraser Broth^{PLUS} (ISO) LAB212.

Formula	g/litre
Peptone mixture	15.00
Yeast extract	5.00
Aesculin	1.00
Disodium hydrogen phosphate	9.60
Potassium dihydrogen phosphate	1.35
Sodium chloride	20.00
Lithium chloride	3.00
Acriflavine	0.0125
Nalidixic acid	0.01

Method for reconstitution

Weigh 55 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C and add 2 vials X211. Mix well and dispense into sterile containers.

Appearance:

Powder: fine, free-flowing, homogeneous, buff
Finished medium: straw opalescent broth with yellow fluorescence (in final medium).

pH: 7.2 ± 0.2

Minimum Q.C. organisms (According to ISO/TS 11133-2):
Listeria monocytogenes ATCC 13932
Escherichia coli ATCC 25922
Enterococcus faecalis ATCC 29212

Storage:

Dehydrated culture media: 10-25°C
Final medium: 14 days at 2-8°C in the dark

Inoculation: Add 25g of sample to 225mL LAB211 and homogenise. Sub-culture 0.1mL of LAB211 into 10mL of LAB212 Fraser Broth^{PLUS} (ISO).

Incubation: 30°C aerobically for 24 hours.

Interpretation: Blackening of the broth indicates the presence of a potential *Listeria* spp. and should be sub-cultured onto a selective *Listeria* isolation medium, e.g. Harlequin™ Listeria Chromogenic Agar (HAL010). All broths should be sub-cultured before discarding irrespective of colour change.

References

Fraser J.A. and Sperber W.H. (1988). Rapid detection of Listeria spp in food and environmental samples by esculin hydrolysis. *J. Food Protect.* 51, No.10, 762-765.

McClain D. and Lee W.H. (1989). FSIS method for isolation of L. monocytogenes from processed meat and poultry products. Lab. Comm.No.57, Revised May 24, (1989). US Dept of Agric.FSIS, Microbiol. Div.

ISO 11290-1:1997 (Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of *Listeria monocytogenes* - part 1, Incorporating Amendment 1.)

ISO/TS 11133-2:2003. Microbiology of food and animal feed stuffs-Guidelines on preparation and production of culture media – Part 2: Practical guidelines on performance testing of culture media.

Hektoen Enteric Agar

LAB110

Description

A medium developed at the Hektoen Institute in Chicago for the enhanced recovery of shigellae from clinical specimens. This medium has high levels of peptones and sugar which counteract some of the toxic effects of bile salts used to make the medium selective. This allows the shigellae to grow as well as the salmonellae. Salicin is fermented by many coliforms including those that do not ferment lactose and sucrose. The medium employs a double indicator system similar to that used in LAB006 C.L.E.D., (Bevis) and an H_2S indicator system similar to that used in LAB032 XLD. Although intended primarily for clinical use this medium is quoted in B.S. 4285 as suitable for the examination of dairy products for salmonellae.

Formula	g/litre
Meat Peptone	12.0
Yeast Extract	3.0
Lactose	12.0
Sucrose	12.0
Salicin	2.0
Bile Salts No. 3	7.0
Sodium desoxycholate	2.4
Sodium chloride	5.0
Sodium thiosulphate	5.0
Ammonium ferric citrate	1.5
Acid fuchsin	0.1
Bromothymol blue	0.065
Agar No. 1	14.0

Method for reconstitution

Weigh 76 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then heat gently and bring to the boil. Cool to 47˚C and pour plates. DO NOT AUTOCLAVE OR OVERHEAT THIS MEDIUM.

Appearance: Green, clear.

pH: 7.5 ± 0.2

Minimum Q.C. organisms: *Salmonella* sp. NCIMB 50076
Shigella sp.
E. coli (some inhibition) NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface plating, streak out to single colonies.

Incubation: 37˚C aerobically for 18-24 hours.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
H_2S +ve *Salmonella*	2-3 Black	CV.E.G.	Green+	
H_2S -ve *Salmonella*	2-3	CV.E.G.	Green	
S. sonnei	2-2.5	CV.E.G.	Green	(Rough)
S. flexneri	1.0-2.5	CV.E.G.	Green	
S. dysenteriae	1-2	CV.E.G.	Green	
E. coli	0.5-2	CV.E.G.	Salmon ppt. around colonies	(Rough) (No growth)
Citrobacter spp.	1.0-2.0	CV.E.G.	Salmon	(Rough)
Klebsiella spp.	0.5-2.0	CV.E.G.	Salmon	(Mucoid)
Proteus spp.	1.0-2.0	CV.E.G.	Green/ Black centre	(No growth) (brownish centre)
Pseudomonas spp.	0.5-1.5	F.Rz.D.	Green	(No growth)

References

King, S. and Metzger, W.I. (1967). A new medium for the isolation of *Salmonella* and *Shigella* species. Bact. Proc. Am. Soc. Microbiol. 77.

King, S. and Metzger, W.I. (1968). A new plating medium for the isolation of enteric pathogens. Hektoen Enteric Agar, Appl. Microbiol., 16(4), 577.

King, S. and Metzger, W.I. (1968). A new plating medium for the isolation of enteric pathogens. II. Comparison of Hektoen Agar with SS and EMB agar. Appl. Microbiol., 16(4), 579.

Speck, M.L. (ed.). (1976). Compendium of Methods for the Microbiological Examination of Food. Washington, D.C.: American Public Health Association.

Hoyle's Medium

LAB027

Description

A highly selective culture medium for the isolation and differentiation of *Corynebacterium diphtheriae* types gravis, mitis and intermedius. Hoyle's medium gives rapid growth of all types of *C. diphtheriae,* which results in most specimens giving adequate growth with overnight incubation.

Formula	g/litre
Beef Extract	10.0
Peptone	10.0
Sodium chloride	5.0
Agar	12.0

Method of Reconstitution

Weigh 37 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, and sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C, add 50ml of lysed horse or sheep blood and 10ml of X027 potassium tellurite solution. Mix well before pouring.

Appearance: Dark Red, clear gel

pH: 7.8 ± 0.2

Inoculation: Spread the entire surface with the swab or sample under investigation. Hoyle's medium is very selective and spreading for single colonies using a wire loop is not necessary. Use of a non-selective blood agar alongside Hoyle's is recommended.

Incubation: 37˚C for 18-48 hrs, aerobically

Storage: Plates – up to 7 days at 2-8˚C

Interpretation

organism	colony size (mm)	shape & surface	colour	other
C. diphtheriae var gravis	1.5-2.5	CV.CR.D (daisy head)	Grey	Colonies difficult to emulsify
C. diphtheriae var mitis	0.5-2.0	CV.E.G.	Grey (dark centre)	Easily emulsified
C. diphtheriae var intermedius	0.5-1.0	CV.E.G.	Grey (dark centre)	
C. Ulcerans	1.0-1.5	CV.E.G.	Grey (dark centre)	Streptococcal appearance in Gram stain
C.hoffmanii	0.5-1.0	CV.E.G.	Black (confluent growth)	White/grey colonies
C. xerosis	0.5-2.5	CV.E.G	Black	
Streptococcus Spp.	pp-1.5	CV.E.G	Black	Enterococci may be larger
H. influenzae	pp-1.5	CV.E.G	Grey/Black	Some strains no growth

Minimum Q.C. Organisms *C. diphtheriae var mitis* (non-toxigenic)
E. coli NCTC 10418 (inhibition)

Reference:

Hoyle L. (1941) A Tellurite Blood Agar Medium for the Rapid Diagnosis of Diphtheria. Lancet 1 175-176

176. Elek S.D. (1948) The Recognition of Toxigenic Bacterial Strains in vitro. Brit. Med. J. 1 493-496.

Kanamycin Aesculin Azide Agar

(K.A.A. Agar)

LAB106

Description

A selective isolation and enumeration medium for enterococci (Lancefield group D streptococci) in food. Sodium azide and kanamycin provide the selective inhibition required whilst aesculin and iron salts form an indicator system for the presumptive identification of enterococci. Incubation at 42˚C will increase the medium's selectivity.

Formula	g/litre
Tryptone	20.0
Yeast Extract	5.0
Sodium chloride	5.0
Sodium citrate	1.0
Aesculin	1.0
Ferric ammonium citrate	0.5
Sodium azide	0.15
Kanamycin sulphate	0.02
Agar No. 1	10.0

Method for reconstitution

Weigh 43 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C, then dispense into Petri dishes.

Appearance: Pale straw, clear.

pH: 7.0 ± 0.2

Minimum Q.C. organisms: *E. faecalis* NCIMB 50030
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface, spread 0.1ml to 0.5ml over entire surface of plate.

Incubation: 37˚C or 42˚C aerobically for 18-24 hours.

Interpretation: Count all white/grey colonies, approx 2mm diameter, surrounded by a black halo to give presumptive enterococcus/faecal streptococcus count.

References

Mossel, D.A.A., Bijken, P.H.G., Eelderink, I. and van Spreekens, K.A. (1978). Streptococci, edited by Skinner, F. A. and Quesnel, L.B. SAB Symposium Series No. 7 Academic Press, London.

Kanamycin Aesculin Azide Broth

(K.A.A. Broth)

LAB107

Description

An enrichment and isolation medium for enterococci. The medium can be used with the M.P.N. technique to enumerate enterococci in food. This broth is identical to LAB106 K.A.A. agar with the omission of the agar.

Formula	g/litre
Tryptone	20.0
Yeast Extract	5.0
Sodium chloride	5.0
Sodium citrate	1.0
Aesculin	1.0
Ferric ammonium citrate	0.5
Sodium azide	0.15
Kanamycin sulphate	0.02

Method for reconstitution

Weigh 33 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, warm gently to dissolve completely then disperse into tubes or bottles. Sterilise by autoclaving at 121˚C for 15 minutes.

Appearance: Light straw, clear.

pH: 7.0 ± 0.2

Minimum Q.C. organisms: *E. faecalis* NCIMB 50030
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation: Inoculate tubes with decimal dilutions of food suspension.

Incubation: 37˚C or 42˚C aerobically for 18-24 hours.

Interpretation: Blackening of the medium suggests the presence of enterococci/faecal streptococci.

References

Mossel, D.A.A., Bijken, P.H.G., Eelderink, I. and. van Spreekens. K.A. (1978). Streptococci, edited by Skinner, F.A. and Quesnel, L.B. SAB Symposium Series No. 7 Academic Press, London.

Kligler Iron Agar

LAB059

Description

A differential medium for the recognition of enteric pathogens by their ability to ferment glucose and/or lactose, and liberate sulphides. Fermentation liberates acid, with or without gas, turning phenol red indicator yellow. Fermentation of glucose only, is followed by reversion in pH on the slope, from initial acidity to final alkalinity (red colour), but not in the anaerobic conditions of the butt, which remains acid (yellow). Fermentation of lactose as well as glucose, produces acidity in both slope and butt (yellow). Liberation of sulphide results in the formation of iron sulphide (blackening of either slope or butt).

Formula	g/litre
Peptone	20.0
Lactose	10.0
Glucose	1.0
Sodium chloride	5.0
Ferric ammonium citrate	0.5
Sodium thiosulphate	0.3
Phenol red	0.025
Agar No. 2	12.0

Method of reconstitution

Weigh 49 grams of powder and mix with 1 litre of distilled water. Bring to the boil with frequent stirring to dissolve completely. Dispense into tubes and sterilise for 15 minutes at 121˚C. Cool in a slanted position such that slopes are formed over deep butts approx. 3cm in depth.

Appearance: Reddish brown agar.

pH: 7.4 ± 0.2

Minimum Q.C. Organisms *Salmonella typhimurium* NCIMB 50076
Pseudomonas aeruginosa NCIMB 50067

Inoculation

Subcultures for further identification are picked from the centre of isolated colonies on selective media and streaked across the slant and stabbed deep into the butt of tubes of Kligler Iron Agar.

Incubation: 37˚C aerobically for 18-24 hours.

Interpretation

Organism	Butt	Slope	Sulphide
Salmonella typhi	Acid	Alkaline	+
S. paratyhi A+ B	Acid	Alkaline	-
Other *Salmonella*	Acid/gas	Alkaline	+
E. coli	Acid/gas	Acid	-
Proteus spp	Acid/gas	Alkaline	+
Shigella sonnei	Acid	Alkaline	-
S. flexneri	Acid	Alkaline	-

Storage: Tightly capped containers - up to 3 months at 15-20˚C in the dark.

References:

Kligler, I.J. (1917). A Simple Medium for the Differentiation of Members of the Typhoid - Paratyphoid Group. Am. J. Publ. Hlth, 7:1042-1044.

Bailey, S.F. and Lacey, G.R. (1927). A modification of the Kligler Lead Acetate Medium. J. Bact. 13:182-189.

Lactose Broth

LAB126

Description

A medium for the performance and confirmation of the Presumptive Test for members of the coliform group in water and dairy products, recommended by the U.S.P.

Formula	g/litre
Beef Extract	3.0
Gelatin Peptone	5.0
Lactose	5.0

Method for reconstitution

Weigh 13 grams of powder, disperse in 1 litre of deionised water, heat to dissolve then distribute into bottles with Durham tubes. Sterilise by autoclaving at 121˚C for 15 minutes.

Appearance: Straw coloured, clear.

pH: 6.9 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation: See methods for standard techniques.

Incubation: 35˚C aerobically for 48 hours.

Interpretation: Coliforms are presumptively identified by their ability to ferment lactose and produce gas within 48 hours at 35˚C.

References

American Public Health Association. (1975). Standard Methods for the examination of water and waste water, 892. Washington. United States Pharmocopeia, XXI, 1985.

Lauryl Tryptose Broth

(Lauryl Sulphate Broth, LTB, LSB)

LAB196

Description

Lauryl Tryptose Broth is a selective medium for the detection of coliforms in water, dairy products and other foods. The American Public Health Authority (APHA) recommend Lauryl Tryptose Broth for the Most Probable Number Presumptive Test of coliforms in waters, effluent or sewage and as a confirmation test of lactose fermentation with gas production from milk samples and for the detection of coliforms in foods.

Lauryl Tryptose Broth is prepared according to the formulation of Mallmann and Darby. Mallmann and Darby showed that tryptose at a concentration of 2% increased the early logarithmic growth phase when compared to meat peptone. These researchers added phosphate buffers and sodium chloride, which improved gas production by "slow lactose fermenting" organisms. Sodium lauryl sulfate was incorporated as a selective agent for the inhibition of non-coliform organisms.

This medium can also be used with the addition of MUG (4-methylumbelliferyl-β-D-glucuronide) according to the ISO Standard 11866-1 to give enhanced detection of *Escherichia coli*.

Formula	g/litre
Tryptose	20.0
Lactose	5.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	2.75
Potassium dihydrogen phosphate	2.75
Sodium lauryl sulphate	0.1

Method for reconstitution

Weigh 35.6 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and dispense into tubes or bottles containing inverted Durham tubes. Sterilise by autoclaving at 121°C for 15 minutes.

Appearance: straw, clear liquid.

pH: 6.8 ± 0.2

Minimum Q.C. organisms:
Escherichia coli NCIMB 50034
Enterobacter aerogenes NCIMB 50029
Staphylococcus aureus NCIMB 50080 (inhibition)

Storage of Prepared Medium: Store the prepared medium at room temperature (18-22°C), in the dark.

Inoculation: Inoculate the medium in accordance with standard methods or laboratory policy.

Incubation: 35°C ± 2°C for 24 and 48 hours.

Interpretation: After incubation at 35°C for 24 hours examine for turbidity and gas production. If no gas has formed incubate for a further 24 hours and re-examine.

Turbidity in the medium accompanied by the formation of gas within 48 hours is a presumptive result for the presence of coliforms. The results should be confirmed by standard testing methods.

References

American Public Health Association (1980) Standard Methods for the Examination of Water and Wastewater. 15th Edn. APHA Inc. Washington DC.

American Public Health Association (1978) Standard Methods for the Examination of Dairy Products. 14th Edn. APHA Inc. Washington DC.

American Public Health Association (1976) Standard Methods for the Examination of Foods. 15th Edn. APHA Inc. Washington DC.

Mallmann, W.L. and Darby, C.W. (1941) Am. J. Pub. Hlth. 31. 127-134.

ISO Standard 11866-2 Milk and Milk Products –Enumeration of presumptive *Escherichia coli* – part 2: Most probable number technique using 4-methyl umbelliferyl-β-D-glucuronide.

Letheen Agar (AOAC)

(Tryptone Glucose Extract Agar with Lecithin and Polysorbate 80)

LAB185

Description

Letheen Agar is used for evaluating the bactericidal activity of quaternary ammonium compounds, and is used with Letheen Broth to determine the suitability of preservatives for use in cosmetic formulations, as specified by the American Society for Testing and Materials (ASTM), Standard Test Method for Preservatives in Water-Containing Cosmetics. Letheen Agar is a modification of Tryptone Glucose Extract (TGE) Agar, and is formulated to neutralise quaternary ammonium compounds used in testing of germicidal activity, the importance of which was first described by Weber and Black in 1948. The addition of Tween®80 means Letheen Agar also neutralises phenols, hexachlorophene, formalin and ethanol (in the presence of lecithin). Letheen Agar also allows calculation of colony forming units to be assessed, when used with a hygiene swabbing protocol and will ensure against disinfectant carry-over from the swabbing diluent/medium.

Formula	g/litre
Dextrose	1.0
Tryptone	5.0
Beef extract	3.0
Lecithin	1.0
Tween®80	7.0
Agar	15.0

Method for reconstitution

Weigh 32.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix, and then sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and pour into sterile Petri dishes and allow the medium to set.

Appearance: Straw, opalescent gel.

pH: 7.0 ± 0.2

Minimum Q.C. organisms:
Escherichia coli NCIMB 9517
Staphylococcus aureus NCIMB 9518

Storage of Dehydrated Medium: Store at 2-8°C in the dark. Formulation is very hygroscopic, keep container tightly closed after use.

Storage of Prepared Medium: Plates – up to 7 days at 2-8°C in the dark.

Inoculation: Preservative testing - From the dilutions of product in Letheen Broth, subculture to Letheen Agar using a pour plate technique, or surface inoculation.

Hygiene swabbing – Subculture from the swab diluent using a pour plate or surface inoculation to allow calculation of the colony forming units (cfu) for the area swabbed.

Incubation: 37°C aerobically for 24-48 hours.

Interpretation: Count all colonies and calculate the number of cfu per ml of sample allowing for dilution factors, or the cfu of the area swabbed (typically 25cm²).

References

Weber, G.R. and Black, L.A. (1948). Relative efficiencies of quaternary inhibitors. Soap and Sanit. Chem. 24: 134-139.

American Society for Testing Materials. (1998). Standard Test Method for Preservatives in Water-Containing Cosmetics. E640-78. Annual Book of ASTM Standards, Philadelphia, PA.

Association of Analytical Chemists. (1995). Official methods of analysis, 16th edition, section 6.Association of Official Analytical Chemists, Washington, D.C.

Roberts, D., Hooper, W., and Greenwood, M. (1995). Methods for the examination of food for micro-organisms of public health significance, 2nd edition, section 5.10, Practical Food Microbiology. Butler & Tanner. ISBN 0 901144 36 3.

Letheen Broth (AOAC)

LAB184

Description

Letheen Broth is primarily used for the assessing the bactericidal activity of quaternary ammonium compounds, and for determining the phenol co-efficient of cationic surfactants as recommended by the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC). It is also used in hygiene swabbing protocols where it is necessary to neutralise quaternary ammonium compounds. A modification of FDA Broth, Letheen Broth contains lecithin to neutralise quaternary ammonium compounds and Tween®80 to neutralise phenols, hexachlorophene, formalin and (with lecithin) ethanol. Letheen Broth is easily prepared and has a clear appearance aiding in visual inspection for growth. The American Society for Testing Materials (ASTM) specifies the use of Letheen Broth in the Standard Test Method for Preservatives in Water Containing Cosmetics.

Formula	g/litre
Peptone	10.0
Beef extract	5.0
Sodium chloride	5.0
Lecithin	0.7
Tween®80	5.0

Method for reconstitution

Weigh 25.7 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix, and then sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and pour into sterile Petri dishes and allow the medium to set.

Appearance: Straw, clear liquid.

pH: 7.0 ± 0.2

Minimum Q.C. organisms:
Escherichia coli NCIMB 9517
Staphylococcus aureus NCIMB 9518

Storage of Powder: Store at 2-8°C in the dark. Formulation is very hygroscopic, keep container tightly closed after use.

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20°C in the dark.

Inoculation: There are a variety of methods which use Letheen Broth and the appropriate references should be consulted. For example:

Phenol co-efficient testing – Subculture from disinfectant dilutions into 10ml volumes of Letheen Broth

Hygiene swabbing – Swab measured area or specific equipment and place in 10ml volume of Letheen Broth. Area to be swabbed and volume of medium may vary depending upon swabbing protocol used.

Incubation: 37°C aerobically for 24-48 hours.

Interpretation: Examine all tubes for turbidity or as stipulated in the method.

References

American Society for Testing Materials, (1998). Standard Test Method for Preservatives in Water-Containing Cosmetics. E640-78. Annual Book of ASTM Standards, Philadelphia, PA.

Association of Analytical Chemists, (1995). Official methods of analysis, 16th edition, section 6. Association of Official Analytical Chemists, Washington, D.C.

Roberts, D., Hooper, W. and Greenwood, M. (1995). Methods for the examination of food for micro-organisms of public health significance, 2nd edition, section 5.10, Practical Food Microbiology. Butler & Tanner. ISBN 0 901144 36 3.

Listeria Enrichment Broth

LAB138

Description

A medium for the selective enrichment of food and environmental samples for *Listeria* spp. first described in 1987 by J. Lovett. The medium offers more rapid enrichment than the low temperature enrichment techniques. This medium is now recommended by the Commission of European Communities and the International Dairy Federation for the examination of soft cheeses for *Listeria monocytogenes*. The medium is incubated at 30˚C and utilises acriflavine, nalidixic acid and cycloheximide as selective agents.

Formula	g/litre
Tryptone	17.0
Soy Peptone	3.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	2.5
Glucose	2.5
Yeast Extract	6.0

Method for reconstitution

Weigh 36 grams of powder and add to 1 litre of deionised water. Allow to soak for 10 minutes then swirl to mix and sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C and add 2 vials of reconstituted X138 (X139 can be used as an alternative). Aseptically dispense into sterile tubes or bottles.

Appearance: Yellow, clear.

pH: 7.3 ± 0.2

Minimum Q.C. organisms: *L. monocytogenes* NCIMB 50007
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Capped containers – up to 14 days at 2-8˚C in the dark.

Inoculation: Add 25 grams of sample to 225mls of Listeria Enrichment Broth and homogenise.

Incubation: 30˚C aerobically for up to 48 hours.

Subculture: After 24 and 48 hours onto Listeria Isolation Medium – LAB122.

References

Lovett, J. Frances, D.W. Hunt, J.M. J. Food Protect. 50: 188-192.
Bolton, F. J. Personal Communication Public Health Laboratory, Preston U.K.

Listeria Isolation Medium

(Oxford Formulation)

LAB122

Description

A selective identification medium for the isolation of *Listeria monocytogenes* from food and clinical material. Columbia agar is the nutrient base to which selective inhibitors have been added. Lithium chloride is used to inhibit enterococci and acriflavine to inhibit some Gram negative and Gram positive species. Further selective agents may be added after autoclaving to increase the selectivity; these are colistin, fosfomycin, cefotetan and cyclohexamide. Aesculin is included in the formula as a differential indicator. *L. monocytogenes* will hydrolyse aesculin to aesculutin which reacts with the iron salt to give a black precipitate around the colonies.

Lab M's formulation has been used to successfully isolate *Listeria* from such diverse products as chicken giblets and dairy cheeses. The advisability of using this medium at two levels of selectivity has been recognised.

Formula	g/litre
Columbia Agar Base	41.0
Aesculin	1.0
Ferric ammonium citrate	0.5
Lithium chloride	15.0

Method for reconstitution
Weigh 57.5 grams of powder. Add to 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Allow to cool to 47˚C, add 2 vials of selective supplement X122 (X123 can be used as an alternative), mix well and pour plates.

Appearance: Pale yellow, slightly opaque gel.

pH: 7.2 ± 0.2

Minimum Q.C. organisms: *L. monocytogenes* NCIMB 50007
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface, streak out to single colonies. This medium is highly selective, a heavy inoculum can be used.

Incubation: 30˚C aerobically for 24-48 hours.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
L. monocytogenes	0.5-1.0	CV.E.G.	Grey/ Green	Black/brown around colonies diffusion
Gram-ve Bacilli	No growth			
Enterococci	p.p. - 0.5	CV.E.G.	Black	Usually no growth

References

Garayzabal, J.F.F., Rodriguez, L.D., Boland, J.A.V., Cancelo, J.L.B., Fernandez, G.S. (1986). *Listeria monocytogenes* dans le lait pasteurise. Can. J. Microbiol. 32: 149-150.

Donnelly. C.W., Gregory J. Baigent (1986). Method for flow cytometric detection of *Listeria monocytogenes* in milk. Appl. & Environ. Microbiol.Oct. 689-695.

Bolton, C.F.J. Preston P.M.L. Personal communication. Lovett, J. Francis, D.W. Hunt. J.M. (1987). *Listeria monocytogenes* in raw milk: Detection, Incidence and Pathogenicity. Journ. Food Protect. Vol. 50. No. 3: 188-192.

Van Netten, P., Van de Van, A., Perales, I., Mossel, P.A.A. (1988). A selective and diagnostic medium for use in enumeration of *Listeria* spp. in foods. International Journal of Food Microbiology 6:187-198.

Listeria Isolation Medium, Oxford (ISO)

LAB206

Description
A selective identification medium for the isolation of *Listeria monocytogenes* from food and clinical material. This media is formulated according to ISO 11290 and tested in accordance with CEN ISO/TS 11133-2. Columbia agar is the nutrient base to which selective inhibitors have been added. Lithium chloride is used to inhibit Enterococci, whilst acriflavine inhibits some Gram-negative and Gram-positive species. Further selective agents may be added after sterilisation to increase the selectivity.

Formula	g/litre
Columbia agar base	41.0
Aesculin	1.0
Ferric ammonium citrate	0.5
Lithium chloride	15.0

Method for reconstitution
Weigh 57.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C, and add 2 vials of either X122 or X123. Mix well before dispensing into sterile Petri dishes.

Appearance:
Powder: Fine, free-flowing, homogeneous, buff.

Final medium: Straw-yellow gel.

pH: 7.0 ± 0.2

Hazard classification
Xn - Harmful

Minimum Q.C. organisms:
Listeria monocytogenes ATCC 19111
Listeria monocytogenes NCTC 10527
Escherichia coli ATCC 25922
Enterococcus faecalis ATCC 29212
Candida albicans ATCC 10231

Storage:

Dehydrated culture media: 10-25°C

Poured plates: 7 days at 2-8°C in the dark

Inoculation: Surface inoculation as per user's validated methods.

Incubation: Incubate for 24-48 hours at 30, 35 or 37°C according to user's validated methods.

References

ISO/TS 11133-2:2003 Microbiology of food and animal feeding stuffs - Guidelines on preparation and production of culture media - Part 2: Practical guidelines on performance testing of culture media (ISO/TS 11133-2:2003).

ISO 11290-1:1996 Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection method.

Listeria Monocytogenes Blood Agar

(LMBA)

LAB172

Description

Listeria monocytogenes Blood Agar (LMBA) has been developed for the specific detection and enumeration of *L. monocytogenes* in food samples. This medium has been shown to improve the isolation rate of *L. monocytogenes* in ready to eat foods by up to 22%.

L. monocytogenes is the only important human pathogen among the species of Listeria currently recognised. It is distinguished from *L. innocua* on LMBA using colonial appearance and haemolysis. Studies have shown that the most commonly isolated species of listeria, other that *L. monocytogenes* from food and processing environments is *L. innocua*.

L. monocytogenes can be found in all main categories of products e.g. dairy, meat and poultry. The symptoms of infection with this organism include fever, generalised aches and pains, sore throat, diarrhoea and abdominal pains. In severe cases pneumonia, septicaemia and meningitis may develop. Pregnant women are particularly susceptible to listeriosis, due to immune suppression. *L. monocytogenes* can cross the placenta causing abortion, still birth, or meningitis of the new born.

LMBA contains lithium chloride in concentrations that inhibit the growth of enterococci yet allow good haemolysis by *L. monocytogenes*. LBMA is supplemented by polymyxin plus ceftazidine (X072) and nalidixic acid (X072N) to suppress competing flora such as members of the bacillus group and staphylococci.

The addition of donated sheep blood (defibrinated with sodium citrate) to LMBA allows differentiation between haemolytic and non-haemolytic stains of *Listeria*. The use of sheep blood is standard methodology for *Listeria* testing. However, ingredients in selective agars can result in partial lyses or darkening of the blood supplement. The use of citrated sheep blood prevents this and allows differentiation of *L. monocytogenes* from other haemolytic *Listeria* species e.g. *L. seeligeri* and *L. ivanovii*, due to its distinctive haemolytic pattern. *L. seeligeri* is rarely isolated from foods and produces very weak haemolysis, whilst *L. ivanovi*i produces wide zones of haemolysis compared to the narrow zone of *L. monocytogenes* and is an animal rather than human pathogen.

LBMA is a cost effective method by which to specifically isolate and enumerate *L. monocytogenes*

Formula	g/litre
Tryptone	15.0
Soy peptone	5.0
Sodium chloride	5.0
Lithium chloride	10.0
Magnesium sulphate (3/4H_2O)	3.8
Agar	15.0

Method for Reconstitution

Weigh 53.8 grams of powder and disperse into 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix then sterilise at 121°C for 15 minutes. Cool to 47°C, and aseptically add citrated sheep blood to 5%, 2 vials of X072 supplement and 2 vials of X072N supplement. Mix well, and pour into sterile Petri dishes and allow to set.

Appearance: Opaque and blood red.

pH: 7.2 ± 0.2

Minimum Q.C. organisms:
Listeria monocytogenes NCIMB 50007.
Listeria innocua NCTC 11288.
Escherichia coli NCIMB 50034.
Enterococcus faecalis NCIMB 50030.

Storage of Prepared Medium: Plates can be stored up to 7 days at 2-8°C in the dark.

Inoculation: Surface plating, streaking out to single colonies, from the enrichment broth. For enumeration, 0.1ml of neat or 10-1 dilution of the food sample is spread over the entire surface of the plate. Use multiple plates if the volume required is greater than 0.1 ml.

Incubation: Aerobically at 37°C for 24-48 hours.

Interpretation:

Organism	Shape and Surface	Colour & Haemolysis
L. monocytogenes	C.V.E.G.	Cream, narrow zone of β haemolysis
L. ivanovii	C.V.E.G.	Cream, wide zone of β haemolysis
L. innocua	C.V.E.G.	Cream, no haemolysis
L. seeligeri	C.V.E.G.	Cream, very weak β haemolysis
Enterococcus faecalis		No growth
Escherichia coli		No growth

References

Johansson, T (1998). Enhanced detection and enumeration of *Listeria monocytogenes* from foodstuffs and food processing environments. International Journal of Food Microbiology, 40; 77-85.

Jay, J.M. (1996). Prevalence of *Listeria* spp. in meat and poultry products. Food Control, 7; 209-214.

Kozak, J., Balmer, T., Byrne, R. and Fisher, K. (1996). Prevalence of *Listeria monocytogenes* in foods: incidence in dairy products. Food Control, 7; 215-222.

Lysine Agar

LAB201

Description

Originally described by Morris and Eddy, this complex synthetic medium is designed for the isolation and enumeration of wild yeasts in pitching yeast. Lysine is utilised by wild yeasts, but not by *Saccharomyces cerevisiae*, *S. carlsbergensis* and *S. pastorianus*. Lab M Lysine Agar is made to the Morris and Eddy published formulation.

Formula	g/litre
Peptone tryptic digest of meat	10.0
Meat extract	10.0
Yeast extract	1.5
Starch	1.0
Hydrated sodium acetate	5.0
Glucose	1.0
L-Cysteine hydrochloride	0.5
Sodium sulphite	0.4
Iron (III) citrate	0.7

Method for reconstitution

Disperse 30.1g of powder in 1 litre of distilled water. Allow to soak for 10 minutes, swirl to mix and dispense into final containers. Sterilise by autoclaving for 15 minutes at 121°C.

Medium should be used on day of preparation. If medium is stored, tubes should be reheated to deoxygenate the medium. Tubes should not be reheated more than once.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, straw liquid

pH: 7.1 ± 0.2

Hazard classification

NR – Not regulated

Storage:

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

Inoculation (as per BS EN 26461-1:1993): Before the test, the sample of water should be heated in a water bath at 75 ± 5°C for 15 minutes from the time it reaches that temperature.

Add 50ml of sample to 50ml double strength medium (x5).
Add 10ml of sample to 10ml double-strength medium (x5).
Add 1ml of sample to 25ml single-strength medium (x5).

If required add 1ml of a 1 in 10 dilution of the sample to 25ml single-strength medium (x5).

To qualitatively examine 100ml drinking/bottled water without performing MPN, add 100ml sample to 100ml double-strength medium.

If required, top up all bottles with single-strength medium to bring the volume of liquid level with the neck of the bottle, and to ensure that only a very small volume of air remains. Seal the bottles hermetically, or incubate under anaerobic conditions.

Incubation (as per BS EN 26461-1:1993): Incubate aerobically with lids uppermost at 37°C ± 1°C for 44 ± 4 hours.

Large volumes of culture in hermetically sealed glass bottles may explode due to gas production. The addition of iron wire, heated to redness and placed into the medium before inoculation, may aid anaerobiosis

Interpretation (as per BS EN 26461-1:1993): Bottles in which blackening is observed, as a result of the reduction of sulphite and the precipitation of iron (II) sulphide, shall be regarded as positive.

References

BS EN 26461-1:1993 / BS 6068-4.8:1993 / ISO 6461-1:1986. Water quality – Detection and enumeration of he spores of sulfite-reducing anaerobes (clostridia) – Part 1: Method by enrichment in a liquid medium.

Freame, B. & Fitzpatrick, B.W.F. (1967). The use of Differential Reinforced Clostridial Medium for the isolation and enumeration of Clostridia from foods. *The Society for Applied Microbiology Technical Series n. 5: Isolation of Anaerobes,* ed. Shapton, D.A. & Board, R.G. **Vol. 5.** London Academic Press. 49-55.

Gibbs, M.B. (1973). The detection of Clostridium welchii in the Differential Reinforced Clostridial Medium technique. *J. Appl. Bact.* **36**. 23-33.

Gibbs, M.B. & Freame, B. (1965). Methods for the recovery of clostridia from foods. *J. Appl. Microbiol.* **28**. 95-111.

Gibbs, M.B. & Hirsch, A. (1956) Spore formation by Clostridium species in an artificial medium. *J. Appl. Bact.* **19**. 129-141.

Hirsch, A. & Grinstead, E. (1954). Methods for the growth and enumeration of anaerobic spore-formers from cheese, with observations on the effects on nisin. *J. Dairy Res.* **21**. 101-110.

Methods for the Examination of Waters and Associated Materials. The Microbiology of Drinking Water (2002) – Part 1 – Water Quality and Public Health.

Lysine Iron Agar

LAB054

Description

This is a differential medium for the detection of salmonellae and other enteric pathogens, by means of lactose fermentation, lysine decarboxylase activity and hydrogen sulphide production. *Salmonella* strains (including *Salmonella arizona*) which ferment lactose and produce black colonies on Bismuth Sulphite Agar (LAB013) can be recognised by the alkaline reaction (purple colour) produced throughout the medium, together with blackening due to sulphide production. Enteric organisms that do not decarboxylate lysine yield an alkaline slant over an acid butt (yellow). Thus no distinction between *Shigella* and *E. coli* is possible and Triple Sugar Iron Agar (LAB053) is recommended in parallel. *Proteus* and *Providencia* cultures characteristically produce a distinctive red slant over an acid butt since these organisms deaminate lysine but without sulphide production. *Salmonella arizona* strains which produce pink to red colonies on bile salt media are often overlooked in outbreaks of food poisoning, however the use of Bismuth Sulphite Agar with subculture into Lysine Iron Agar allows determination of their presence.

Formula	g/litre
Balanced Peptone No. 1	5.0
Yeast Extract	3.0
Glucose	1.0
L-Lysine	10.0
Ferric Ammonium Citrate	0.5
Sodium thiosulphate	0.04
Bromocresol Purple	0.02
Agar No. 2	12.0

Method for reconstitution

Weigh 31.5 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and bring to the boil, with frequent stirring to dissolve completely. Dispense into tubes and sterilise by autoclaving at 121°C for 15 minutes. Cool in a slanted position such that slopes are formed over deep butts approx. 3cm in depth.

Appearance: Clear purple gel.

pH: 6.7 ± 0.2

Storage of Prepared Medium: Tightly capped containers – up to 3 months at 15-20°C in the dark.

Inoculation: Subcultures for further identification are picked from the centre of isolated colonies on selective media and streaked across

the slant and stabbed into the butt of tubes of Lysine Iron Agar.

Incubation: 37°C aerobically for 18-24 hours.

Growth Characteristics

Organism	Butt	Slant	Sulphide Production
Salmonella arizona	Alkaline	Alkaline	+
Salmonella	Alkaline	Alkaline	+
Salmonella paratyphi	Acid	Acid	-
Enterobacter aerogenes)			
Klebsiella)	Alkaline	Alkaline	-
Hafnia)			
Serratia)			
Citrobacter	Acid	Alkaline	+
Escherichia coli	Acid (NC)	Alkaline	-
Shigella	Acid	Alkaline	-
Proteus	Acid	'red'	-
Providencia	Acid	'red'	-

References

Edwards, P.R. and Fife, M.A. (1961). Lysine iron agar in the detection of Arizona cultures. Appl. Microbiol. 9:478-480.

Edwards, P.R. and Ewing, W.H. (1964). Identification of Enterobacteriaceae. Burgess Publishing Co. Minn.

M17 Agar

LAB092

Description

A medium for the enumeration of lactic streptococci *(Streptococcus lactis, Streptococcus cremoris, Streptococcus diacetylactis)* in dairy products. The medium can also be used to investigate the bacteriophage susceptibilities of these organisms. Another application is for the enumeration of *Streptococcus thermophilus* in yoghurts.

Formula	g/litre
Balanced Peptone	5.0
Soy Peptone	5.0
Yeast Extract	2.5
Beef Extract	5.0
Lactose	5.0
Sodium glycerophosphate	19.0
Magnesium sulphate	0.25
Ascorbic acid	0.5
Agar No. 2	15.0

Method for reconstitution

Weigh 57.2 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then bring to boil to dissolve agar before dispensing in 15ml aliquots. Sterilise by autoclaving at 115˚C for 20 minutes.

Appearance: Pale straw, translucent agar.

pH: 7.1 ± 0.2

Minimum Q.C. organisms: *S. lactis*

Storage of Prepared Medium: Capped containers – up to 1 month at 15-20˚C in the dark.

Inoculation: Pour plate technique.

Incubation: 30˚C for 48-72 hours for mesophilic streptococci, 37˚C for 48 hours for *Streptococcus thermophilus*.

Interpretation: Count all colonies. Streptococci form colonies of 1-2mm in diameter.

References

Terzaghi, B.E. Sandine, W.E.. (1975). Improved medium for lactic Streptococci and their Bacteriophages. Appl. Microbiol. 29 No. 6 pp 807-813.

MacConkey Agar

(With Salt)

LAB030

Description

A selective medium for the isolation of bile tolerant organisms from faeces, urine, sewage and foodstuffs. Bile-tolerant Gram positive organisms as well as Gram negative organisms will grow on this medium. This formula is recommended by W.H.O. and other bodies for the examination of water and milk. Some strains of *Proteus* spp. will spread on this medium making interpretation difficult, for this reason LAB002 MacConkey Agar (without salt) may be preferred as it is less prone to this phenomenon.

Formula	g/litre
Peptone	20.0
Lactose	10.0
Bile Salts	5.0
Sodium chloride	5.0
Neutral red	0.05
Agar No. 2	12.0

Method for reconstitution

Weigh 52 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C and mix well before pouring into Petri dishes.

Appearance: Pink/red, clear

pH: 7.4 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface plating, streaking out to single colonies.

Incubation: 37˚C aerobically for 24 hours.

Growth Characteristics

organism	approx. size (mm)	shape & surface	colour	other
E. coli	2.0-3.0	CV.E.G.	Red	(ppt around colony)
K. aerogenes	2.0-4.0	CV.E.G.	Pink/Red	(mucoid)
Citrobacter spp.	2.0-4.0	CV.E.G.	Pink/Red	(ppt around colony)
Proteus spp.	1.5-2.5	CV.E.G. (spreading)	Yellow	
Salmonella spp.	1.5-2.5	CV.E.G.	Colourless	
Shigella spp.	1.0-2.0	CV.E.G. (pink) Transp.	Colourless	
S. aureus	0.5-2.0	CV.E.G.	White/ Pink Orange Opaque	(dependent on lactose fermentation and pigment production)
Enterococcus spp.	P.P.-0.5	CV.E.G.	Pink/Deep Red Opaque	
P. aeruginosa	1.0-3.0	F.CR.D.	Transp. Pinkish	(colonial-variation)

References

Environment Agency: The Microbiology of Drinking Water (2002). Methods for the Examination of Water and Associated Materials.

World Health Organisation (1971). International Standards for Drinking Water. 3rd Edn. W.H.O., Geneva.

Taylor, E.W. (1958). The Examination of Water and Water Supplies. 7th Edn. Churchill, London.

Cruikshank, R. (1973). A Guide to the Laboratory Diagnosis and Control of Infection. Medical Microbiology. 12th Edn. Churchill.

MacConkey Agar

(without salt)

LAB002

Description

A medium first introduced by MacConkey in 1905 for the isolation and differentiation of lactose and non lactose fermenting enteric bacteria. The medium has since been modified to improve the recovery of staphylococci and enterococci, it is used for culturing a wide range of clinical material and has applications in food, water and dairy bacteriology.

Formula	g/litre
Mixed Peptones	20.0
Lactose	10.0
Bile	5.0
Neutral red	0.05
Agar No. 2	13.5

Method for reconstitution

Weigh 48.5 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving for 15 minutes at 121˚C. Cool to 47˚C and mix well before pouring plates. Prior to inoculation, dry the surface of the agar by partial exposure at 37˚C.

Appearance: Pink/red, clear.

pH: 7.4 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034
S. aureus NCIMB 50080

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface inoculation, streaking for single colonies.

Incubation: 37˚C aerobically for 24 hours.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
E. coli	2.0-3.0	CV.E.G.	Red	(non lactose fermenting yellow)
K. aerogenes	3.0-4.0	CV.E.G.	Pink-Red	(mucoid)
Proteus spp.	2.0-3.0	CV.E.G.	Yellow	fishy odour
Ps. aeruginosa	2.0-4.0	F.CR.D.	Pink-Yellow-(Green)	characteristic odour if green
Shigella spp.	1.0-3.0	CV.E.G.	Yellow	
Salmonella spp.	2.0-3.0	CV.E.G.	Yellow	
S. aureus	0.5-1.0	CV.E.G.	Pink-Orange	(lactose-negative)
Enterococcus spp.	0.5	CV.E.G.	Pink-Deep Red	

References

MacConkey, A.T. (1905) Lactose-fermenting bacteria in faeces. J.Hyg. (Camb), 5: 333-379.

MacConkey, A. T. (1908) Bile salt media and their advantages in some bacteriological examinations, J.Hyg. (Camb.), 8: 322-341.

Environment Agency: The Microbiology of Drinking Water (2002). Methods for the Examination of Water and Associated Materials.

World Health Organisation (1971), International Standards for Drinking Water, 3rd Edn. W.H.O., Geneva. Taylor, E.W. (1958). The Examination of Water Supplies, 7th Edn. Churchill, London

MacConkey Agar No.2

NEW **LAB216**

Description

MacConkey Agar No.2 is a modification of MacConkey Agar which contains bile salts No. 2 for the recognition of enterococci. This is especially useful when looking for enterococci in the presence of coliforms and non-lactose fermenters from water, sewage and food products. Enterococci are frequently sought as an index of faecal pollution and appear on this medium as small, intensely coloured red-purple colonies. Non-lactose fermenters appear colourless, whilst bile tolerant Gram-positive organisms, such as staphylococci and non-faecal streptococci, are completely inihibited.

Formula	g/litre
Peptone	20.0
Lactose	10.0
Bile salts No.2	1.5
Sodium chloride	5.0
Neutral red	0.05
Crystal violet	0.001
Agar	15.0

Method for reconstitution

Weigh 51.6 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C and mix well before dispensing into Petri dishes. Dry the agar surface prior to use.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, red-purple gel

pH: 7.2 ± 0.2

Hazard classification

NR – Not regulated

Minimum Q.C. organisms:
Enterococcus faecalis ATCC 29212
Escherichia coli ATCC 25922
Salmonella typhimurium NCIMB 13284
Staphylococcus aureus ATCC 25923 (inhibited)

Storage:

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

Inoculation: Surface inoculation as per user's validated methods.

Incubation: Incubate at 37°C + 1°C for 18 - 48 hours.

Interpretation: After incubation the plate should be assessed for typical colonies.

Interpretation

organism	colony size (mm)	shape & surface	colour
Enterococcus faecalis	0.5mm	Convex, entire, glossy	Red-purple
Escherichia coli	3 - 4mm	Convex, entire, glossy	Red-purple
Salmonella spp.	2 – 3mm	Convex, entire, glossy	Translucent
Shigella spp.	2 – 3mm	Convex, entire, glossy	Translucent
Staphylococcus aureus	No growth		

MacConkey Agar No. 3

LAB045

Description

A modification recommended by the W.H.O. and the American Public Health Association for the isolation of *Enterobacteriaceae* from waters and sewage. The medium has been made more selective than MacConkey's original formula by the use of crystal violet as well as bile salts. Gram positive organisms will not grow on this medium.

Formula	g/litre
Peptone	20.0
Lactose	10.0
Bile Salts No. 3	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar No. 2	15.0

Method for reconstitution

Weigh 51.5 grams of powder and add to 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise for 15 minutes at 121˚C. Cool to 47˚C and pour into Petri dishes. Dry the surface before inoculation.

Appearance: Pale red slight violet tinge.

pH: 7.1 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034
Ent. faecalis (inhibition) NCIMB 50030

Storage of prepared medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface, streaking for single colonies.

Incubation: 37˚C aerobically for 18-24 hours.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
E. coli	3.0-4.0	CV.E.G. (D)	Red	(red ppt around colony)
Kleb. aerogenes	4.0-5.0	CV.E.G.	Pink-red	(mucoid)
Proteus spp.	3.0-4.0	CV.E.G.	Pale-yellow	(fishy odour)
Ps. aeruginosa	1.0-1.5	F.CR.D.	Yellow-green	
Shigella spp.	2.0-3.0	CV.E.G. (D)	Pale-yellow	(pink)
Staph. aureus	No growth			
Other *Staphylococcus* spp.	No growth			
Enterococcus spp.	No growth			

References

American Public Health Association (1950). Diagnostic Procedures and Reagents. 3rd edn. A.P.H.A., New York.

American Public Health Association (1946). Standard Methods for the examination of Water and Sewage. 9th edn. A.P.H.A., New York

MacConkey Broth

(Purple)

LAB005

Description

This medium is used in the detection and enumeration of faecal coliforms (37˚C) and *E. coli* (44˚C). The replacement of neutral red used in the original formulation by bromocresol purple makes the colour change caused by acid producing organisms easier to read.

Formula	g/litre
Peptone	20.0
Lactose	10.0
Bile Salts	5.0
Bromocresol purple	0.01

Method for reconstitution

Weigh 35 grams of powder, disperse in 1 litre of deionised water. Mix well and dispense into tubes or bottles with inverted Durham tubes. Sterilise by autoclaving for 15 minutes at 121˚C. Prepare double strength broth (70g/l) if 50ml or 10ml amounts of inoculum are to be added to equal volumes of broth. Prepare single strength broth (35g/l) if 1ml or 0.1ml amounts of inoculum are to be added to 10ml of broth.

Appearance: Purple, clear.

pH: 7.3 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034
B. subtilis (inhibition)

Storage of Prepared Medium: Capped containers – up to 1 month at 15-20˚C in the dark.

Incubation: 37˚C aerobically for coliforms, 44˚C aerobically for *E. coli*. Use Durham tubes to detect gas production for *E. coli*.

Growth Indicators: Turbidity, gas production. Lactose-fermenting organisms cause a colour change from purple to yellow.

References

Ministry of Health (1937). Bacteriological Tests for Graded Milk, Memo 139/Foods. H.M.S.O., London.

Minister of Health, Public Health Laboratory Service Water Committee (1969). The Bacteriological Examination of Water Supplies, 4th Edn. report No. 71. H.M.S.O., London.

World Health Organisation (1971). International Standards for Drinking Water, 3rd Edn, W.H.O., Geneva.

Malt Extract Agar

LAB037

Description

An acidic medium which will support the growth of most yeasts and moulds whilst inhibiting most bacteria. It was first described by Thom and Church in 1926 in a study of *Aspergillus* spp. claiming the high carbohydrate content ensured rapid growth. Selectivity can be increased by further lowering the pH with the addition, after sterilisation, of X037 Lactic Acid. It should be noted that excess heating of this medium together with its low pH can easily result in hydrolysis of the agar gel producing soft plates.

Formula	g/litre
Malt Extract	30.0
Mycological Peptone	5.0
Agar No. 2	15.0

Method for reconstitution

Weigh 50 grams of powder, disperse in 1 litre of deionised water, allow to soak for 10 minutes, swirl to mix then sterilise at 115˚C for 10 minutes. If the addition of X037 Lactic Acid is required this should be done after sterilisation. One 5ml vial of X037 will lower the pH of 250ml of medium to 3.5-4.0. Cool to 47˚C before making additions and pouring plates.

Appearance: Pale brown/straw, clear.

pH: 5.4 ± 0.2 (if X037 is added pH 3.5-4.0)

Minimum Q.C. organisms: *Candida* spp. NCIMB 50010

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark. Capped container – up to 1 month at 15-20˚C in the dark.

Inoculation: Pour plate technique or surface streaking for single colonies.

Incubation: 25˚C aerobically for 5 days.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
Candida albicans	4	CV.E.D.	White	
Candida krusei	10	F.CR.D.	White	
Penicillium notatum	25		Green velvet	(white/yellow - strain dependent)
Aspergillus niger	25		White border, black centre	(yellow/black centre)

References

Galloway, L.D. and Burgess, R. (1952). Applied Mycology and Bacteriology, Leonard Hill, London. Thom and Church, 1926. The Aspergilli.

Malt Extract Broth

LAB159

Description

A liquid medium of low pH for the growth of yeasts and moulds, typically employed as part of sterility testing protocols for various products. The high carbohydrate content of the medium ensures rapid growth of yeasts and moulds.

Formula	g/litre
Malt Extract	17.0
Mycological Peptone	3.0

Method for reconstitution

Weigh 20g of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to dissolve and dispense into final containers. Sterilise by autoclaving at 115˚C for 10 minutes.

Appearance: Pale brown/straw, clear broth.

pH 5.4 ± 0.2

Inoculation: Inoculate samples direct into tubes of broth according to the particular method being employed.

Incubation: 25˚C (or 37˚C) for up to 7 days aerobically, depending upon protocol used. Subculture turbid tubes onto solid media for identification of growth.

Minimum QC organism: *Candida albicans* NCIMB 50010
Aspergillus niger NCIMB 50097

References

Galloway, L.D. and Burgess, R. (1952) Applied Mycology and Bacteriology, 3rd ed, Leonard Hill, London pp 54 & 57.

Mannitol Salt Agar

LAB007

Description

Mannitol Salt Agar is a medium for *Staphylococcus aureus* which is selective because the high sodium chloride level inhibits most other species with the exception of halophilic Vibrios. The majority of *S. aureus* ferment mannitol producing yellow colonies, occasional strains of coagulase-negative staphylococci may also ferment mannitol. It is necessary to confirm the identity of presumptive *S. aureus* colonies by other means e.g. coagulase, protein A, DN'ase, thermonuclease or latex agglutination. This medium is recommended in the European Pharmacopeia.

Formula	g/litre
Beef Extract	1.0
Balanced Peptone No. 1	10.0
Sodium chloride	75.0
D-Mannitol	10.0
Agar No. 2	12.0
Phenol Red	0.025

Method for reconstitution

Weigh 108 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C before pouring into Petri dishes.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, red gel

pH: 7.4 ± 0.2

Minimum Q.C. organisms: *S. aureus ATCC 6538*
E. coli ATCC 8739 (inhibition)

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation Method: Surface plating, streak out for single colonies.

Incubation: In accordance with European Pharmacopeia, incubate aerobically at 30-35°C for 18-72 hours. The product may also be used for other applications at 37°C for 48 hours.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
S. aureus	1.5-2.0	CV.E.G.	Bright Yellow	
Other Staphylococci	1.0-1.5	CV.E.G.	White or Yellow	(some ferment mannitol)
Enterobacteriaceae no growth				
Vibrio spp. & other halophiles	1.5-4.0	various	usually Pink	(yellow if mannitol +ve)

References

American Public Health Association (1966). Recommended Methods for Microbiological Examination of Foods, 2nd Edn. (ed. J.M. Sharf) A.P.H.A. Washington.

Davis, J.G., (1959). Milk Testing 2nd edn, Dairy Industries, London.

European Pharmacopeia 6th Edition Supplement 6.3. Council of Europe European (COE) - European Directorate for the Quality of Medicines (EDQM). June 2008

Maximum Recovery Diluent

(Peptone/Saline diluent)

LAB103

Description

An osmotically controlled solution which is an alternative to, and a replacement for, 1/4 strength Ringer's Tablets (LAB100Z). The presence of a low level of peptone lessens the physiological shock normally experienced by bacterial cells when they are introduced to a diluent such as Ringer's Solution. The level of peptone is such that multiplication of the organisms is not possible in the time in which the sample will be present in the diluent (1-2 hours). This formula is recommended by ISO 6887: BS5763.

Formula	g/litre
Peptone	1.0
Sodium chloride	8.5

Method for reconstitution

Weigh 9.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and if required, heat gently to dissolve. Distribute into final containers. Sterilise by autoclaving for 15 minutes at 121°C.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, colourless liquid

pH: 7.0 ± 0.2

N.B. The absence of any buffer results in the product taking on the pH of any sample added.

Minimum Q.C. organisms: *E. coli ATCC 25922*
S. aureus ATCC 25923

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20˚C in the dark.

References

Straka, R.P. and Stokes, J.L. (1957). Rapid destruction of bacteria in commonly used diluents and its elimination. Appl. Microbiol. 5: 21-25.

ISO 6887-1:1999 Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 1: General rules for the preparation of the initial suspension and decimal dilutions.

Membrane Lauryl Sulphate Broth

LAB082

Description

This medium superseded Membrane Enriched Teepol broth when Shell Chemicals withdrew Teepol 610 from sale. Sodium lauryl sulphate was found to be an adequate reproducible substitute and this medium is recommended for the enumeration of coliform and organisms in water and sewage.

Formula	g/litre
Peptone	39.0
Yeast Extract	6.0
Lactose	30.0
Phenol red	0.2
Sodium lauryl sulphate	1.0

Method for reconstitution

Weigh 76.2 grams of powder, disperse in 1 litre of deionised water. Distribute into screw cap containers and sterilise by autoclaving at 115˚C for 10 minutes.

Appearance: Red, clear solution.

pH: 7.4 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation: *E. coli* and coliform counts should be made on separate samples of water. The volumes should be chosen so as the number of colonies on the membrane lies between 10 and 100. With waters expected to contain less than 1 coliform per ml, a sample of 100ml should be filtered. The membrane filter should be placed face upwards on a pad soaked in Membrane Lauryl Sulphate Broth, after filtration. These membranes should be incubated in a container which does not allow evaporation to occur. Water tight metal containers placed in an accurate water bath are required for incubation of membranes at 44˚C.

Incubation: *E. coli* 4 hours at 30˚C 14 hours at 44˚C; Coliforms 4 hours at 30˚C 14 hours at 35˚C.

Interpretation: No colonies:- assume a nil count. Small colonies of an intermediate colour:- return to incubation for a full period.

E. coli: Yellow-coloured colonies from membranes incubated at 44˚C should be subcultured to Lactose Broth LAB126 and Tryptone Water, LAB129 to confirm gas and indole production respectively, after 24 hours incubation at 44˚C.

Coliform organisms: Yellow colonies from membranes incubated at 35˚C or 37˚C should be subcultured into Lactose Broth LAB126. After 48 hours incubation at 37˚C a result should be obtained regarding the production of gas.

Full details of the methodology can be found in The Bacteriological Examination of Water Supplies 71, 1969.

References

Burnham, N.P. (1967). Proc. Soc. Wat. Treat, Exam. 16:40.

Environment Agency: The Microbiology of Drinking Water (2002). Methods for the Examination of Water and Associated Materials.

Windle Taylor, E. (1961) Glutamic acid medium, 40th Ann Rep. Div. Water Exam. Met. Water Board London pp 18-22.

Microbial Content Test Agar (MCA)

(Tryptone Soy Agar with Lecithin and Tween®80 (TSALT))

(Casein Soy Peptone Agar with Lecithin and Polysorbate 80)

LAB189

Description

The use of Microbial Content Test Agar (MCA) is recommended for the detection of microorganisms on surfaces sanitised with quaternary ammonium compounds, phenolic compounds and formalin. The medium is a modification of Tryptone Soy Agar with added neutralising compounds lecithin and Tween®80. It is recommended for determining the hygiene status of containers, equipment and work areas treated with disinfectants or other sanitisers. The addition of Lecithin and Tween®80 in the formula inactivates some preservatives that may inhibit bacterial growth, reducing "preservative carryover". The formulation is recommended for Aerobic Plate Count (Microbial Limit Test) for water miscible cosmetic products containing preservatives. Lecithin is included to neutralise quaternary ammonium compounds and Tween®80 is incorporated to neutralise phenols, hexachlorophene, formalin and with lecithin, ethanol.

Formula	g/litre
Tryptone	15.0
Soy Peptone	5.0
Sodium Chloride	5.0
Tween®80	5.0
Lecithin	0.7
Agar No.2	15.0

Method for Reconstitution

Weigh 45.7 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and pour into sterile Petri dishes and allow the medium to set.

Appearance: Straw opalescent gel.

pH: 7.3 ± 0.2

Minimum Q.C. organisms:
Escherichia coli ATCC 11229
Staphylococcus aureus ATCC 6538P

Storage of Powdered Medium: Store at 2-8°C in the dark. Formulation is very hygroscopic, keep container tightly closed after use.

Storage of Prepared Medium: Plates – up to 7 days at 2-8°C in the dark.

Inoculation: Consult the appropriate references as this product is used in several procedures.

Incubation: 37°C aerobically for 24-48 hours.

Interpretation: Count all colonies and calculate the number of colony forming units, (cfu) per ml of sample allowing for dilution factors.

References

Orth, D.S. (1993) Handbook of Cosmetic Microbiology. Marcel Dekker, Inc., New York, NY.

Brummer, B. (1976). Influence of possible disinfectant transfer on Staphylococcus aureus plate counts after contact sampling. App. Environ. Microbiol. 32:80-84.

Milk Agar

LAB019

Description

An approved formulation for the enumeration of micro-organisms in milk, rinse waters and dairy products. With the addition of a further 5 g/l Agar No. 1 the medium is suitable for the preparation of Roll-Tubes using established mechanical equipment. Also see Milk Plate Count Agar LAB115. Milk Agar can also be used with the P-INC supplement (X019, X219) for accelerated shelf-life determination of dairy products.

Formula	g/litre
Yeast Extract	3.0
Peptone	5.0
Antibiotic Free Skim Milk Powder	1.0
Agar No. 1	15.0

Method for reconstitution

Weigh 24 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 15 minutes, swirl to mix then sterilise for 15 minutes at 121˚C. Cool to 45˚C before mixing with sample dilutions.

Appearance: White, opalescent gel.

pH: 7.2 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034
S. aureus NCIMB 50080

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation: Pour plate technique.

Incubation: Aerobically at 30˚C for 72 hours.

References

Ministry of Health (1937). Bacteriological Tests for Graded Milk. Memo 139/Foods H.M.S.O., London. British Standard 4285: Methods of Microbiological Examination for Diary Purposes.

Milk Plate Count Agar

LAB115

Description

A medium recommended by the British Standards Institute and the International Organisation for Standardisation for the enumeration of viable bacteria in milk and other dairy products.

Formula	g/litre
Tryptone	5.0
Yeast Extract	2.5
Dextrose	1.0
Antibiotic Free Skim Milk Powder	1.0
Agar No. 1	10.0

Method for reconstitution

Weigh 19.5 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then bring to the boil to dissolve the agar. Allow to cool to 47˚C and dispense into suitable containers. Sterilise by autoclaving at 121˚C for 15 minutes.

Appearance: Pale cream, opalescent gel.

pH: 6.9 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034
S. aureus NCIMB 50080

Storage of Prepared Medium: Capped container – up to 3 months at 15-20˚C in the dark.

Inoculation: Pour plate technique.

Incubation: 30˚C aerobically for 72 hours.

Interpretation: Count all colonies. Calculate back to determine viable organisms per ml

References

British Standards Institute. (1984). BS 4285 Section 1.2. International Organisation for Standardisation Draft International Standard. (1982) ISO/DIS 6610. D.I.N. 10192.

Minerals Modified Glutamate Medium

LAB080A & LAB080B

Description

This medium was developed for use with the Most Probable Numbers Technique (M.P.N.) for the enumeration of coliforms in water supplies. The medium is an improved version of the chemically defined glutamic acid medium described by Gray in 1964. The product is supplied in two parts because it has been shown that separating the sodium glutamate from the base improves its stability.

Formula	g/litre
LAB080A	**(double strength)**
Lactose	20.0
Sodium formate	0.5
L-Cystine	0.04
L(-) Aspartic acid	0.048
L(+) Arginine	0.04
Thiamine	0.002
Nicotinic acid	0.002
Pantothenic acid	0.002
Magnesium sulphate ($MgSO_4.7H_20$)	0.2
Ferric ammonium citrate	0.02
Calcium chloride ($CaCl_2.2H_2O$)	0.02
Dipotassium hydrogen phosphate	1.8
Bromocresol purple	0.02
LAB080B	
Glutamic acid (sodium salt)	12.7

Method for reconstitution

Double strength: Dissolve 22.7 grams of base medium (LAB080A) together with 12.7 grams of sodium glutamate (LAB080B) in 1 litre of deionised water containing 5 grams of ammonium chloride eg BDH cat no. 27149. Dispense 10ml and 50ml volumes into tubes with inverted Durham tube.

Single strength: Dissolve in 11.35 grams of base medium (LAB080A) together with 6.35 grams of sodium glutamate (LAB080A) in 1 litre of distilled water containing 2.5 grams ammonium chloride. Dispense 5ml volumes into tubes with inverted Durham tubes.

Sterilise by autoclaving for 10 minutes at 115˚C, alternatively heat to 100˚C for 30 minutes on three successive days.

Appearance: Purple, clear solution.

pH: 6.7 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation: Use the Most Probable Number technique. With 10ml and 50ml of sample add to equal volumes of double strength medium. With 1ml volumes of sample add to 5ml of single strength medium. Ensure the Durham tube is free of bubbles.

Incubation: 37˚C for 18-24 hours aerobically.

Interpretation Tubes showing the production of acid (medium turns yellow) and gas in the Durham's tube are considered presumptive positive. Each presumptive positive tube should be subcultured to Brilliant Green Bile Broth LAB051 with Durham tube and incubated at 44˚C for 24 hours and examined for gas production. A tube of Tryptone Water LAB129 should also be inoculated and incubated at 44˚C for 24 hours for the production of indole. The production at 44˚C of gas from lactose and the formation of indole are evidence of *E. coli*.

References

Gray, R.D. (1964). An improved formate lactose glutamate medium for the detection of *Escherichia coli* and other coliform organisms in water. J. Hyg. Camb. 62: 495-508.

PHLS Water Sub-Committee. (1958). A comparison between MacConkey broth and Glutamic acid media for the detection of coliform organisms in water. J. Hyg. Camb. 56: 377-388.

PHLS Standing Committee on Bacteriological Examination of Water Supplies. (1968). Comparison of MacConkey Broth, Teepol Broth and Glutamic Acid Media for the enumeration of Coliform organisms in water. J. Hyg. Camb. 66: 67-87.

Environment Agency: The Microbiology of Drinking Water (2002). Methods for the Examination of Water and Associated Materials.

M.L.C.B. Agar

(Mannitol Lysine Crystal Violet Brilliant Green Agar)

LAB116

Description

A medium for the selective isolation of *Salmonella* spp. (with the exception of *S. typhi* and *S. paratyphi A*) from food and faeces. *Salmonella* colonies are recognised by distinctive colonial appearance and H_2S production and like the Bismuth Sulphite Agar of Wilson & Blair, this medium will detect lactose and sucrose fermenting strains. Some problems may occur with H_2S negative strains, eg *S. pullorum, S. senftenberg, S. sendai* and *S. berta.* This medium should not be used to detect *S. typhi* and *S. paratyphi A,* as these strains are more susceptible to the brilliant green dye.

Formula	g/litre
Yeast Extract	5.0
Tryptone	5.0
Meat Peptones	7.0
Sodium chloride	4.0
Mannitol	3.0
L-Lysine HCL	5.0
Sodium thiosulphate	4.0
Ferric ammonium citrate	1.0
Brilliant green	0.012
Crystal violet	0.01
Agar No. 2	15.0

Method for reconstitution

Weigh 49 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then bring to the boil with frequent agitation to completely dissolve the powder. Cool to 47˚C and pour plates. DO NOT AUTOCLAVE OR OVERHEAT.

Appearance: Pale purple, translucent gel.

pH: 6.8 ± 0.2

Minimum Q.C. organisms: *Salmonella* spp. NCIMB 50076
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface plating, streaking for single colonies. Inoculation can be carried out directly, or from enrichment broths. Because of the low selectivity of this medium the inoculum should not be heavy, and it is recommended that this medium should be used in conjunction with other more selective media.

Incubation: 37˚C aerobically for 24 hours.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour
Salmonella spp.	2.0-3.0	CV.E.G.	Black
Salmonella spp. (H_2S negative)	2.0-3.0	CV.E.G.	Pale
Proteus spp.	1.0-2.0	CV.E.G.	Grey brown
Shigella spp.			mainly inhibited
Citrobacter spp.	0.5-2.5	CV.E.G.	Mainly inhibited pale may have black centre
E. coli	mainly inhibited		
Klebsiella spp	mainly inhibited		
Gram positive organisms	mainly inhibited		

References

Inove *et al.* 66th meeting of the Japanese Vet. Medicine Society.

MLSTB-MT (ISO)

Modified Lauryl Sulphate Tryptose Broth with MUG & Tryptophan (ISO)

NEW **LAB077**

Description

Modified Lauryl Sulphate Tryptose Broth with MUG & Tryptophan (ISO) is used for the presumptive enumeration of *Escherichia coli* from milk and milk products using the Most Probable Number (MPN) technique according to ISO 11866-1:2005.

The original Lauryl Tryptose Broth described by Mallmann and Darby (1941) has been modified to incorporate 4-Methylumbelliferyl-β-D-glucuronide (MUG) and tryptophan allowing presumptive *E. coli* to be enumerated from presumptive coliforms. The addition of MUG to the medium allows the positive discrimination of *E. coli* strains. As the majority of *E. coli* produce the β-glucuronidase enzyme, they are able to hydrolyse MUG, releasing a fluorogenic compound. Tryptophan acts as a substrate for the indole test. Tubes which fluoresce under UV are confirmed for *E. coli* by a positive indole reaction when indole (Kovac's) reagent is added to the tube. Tubes showing gas formation are identified as being positive for presumptive coliforms.

Phosphate buffers and sodium chloride improve gas production by slow lactose fermenting organisms whilst sodium lauryl sulphate acts as a selective agent for the inhibition of non-coliform organisms.

Modified Lauryl Sulphate Tryptose Broth with MUG & Tryptophan (ISO) is described in ISO 11866-1:2005 and is used in the HPA National Standard Method D5 for Enumeration of coliforms and presumptive *Escherichia coli* by the Most Probable Number (MPN) technique.

Formula	g/litre
Tryptose	20.0
Lactose	5.0
Dipotassium hydrogen phosphate	2.75
Potassium dihydrogen phosphate	2.75
Sodium chloride	5.0
Sodium lauryl sulphate	0.1
4-Methylumbelliferyl-β-D-glucuronide (MUG)	0.1
Tryptophan	1.0

Method for reconstitution

Disperse 36.7g of powder in 1 litre of distilled water. Allow to soak for 10 minutes, swirl to mix and heat gently to dissolve. Dispense 10 ml volumes into test tubes (e.g. 16mm x 160mm tubes of non-autofluorescent glass) containing inverted Durham's tubes. Sterilise at 121ºC for 15 minutes. This medium may also be used at double-strength (73.4g/l).

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, straw liquid

pH: 6.8 ± 0.2

Hazard classification

NR – Not regulated

Minimum Q.C. organisms:
Escherichia coli ATCC 25922
Enterococcus faecalis ATCC 29212 (inhibition)

Storage:

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 1 month at 2-8°C in the dark.

Inoculation: Add 1ml of test sample per tube of single-strength medium. For double-strength medium add 10ml of test sample per tube. Repeat for each further dilution.

Incubation: Incubate all inoculated tubes at 30°C for 24 hours (± 2 hours). Incubation may be extended for up to 48 hours (± 2 hours) if gas formation and/or turbidity not observed at 24 hours (± 2 hours).

After incubation, add 0.5ml sodium hydroxide solution to all tubes. Examine for fluorescence under a long-wave (360-366nm) UV lamp. Add 0.5ml indole (Kovac's) reagent to all tubes showing fluorescence. Mix & observe after 1 minute. The presence of indole (positive tube) is indicated by the formation of a red colour in the alcoholic phase.

Interpretation: Presumptive *E. coli* are indicated by those tubes showing fluorescence and a positive indole reaction.

Presumptive coliforms are indicated by those tubes which show gas formation.

Counts should then be performed according to Most Probable Number (MPN technique).

References

Health Protection Agency National Standard Method. Enumeration of coliforms and presumptive *Escherichia coli* by the Most Probable Number (MPN) technique. Reference number D5i2.4; issued May 2005.

ISO 11866-1:2005 Milk and milk products – Enumeration of presumptive *Escherichia coli* – Part 1: Most probable number technique using 4-methylumbelliferyl-β-D-glucuronide (MUG).

Mallmann, W.L. and Darby, C.W. (1941). *Am. J. Pub. Hlth*. **31**: 127-134.

Modified Giolitti and Cantoni Broth (ISO)

NEW **LAB219**

Description

Modified Giolitti and Cantoni Broth (ISO) is used for the detection and enumeration of coagulase-positive staphylococci from food and animal feeding stuffs using the Most Probable Number (MPN) technique according to ISO 6888-3:2003.

Originally described by Giolitti and Cantoni as a medium for the enrichment of staphylococci from foodstuffs, Mossel later applied the medium to use with samples from dried milk and infant food.

Optimised for use in samples where staphylococci may be stressed and/or in low numbers, growth of the target organisms is promoted by sodium pyruvate, Glycine and the high concentration of mannitol. Selectivity is achieved via lithium chloride, which inhibits Gram-negative bacilli, and potassium tellurite, which inhibits Gram-positive organisms other than staphylococci. Further selectivity is achieved by use of anaerobiosis either by pouring a plug of agar/paraffin or by incubation in a jar or incubator under anaerobic conditions. Anaerobiosis particularly inhibits the growth of *Micrococcus* spp.

The presence of coagulase-positive staphylococci is indicated by the reduction of tellurite, resulting in a blackening of the broth or a black precipitate. Coagulase-positive staphylococci are principally *Staphylococcus aureus* but may also include the species *Staphylococcus intermedius* and *Staphylococcus hyicus*.

Formula	g/litre
Enzymatic digest of casein	10.0
Meat extract	5.0
Yeast extract	5.0
Lithium chloride	5.0
Mannitol	20.0
Sodium chloride	5.0
Glycine	1.2
Sodium pyruvate	3.0

Method for reconstitution

For single-strength media: disperse 54.2g of powder in 1 litre of distilled water. Allow to soak for 10 minutes, swirl to mix and add 1g polyoxyethelene sorbitan mono-oleate (Tween 80). Swirl to disperse and heat gently to dissolve. Dispense the medium in appropriate quantities into tubes of suitable dimensions e.g. 9ml in 16mm x 160mm tubes. Sterilise at 121ºC for 15 minutes.

For double-Strength media: disperse 108.4g of powder in 1 litre of distilled water. Allow to soak for 10 minutes, swirl to mix and add 2g polyoxyethelene sorbitan mono-oleate (Tween 80). Swirl to disperse and heat gently to dissolve. Dispense the medium in appropriate quantities into tubes of suitable dimensions e.g. 10ml in 20mm x 200mm tubes. Sterilise at 121ºC for 15 minutes.

If product is to be used on day of preparation, allow to cool to 44-47ºC and use immediately.

If the medium is not used as above then the medium must be re-heated to 100ºC for 15 minutes to expel any dissolved oxygen and cooled to 44-47ºC.

Prior to use add X043 1% Potassium Tellurite to give a final concentration of 0.1g/L, e.g. add 0.1ml X043 to 9ml of single strength base or add 0.2ml X043 to 10ml of double strength base. DO NOT REHEAT MEDIA CONTAINING POTASSIUM TELLURITE.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, straw liquid (will be darker if prepared at double-strength)

pH: 6.9 ± 0.2

Hazard classification

NR – Not regulated

Minimum Q.C. organisms:
Staphylococcus aureus ATCC 6538P
Staphylococcus aureus ATCC 25923
Escherichia coli ATCC 25922
Escherichia coli ATCC 8739

Storage:

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: use immediately after preparation. The sterilised base medium can be stored at 2-8°C for 1 week but prior to use heat the base medium at 100ºC for 15 minutes to expel any dissolved oxygen, cool to 44-47ºC and aseptically add X043 as per directions for use.

Inoculation:

Detection method: add 1g/1ml of sample to 9ml/9g single-strength medium or 10g/10ml of sample to 10ml/10g double-strength broth.

Enumeration method: add 1ml of sample to each of three tubes of single-strength medium or 10ml of sample to each of three tubes of double-strength medium. Repeat for any subsequent dilutions.

Incubation: Incubate anaerobically (either by agar/paraffin plug in each tube or under anaerobic conditions in a gas jar or anaerobic workstation) at 37°C for 24 to 48 hours (± 2 hours).

Interpretation: Formation of a black precipitate or the blackening of the broth indicates the presence of coagulase-positive staphylococci.

Sub-culture:

24 hours: tubes suspected as positive for coagulase-positive staphylococci after 24 hours should be confirmed by sub-culture on to either Baird-Parker Medium (LAB285+X085) or Rabbit Plasma Fibrinogen Agar (LAB285+X086). Suspected positive tubes should then be reincubated for full 48 hours.

48 hours: tubes suspected as positive at 48 hours should be confirmed by sub-culture on to either Baird-Parker Medium (LAB285+X085) or Rabbit Plasma Fibrinogen Agar (LAB285+X086).

Tubes showing a presumptive negative result after 48 hours incubation should also be sub-cultured on to either Baird-Parker Medium or RPF Agar.

References

Giolitti, G. and Cantoni, C. (1966). A medium for the isolation of staphylococci from foodstuffs. J. Appl. Bacteriol. 29:395-398.

Mossel, D.A.A., Harrewijn, G.A. and Elzebroek, J.M. (1973). UNICEF.

ISO 6888-3:2003 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 3: Detection & MPN technique for low numbers.

M.R.S. Agar

(de Man, Rogosa and Sharpe Agar)

LAB093

Description

MRS Agar is a medium for the cultivation and enumeration of *Lactobacillus* spp.

Originally developed in 1960 by de Man, Rogosa & Sharpe, the medium is suitable for most lactic acid bacteria and is intended as a substitute for Tomato Juice Agar.

When acidified to pH 5.4 M.R.S. Agar can be used to enumerate *Lactobacillus bulgaricus* in yoghurts.

Nutrition is provided by a mixture of carefully selected peptones, glucose, beef & yeast extracts whilst Tween® 80, magnesium and manganese sulphates act as growth stimulants. Selectivity against streptococci & moulds is provided by ammonium citrate and sodium acetate. Used at low pH, ammonium citrate allows growth of lactobacilli whilst inhibiting a number of other organism groups.

Occasionally, sterilisation of this medium at 121˚C for 15 minutes, in some autoclaves, may cause the pH to fall outside of the specified pH limits 6.4 +/- 0.2. In these rare cases, adjustment of the medium using acetic acid or sodium hydroxide is recommended.

Formula	g/litre
Mixed Peptones	10.0
Yeast Extract	5.0
Beef Extract	10.0
Glucose	20.0
Dipotassium phosphate	2.0
Sodium acetate	5.0
Triammonium citrate	2.0
Magnesium sulphate	0.2
Manganese sulphate	0.05
Tween® 80	1.08
Agar No. 1	15.0

Method for reconstitution

Weigh 70 grams of powder and add 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and pour into sterile Petri dishes. If acidified medium is required, adjust pH prior to pouring. Dry the agar surface before use.

Appearance:

Powder: fine, slightly cohesive, light tan powder with some lumps
Finished medium: Light amber, clear gel

pH: 6.4 ± 0.2

Hazard classification: NR – Not regulated

Minimum Q.C. organisms:
Lactobacillus casei subsp. *rhamnosus* ATCC 7469
Lactobacillus plantarum ATCC 8014
Lactobacillus delbrueckii subsp. lactis ATCC 4797

Storage of Prepared Medium:

Dehydrated culture media: 10-25˚C.

Final medium: Plates - 7 days at 2-8˚C.

Capped containers – up to 1 month at 15-20˚C in the dark.

Inoculation: Surface, spread to cover surface, or use pour plate technique.

Incubation: 25˚C microaerobically for 2-5 days.

Interpretation: Count all colonies exhibiting typical morphology.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour
Lactobacillus acidophilus	0.5-2.5	F.E.G.	White Rough
Lactobacillus sake	0.5-1.0	F.E.G.	White
Lactococcus lactis subsp. lactis	0.5-1.5	CV.E.G.	White
Lactobacillus bulgaricus	1.0-1.3	CV.E. G.	White

References

de Man, J.C.,. Rogosa, M and Sharpe, M.E. (1960). A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. 23: 130-135.

M.R.S. Broth

(de Man, Rogosa and Sharpe Broth)

LAB094

Description

MRS Broth is a medium for the cultivation and enumeration of *Lactobacillus* spp. This product has the same formulation as LAB093 MRS Agar with the omission of agar.

Originally developed in 1960 by de Man, Rogosa & Sharpe, the medium can be used for confirmatory tests on organisms isolated on MRS Agar. The medium can also be used for enumeration by the Miles and Misra technique.

Nutrition is provided by a mixture of carefully selected peptones, glucose, beef & yeast extracts whilst Tween® 80, magnesium and manganese sulphates act as growth stimulants. Selectivity against streptococci & moulds is provided by ammonium citrate and sodium acetate.

Occasionally, sterilisation of this medium at 121˚C for 15 minutes, in some autoclaves, may cause the pH to fall outside of the specified pH limits 6.4 +/- 0.2. In these rare cases, adjustment of the medium using acetic acid or sodium hydroxide is recommended.

Formula	g/litre
Mixed Peptones	10.0
Yeast Extract	5.0
Beef Extract	10.0
Glucose	20.0
Potassium phosphate	2.0
Sodium acetate	5.0
Magnesium sulphate	0.2
Manganese sulphate	0.05
Tween® 80	1.08
Ammonium citrate	2.0

Method for reconstitution

Weigh 55 grams of powder and add 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then warm to completely dissolve solids. Dispense into suitable tubes or bottles then sterilise by autoclaving at 121°C for 15 minutes.

Appearance:

Powder: fine, slightly cohesive, light tan powder with some lumps
Finished medium: Light amber, clear

pH: 6.4 ± 0.2

Hazard classification: NR – Not regulated

Minimum Q.C. organisms:
Lactobacillus casei subsp. *rhamnosus* ATCC 7469
Lactobacillus plantarum ATCC 8014
Lactobacillus delbrueckii subsp. lactis ATCC 4797

Storage of Prepared Medium:

Dehydrated culture media: 10-25°C.

Final medium: capped containers – up to 3 months at 15-20°C in the dark.

Inoculation: Either with suspect colonies from M.R.S. agar or with serial dilutions of test material.

Incubation: 25˚C microaerobically for 2-5 days.

Interpretation: For enumeration purposes count tubes showing signs of growth as positive.

References

de Man, J.C., Rogosa, M. and Sharpe, M.E. (1960). A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. 23, 130-135.

MSRV

(Semi-solid Rappaport Medium)

LAB150

Description

MSRV was developed in 1986 by De Smedt, Bolderdijk and Rappold as a rapid means of *Salmonella* detection. The medium, based upon Rappaport Vassiliadis broth, is inoculated directly from the pre-enrichment medium, in the centre of the plate. Motile organisms spread from the centre in the semi-solid agar, but non-salmonellas are inhibited by the selective agents.

After overnight incubation the use of polyvalent salmonella antisera or a latex kit can confirm the presence of a *Salmonella.* Alternatively, a paper disc wetted with polyvalent H antiserum can be placed 1/3 of the way from the edge of the dish, and will signal the presence of a *Salmonella* by inhibiting the mobility of the organism around the disc.

Using this medium De Smedt and Bolderdijk have reported the possibility of detecting *Salmonella* in 24hrs (1987)

Formula	g/litre
Tryptone	2.3
Meat Peptone	2.3
Acid Hydrolysed Casein	4.7
Sodium chloride	7.3
Potassium dihydrogen phosphate	1.5
Magnesium chloride	10.9
Malachite green	0.037
Agar No. 1	2.5

Method for reconstitution

Weigh 31.5 grams of powder and disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix and bring to the boil. Cool to 47˚C. and add 2 vials of X150 novobiocin supplement (10mg/vial). Mix well before dispensing.

Appearance: Turquoise/blue, clear, soft gel.

pH: 5.2 ± 0.2

Minimum Q.C organisms: *Salmonella typhimurium* NCIMB 50076
E. coli (inhibition) NCIMB 50034

Storage of prepared medium: Plates – up to 7 days at 4˚C.

Inoculation: From pre-enrichment broth (6-24hrs) adding 0.1ml to the centre of the plate.

Incubation: 37˚C. or 42 ± 0.5˚C. for 18-24 hours. Keep lid uppermost at all times.

Interpretation: A spreading growth indicates a *Salmonella* may be present, substantiated if a disc with polyvalent H antiserum has been added and is inhibiting the zone. This should be confirmed by subculturing from the edge of the mobility zone onto XLD and brilliant green agar and performing biochemical and serological tests. Direct latex agglutination may be carried out from the edge of the mobility zone.

References

De Smedt, J.M. and Bolderdijk, R.F. (1987): 'One Day Detection of *Salmonella* from Foods and Environmental Samples by Mobility Enrichment'. Fifth International Symposium on Rapid Methods and Automation in Microbiology and Immunology, Florence (1987). Brixia Academic Press.

De Smedt, J.M. and Bolderdijk, R.F., Rappold H. and Lautenschlaeger, D. Rapid Salmonella Detection in Foods in Mobility Enrichment on a Modified Semi-Solid Rappaport- Vassiliadis Medium. Journal of Food Protection 49 510-514. (1986).

De Smedt, J.M. and Bolderdijk, R.F. Dynamics of Salmonella Isolation with Modified Semi-Solid Rappaport-Vassiliadis Medium. Journal of Food Protection 50 658-661. (1987).

De Smedt, J.M. and Bolderdijk, R.F. Collaborative Study of the International Office of Cocoa. Chocolate and Sugar Confectionery on the Use of Mobility Enrichment for Salmonella Detection in Cocoa and Chocolate. Journal of Food Protection 53 659-664. (1990).

Goossens, H., Wauters, G., De Boeck, M., Janssens, M., and Butzler, J.P. Semi-solid selective mobility enrichment medium for isolation of Salmonella from faecal specimens J. Clin. Microbiol 19 940-941. (1984).

Mueller Hinton Agar

LAB039

Description

A medium for antimicrobial sensitivity testing by the disc diffusion method. This medium, used in the technique of Bauer and Kirby, has been adopted by the National Committee for Clinical Laboratory Standards (NCCLS) in the USA as the definitive method for susceptibility testing. The medium has a very low thymine and thymidine content, making it suitable for trimethoprim and sulphonamide testing, controlled to ensure correct zone sizes with aminoglycoside and tetracyline antibiotics. The medium was originally formulated as a heat labile protein free medium for the isolation of pathogenic *Neisseriaceae.*

Formula	g/litre
Beef Extract	2.0
Acid Hydrolysed Casein	17.5
Starch	1.5
Agar No. 1	17.0
Calcium ions	50-100mg/litre
Magnesium ions	20-35mg/litre

Method for reconstitution

Weigh 38 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise at 121˚C for 15 minutes. Cool to 47˚C, mix well and pour plates.

Appearance: Straw coloured, clear gel.

pH: 7.3 ± 0.1

Minimum Q.C. organisms: *E. coli* ATCC 25922
S. aureus (antibiotic sensitivity zones) ATCC 25923

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface, inoculum as described by N.C.C.L.S.

Incubation: As recommended by methodology for particular organisms and antibiotics by NCCLS.

References

Mueller, J.H. and Hinton, J. (1941). Protein-free medium for primary isolation of gonococcus and meningococcus. Proc. Soc. Exp. Biol. and Med., 48: 330-333.

Goodale, W.I., Gould, G. and Schwab, L. (1943). Laboratory Identification of sulphonamide resistant gonococcic infection. J.Am. Med. Ass., 123: 547-549.

American Public Health Association. (1950). Diagnostic Procedures and Reagents. 3rd edn., A.P.H.A., New York.

NCCLS. (1986). Performance standards for antimicrobial susceptibility testing – second informational supplement.

Mueller Hinton Broth

LAB114

Description

This medium is the broth version of Mueller Hinton Agar. It is an antagonist free medium for use in the tube dilution technique for the determination of antibiotic M.I.C. values. The medium is carefully standardised to meet N.C.C.L.S. standards for antimicrobial susceptibility tests on bacteria which grow aerobically.

Formula	g/litre
Beef Extract	2.0
Acid Hydrolysed Casein	17.5
Starch	1.5
Calcium ions	50 mg/litre
Magnesium ions	20 mg/litre

Method for reconstitution

Weigh 21 grams powder, disperse in 1 litre distilled water. Allow to soak for 10 minutes, swirl to mix then heat gently to dissolve. Distribute into tubes or bottles, and sterilise at 121˚C for 10 minutes.

Appearance: Pale straw, clear.

pH: 7.4 ± 0.2

Minimum Q.C. organisms: *S. aureus* ATCC 25923
E. coli ATCC 25922
(M.I.C. values)

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation: Standard inocula are required. As described by NCCLS.

Incubation: As recommended by methodology for particular organisms and antibiotics by NCCLS.

References

MacFaddin, J. (1985). Media for isolation cultivation, identification maintenance of medical bacteria. Williams & Williams, Baltimore.

N.C.C.L.S.-M7-A. (1985). Methods for dilution antimicrobiol susceptibility tests for bacteria that grow aerobically. Approved Standard.

Mueller Kauffman Tetrathionate Broth

LAB042

Description

A selective enrichment broth for salmonellae first described by Mueller in 1923 then modified by Kauffman in 1935 with the addition of Brilliant Green and Ox Bile increasing its selectivity. Organisms which can utilise tetrathionate, such as most *Salmonella,* flourish. However some salmonellas will be missed in this medium either because they are sensitive to Brilliant Green or cannot utilise tetrathionate (included in this group is *S. typhi.)* This medium is used in the standard European Community *Salmonella* Isolation Procedure and in International Standards Organisation (ISO) Methods.

Formula	g/litre
Tryptone	7.0
Soy Peptone	2.3
Sodium chloride	2.3
Calcium carbonate	25.0
Sodium thiosulphate anhydrous	40.7
Ox Bile	4.75

Method for reconstitution

Weigh 82 grams of powder, disperse in 1 litre of deionised water. Bring to the boil and cool to below 45˚C. Prior to use, add 30ml 1N iodine solution (or 19ml of a solution containing iodine 20g, potassium iodine 25g, distilled water 100ml) and 9.5ml of brilliant green 0.1% w/v solution. Mix well and dispense into sterile containers, keeping the chalk in suspension.

Appearance: Green, turbid solution which precipitates on standing.

pH: 7.8 ± 0.2

Minimum Q.C. organisms: *Salmonella* sp. NCIMB 50076
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Capped container – up to 7 days at 2-8˚C in the dark.

Inoculation: 1 part sample to 9 parts medium or 1 part pre-enrichment medium to 10 parts medium.

Incubation: 43˚C aerobically for 24-48 hours.

Subculture: Subculture onto selective agars. e.g. B.G.A. LAB034, XLD LAB032, M.L.C.B. LAB116.

References

Mueller. (1923). Un nouveau milieu d'enrichissment pour la recherche du bacille typhique et des paratyphiques C.R. Soc. Biol (Paris) 89: 434. (1975).

International Organisation for Standardisation. Meat and meat products. Detection of salmonellae (Reference method).

ISO 3565 (E). International Organisation for Standardisation Microbiology-1981 General guidance on methods for the detection of *Salmonella.* ISO 6579(E).

International Organisation for Standardisation. Milk and milk products- 1985. Detection of *Salmonella.* ISO 6785(E).

Edel, W. and Kampelmacher, E.H. (1968). Comparative studies on *Salmonella* isolations in eight European laboratories, Bull. Wld. Hlth.
Org. 41: 297-306.

Kauffman, F., (1935). Weitere Erfahrungen mit dem kombinierten Anreicherungsverfahren fur Salmonellabacillen. Z. Hyg. 117: 26-32.

van Leusden, F.M., van Schothorst, M. and Beckers, H.J. (1982). The standard *Salmonellae* isolation method. In: Isolation and identification methods for food poisoning organisms, edited by Corry, J.E.L., Roberts, D. and Skinner, F.A. SAB Technical Series,
No. 17 35-49. Academic Press, London.

Mueller-Kauffmann Tetrathionate novobiocin Broth (MKTTn)

LAB202

Description

A selective enrichment medium for the isolation of salmonellae from food and animal feeds. This product conforms to BS EN ISO 6579:2002. The recent addition of novobiocin is to inhibit the growth of *Proteus* spp.

Formula	g/litre
Meat Extract	4.3
Enzymatic digest of casein	8.6
Sodium chloride	2.6
Calcium carbonate	38.7
Sodium thiosulphate (anhydrous)*	30.45
Ox bile	4.78
Brilliant green	0.0096

*Equivalent to 47.8g of sodium thiosulphate pentahydrate.

Method for reconstitution

Weigh 89.4 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and bring to the boil. Cool to below 45°C, prior to use add 20 ml of iodine-iodide solution and 4 vials of X150 Novobiocin. Mix well and distribute into sterile containers.

Iodine-iodide solution

Dissolve 25g of potassium iodide in 10 ml of water. Add 20g iodine and dilute to 100ml with sterile deionised water.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: Green turbid solution that precipitates on standing

pH: 8.2 ± 0.2 (base medium)

Hazard classification

NR – Not regulated

Minimum Q.C. organisms (according to CEN ISO/TS 11133-2:2003):
Salmonella Typhimurium ATCC 14028 or
Salmonella Enteritidis ATCC 13076
Escherichia coli ATCC 25922 or ATCC 8739

Storage:

Dehydrated culture media: 10-25°C

Base medium (without supplements): 2 weeks at 10-25°C

Complete medium: use on day of preparation

Inoculation and Incubation: Following pre-enrichment in non-selective liquid media (see ISO 6579:2002), transfer 1ml of the broth to 10ml of MKTTn and 0.1ml to 10ml of RVS broth (LAB086). Incubate LAB202 MKTTn at 37°C ± 1°C for 24h ± 3h and LAB086 RVS broth at 41.5 ± 1°C, for 24h ± 3h. Subculture these selective broths onto XLD agar (LAB032) and a second isolation medium of your choice and incubate for 24h ± 3h. Salmonella should be confirmed by appropriate biochemical and serological techniques.

References

BS EN ISO 6579:2002 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.

ISO/TS 11133-2:2003 Microbiology of food and animal feeding stuffs – Guidelines on preparation and production of culture media – Part 2: Practical guidelines on performance testing of culture media.

Nutrient Agar

LAB008

Description

A general purpose medium for the cultivation of organisms that are not demanding in their nutritional requirements e.g. organisms that can be isolated from air, water, dust etc. Nutrient Agar is suitable for teaching and demonstration purposes, it is isotonic and can be enriched with biological fluids such as sterile blood and egg yolk.

Formula	g/litre
Peptone	5.0
Beef Extract	3.0
Sodium chloride	8.0
Agar No. 2	12.0

Method for reconstitution

Weigh 28 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving for 15 minutes at 121˚C. Cool to 47˚C, mix well then pour plates.

Appearance: Buff, opalescent gel.

pH: 7.3 ± 0.2

Minimum Q.C. organisms: *S. aureus* NCIMB 50080
E. coli NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark. Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation: Surface streaking for single colonies.

Incubation: Temperature and time to suit organisms. Usually aerobic.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
S. aureus	1.0-2.0	CV.E.G.	White-Yellow	
other *Staphylococcus* spp.	0.5-2.0	CV.E.G.	White-Yellow	
Strep. pyogenes	P.P.-0.5	CV.E.G.	Transp.	
E. coli	1.5-2.5	CV.E.G.	Grey	
Proteus spp.	spreading	-	Grey	fishy odour
Klebsiella spp.	2.0-4.0	CV.E.G.	Grey	mucoid
Bacillus spp.	2.0-6.0	various	Grey	may spread
Ps. aeruginosa	2.0-4.0	F.CR.D.	Grey-Green	odour if pigmented

Nutrient Agar (ISO)

LAB214

Description

A general purpose medium for the cultivation of non-fastidious micro-organisms. In microbiological terms this is a relatively basic composition enabling use with a wide range of organisms. This version of Nutrient Agar is formulated according to ISO 6579:2002, ISO 10273:2003 and ISO 16654:2001 and tested in accordance with ISO/TS 11133-2:2003.

Formula	g/litre
Meat Extract	4.3
Enzymatic digest of casein	8.6
Sodium chloride	2.6

Method for reconstitution

Weigh 21.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C. Mix well before dispensing into Petri dishes and dry the agar surface prior to use.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: straw, clear gel

pH: 7.0 ± 0.2

Hazard classification

NR – Not regulated

Minimum Q.C. organisms:
Escherichia coli ATCC 25922
Salmonella typhimurium ATCC 14028
Yersinia enterocolitica ATCC 9610

Storage:

Dehydrated culture media: 10-25°C

Final medium: 7 days at 2-8°C in the dark

References

BS EN ISO 6579:2002 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. (*incorporating Corrigendum No. 1*)

ISO 10273:2003 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of presumptive pathogenic *Yersinia enterocolitica*.

ISO 16654:2001 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Escherichia coli* O157.

ISO/TS 11133-2:2003 Microbiology of food and animal feeding stuffs – Guidelines on preparation and production of culture media – Part 2: Practical guidelines on performance testing of culture media.

Nutrient Broth "E"

LAB068

Description

An inexpensive broth for the growth of nutritionally non-demanding organisms. Ideal for teaching purposes.

Formula	g/litre
Beef Extract	1.0
Yeast Extract	2.0
Peptone	5.0
Sodium chloride	5.0

Method for reconstitution

Weigh 13 grams of powder, add to1 litre of deionised water. Heat to dissolve then dispense into bottles or tubes. Sterilise by autoclaving at 121˚C for 15 minutes.

Appearance: Straw coloured, clear.

pH: 7.4 ± 0.2

Minimum Q.C. organisms: *S. aureus* NCIMB 50080
E. coli NCIMB 50034

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation and incubation: To suit chosen organism.

Growth indicator: Turbidity.

Nutrient Broth No. 2 B.P.

LAB014

Description

A general purpose broth which can be used for sterility testing for aerobic organisms as recommended by the British Pharmacopoeia. This broth can also be used as the suspending medium for cooked meat granules for the cultivation of anaerobic organisms.

Formula	g/litre
Beef Extract	10.0
Balanced Peptone No. 1	10.0
Sodium chloride	5.0

Method for reconstitution

Weigh 25 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then dispense into tubes or bottles, and sterilise for 15 minutes at 121˚C.

Appearance: Pale straw, clear.

pH: 7.3 ± 0.2

Minimum Q.C. organisms: *S. aureus* NCIMB 50080
E. coli NCIMB 50034

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20˚C in the dark.

Incubation: 37˚C for 18-24 hours aerobically.

References

British Pharmacopoeia. (1973). H.M.S.O., London. Cruikshank, R. (1972). Medical Microbiology. 11th edn. Livingstone, London.

O157 Broth MTSB

(Modified Tryptone Soy Broth)

LAB165

Description

Modified tryptone soy broth has emerged as the medium of choice for the enrichment of *E. coli* O157:H7 in red meats[1,2]. As concern regarding this organism has grown due to the severity of the disease syndromes caused, and the increase in foodborne infection[3], so too has the need to optimise methods for its efficient isolation. Symptoms start with severe stomach cramps and watery, bloody diarrhoea, and a percentage of individuals infected will develop Haemolytic Uraemic Syndrome (HUS) leading to acute renal failure[4]. In a comparison of 4 different selective broth media, MTSB was the most productive and selective for the isolation of *E. coli* O157:H7. MTSB is made selective for O157:H7 by including bile salts in the dehydrated medium, and the addition of novobiocin supplement (X150).

Formulation	g/litre
Tryptone	17.0
Sodium chloride	5.0
K_2HPO_4	4.0
Dextrose	2.5
Soy Peptone	3.0
Bile Salts No.3	1.5

Method for reconstitution

Weigh 33 grams of powder and add to 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and autoclave at 121˚C for 15 minutes. Cool to 47˚C and add 2 vials of Novobiocin supplement X150. Mix well and distribute aseptically into sterile containers.

Appearance: Clear straw broth

pH: 7.2 ± 0.2

Minimum QC organisms: *E. coli* O157:H7 (non-toxigenic) NCTC 12900
E. coli NCIMB 50034 (inhibition)

Inoculation: Add 25g sample to 225ml of supplemented MTSB and homogenise for 2 minutes.

Incubation: 42˚C aerobically for 24hrs. Subculture onto CT-SMAC (LAB161 plus X161) or SMAC-BCIG (HA006) and examine for non-sorbitol fermenting colonies and/or glucuronidase negative organisms. Some workers[1] recommend the use of an immunomagnetic separation step after 6hrs incubation.

Interpretation: Turbidity in the broth indicates growth. All broths should be subcultured to selective media whether turbid or not.

References

1) Bolton, E.J., Crozier, L., Williamson, J.K. (1995) Optimisation of methods for the isolation of *Escherichia coli* O157 from beefburgers. PHLS Microbiology Digest 12 (2) 67-70.

2) Willshaw, G.A., Smith, H.R., Roberts, D., Thirlwell, J., Cheasty, T., Rowe, B. (1993) Examination of raw beef products for the presence of verocytotoxin producing *Escherichia coli,* particularly those of serogroup O157. J.Appl.Bacteriol. 75 420-426.

3) Sharp, J.C.M., Coia, J.E., Curnow, J., Reilly, W.J. (1994) *Escherichia coli* O157 infections in Scotland. J.Med.Microbiol 40 3-9.

4) Doyle, M.P. (1991) *Escherichia coli* O157:H7 and its significancein foods. Int.J.Food Microbiol. 12 289-302.

Orange Serum Agar

LAB147

Description
A medium developed for the investigation of organisms involved in the spoilage of citrus products including fruit juices and citrus concentrates. The low pH of these products restricts the growth of organisms to those capable of tolerating an acid environment such as yeasts and moulds and bacteria belonging to the genera *Bacillus*, *Lactobacillus*, *Leuconostoc*, *Streptococcus* and *Clostridium*. By having a low pH and incorporating orange extract, Orange Serum Agar is the ideal isolation medium.

Formula	g/litre
Tryptone	10.0
Yeast Extract	3.0
Orange Extract	5.0
Glucose	4.0
Di-potassium phosphate	3.0
Agar No.2	17.0

Method for reconstitution
Weigh 42 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes. Bring to the boil, swirling frequently. Sterilise by autoclaving for 15 minutes at 115°C. Cool to 47°C, mix well and dispense into Petri dishes.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: Amber, slightly opalescent gel

pH: 5.5 ± 0.2

Minimum Q.C. organisms *Lactobacillus acidophilus*
Penicillium roquefortii

Storage of Prepared Medium: Plates – up to 7 days at 2-8°C. in the dark. Capped containers – 10-25°C. in the dark.

Inoculation: Pour plate technique.

Incubation: 3 days at 30°C. for bacteria, 5 days at 30˚C for yeasts and moulds.

Interpretation: Count bacterial colonies and yeasts/moulds separately. Calculate the colony forming units (CFU) per ml of the sample, allowing for dilution factors.

References

Hays, G.L. (1951) The isolation, cultivation and identification of organisms which have caused spoilage in frozen concentrated orange juice. Proc. Florida State Hort. Soc.

Hays, G.L. and Reister, D.W. (1952) The control of 'off-odour' spoilage in frozen concentrated orange juice. Food Tech 6 p386.

Murdock, D.I., Folinazzo, J.F., and Troy, V.S. (1952) Evaluation of plating media for citrus concentrates. Food Tech. 6 p181.

ORSIM

(Oxacillin Resistant Staphylococci Isolation Medium)

LAB192

Description
The over-prescription of therapeutic antibiotics in recent years is thought to be a contributing factor in the rising numbers of multi-resistant bacteria being encountered. One such bacterium, MRSA (multi resistant *Staphylococcus aureus*), is particularly prevalent within the hospital environment, and is well recognised as a pathogen amongst immuno-compromised patients. In this situation, early detection is vital to ensure the individual's survival. This media is an improved version of the highly regarded Mannitol Salt Agar (LAB007) and incorporates an enhanced indicator system using aniline blue and mannitol fermentation. This combination produces intense blue colonies as presumptive MRSA, which are unmistakable amongst mixed cultures and easily visualised against the media background. To complement this, ORSIM possesses a refined selectivity, derived from a reduction in the salt level to 55g/L, and the introduction of Lithium chloride at 5g/L. This chemical mixture still provides the required inhibition towards competing organisms, whilst ensuring optimal recovery of MRSA, even at low numbers. To complete the medium, the selective supplement X192 is included. This contains two antibiotics, oxacillin to inhibit multi sensitive *Staphylococcus aureus* (the cause of false positives), and polymyxin B to suppress other halophillic bacteria such as *Proteus* spp.

Formula	g/litre
Peptone	11.8
Yeast Extract	9.0
Mannitol	10.0
Sodium chloride	55.0
Lithium chloride	5.0
Aniline blue	0.2
Agar	12.5

Method for Reconstitution
Weigh 103.5 grams of powder and disperse into 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix then sterilise at 121°C for 15 minutes. Cool to 47°C, and aseptically add 2 vials of X192 supplement. Mix well, and pour into sterile Petri dishes.

Appearance: Straw/grey gel.

pH: 7.2 ± 0.2

Minimum Q.C. organisms:
Staphylococcus aureus (MRSA Strain) NCTC 11940
Staphylococcus aureus (MSSA Strain) NCIMB 50080 (inhibition).

Storage of Prepared Medium: Plates can be stored up to 7 days at 2-8°C in the dark.

Inoculation: Take a swab sample from a suspected infection and apply the swab end directly to the surface of a supplemented plate of ORSIM and streak out for single colonies.

Incubation: Aerobically at 37°C for 24 and 48 hours.

Interpretation: After incubation for 24 hours, examine the plate for intense blue colonies and confirm using either coagulase/latex agglutination and Penicillin binding protein 2' test (PBP2'). Once confirmed, all positive plates should be discarded safely.
Typical strains of MRSA will be detected within 24 hours on this medium. However, some strains may require longer incubation, so all negative plates should be re-incubated for a further 24 hours.

References

Orth, D.S. (1993) Handbook of Cosmetic Microbiology. Marcel Dekker, Inc., New York, NY.

Brummer, B. (1976). Influence of possible disinfectant transfer on Staphylococcus aureus plate counts after contact sampling. App. Environ. Microbiol. 32:80-84.

Oxytetracycline Glucose Yeast Extract Agar Base

(O.G.Y.E.)

LAB089

Description

A selective medium for the enumeration of yeasts and moulds in food, introduced by Mossel in 1970. Unlike many selective media for yeasts OGYE has a neutral pH and it has been shown to give better recovery rates than those media with a low pH. Oxytetracycline is used to inhibit bacteria, certain high protein foods may reduce the effectiveness of this antibiotic as a selective agent. Rose Bengal Chloramphenicol Agar (LAB036) is recommended in these instances.

Formula	g/litre
Yeast Extract	5.0
Dextrose	20.0
Biotin	0.001
Agar No. 2	12.0

Method for reconstitution

Weigh 37 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 115°C for 10 minutes. Cool to 47°C and aseptically add 2 vials of X089 Oxytetracycline selective supplement. Mix thoroughly and pour into Petri dishes. DO NOT REHEAT THIS MEDIA ONCE PREPARED.

Appearance:

Powder: fine, free-flowing, homogeneous, buff
Finished medium: Clear, straw gel

pH: 7.0 ± 0.2

Hazard classification: NR – Not regulated

Minimum Q.C. organisms: *Aspergillus* sp. ATCC 16404
Saccharomyces cerevisiae ATCC 9763

Storage of Prepared Medium: Dehydrated culture media: 10-25oC
Prepared media: Plates - up to 7 days at 2-8oC, in the dark
Capped containers – up to 1 month at 2-8oC, in the dark

Inoculation: Surface spreading or pour plate.

Incubation: 25˚C aerobically for 5 days.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour
Candida spp.	3.0-4.0	CV.E.D.	Cream
Candida krusei	7.0-8.0	F.CR.D.	White
S. cerevisiae	3.0-4.0	CV.E.D.	White
Pen. notatum	1.5		Green/blue centre
Pen. flavescens	1.5-2.0		Yellow centre
Aspergillus niger	3.0-5.0		Yellow with black centre
Rhizopus spp.	confluent		Aerial hyphae with black conidia

Tetracycline resistant bacteria may grow, colonial morphology dependent upon species.

References

Mossel, D.A.A. *et al*. (1970). O.G.Y.E. for Selective Enumeration of Moulds and Yeast in Foods and Clinical Material. J. Appl. Bact. 35: 454-457.

Banks, J.G., Board, R.G. (1987). Some factors influencing the recovery of yeasts and moulds from chilled foods. Int. J. Food Microbiol. 4: 197-206.

PALCAM Agar Base

(Polymyxin, Acriflavine, Lithium chloride, Ceftazidine, Aesculin, Mannitol)

LAB148

Description

Palcam Agar was developed by Van Netten *et al* in 1989 as an improved selective differential medium for the isolation of *Listeria monocytogenes* from food, clinical and environmental specimens.

Improved selectivity is achieved by the combination of antibiotic supplements and microaerobic incubation, whilst the double indicator system of aesculin hydrolysis and mannitol fermentation aids differentiation of *Listeria* spp from enterococci and staphylococci which can be confused with *Listeria* spp on other types of culture media.

Formula	g/litre
Columbia Peptone Mix	23.0
Sodium chloride	5.0
Corn Starch	1.0
Yeast Extract	3.0
Glucose	0.5
Mannitol	10.0
Aesculin	0.8
Lithium chloride	15.0
Ferric ammonium citrate	0.5
Phenol red	0.08
Agar No. 2	12.0

Method for Reconstitution

Weigh 71 grams of powder and disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C and add 2 vials of P.A.C. Supplement – X144. Mix thoroughly and pour into Petri dishes.

Appearance: Red, translucent

pH: 7.2 ± 0.2

Minimum Q.C. organisms *L. monocytogenes* NCIMB 50007
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 4˚C in the dark.

Inoculation: 0.1ml of sample selectively enriched in Palcam Broth (or other enrichment medium) spread over surface of plate.

Incubation: 30˚C aerobically or microaerobically for 24-48 hours.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
L. monocytogenes	1.5-2.0mm	F.E.D.	Grey/green	Black halo, (draughtsman colonies).
Other *Listeria* spp.	0.5-2.0mm	F.E.D.	Grey/green	Black halo, (draughtsman colonies).
Enterococci	Inhibited	-	-	(Small yellow colonies with yellow/ green halo).
Staphylococci	Inhibited	-	-	(Small white/ yellow colonies with yellow/ green halo)
Bacillus spp.	Inhibited	-	-	-

References

Van Netten, P., Perales, I., Curtis, G.D.W., Mossel, D.A.A. (1989) Liquid and solid selective differential media for the enumeration of *L. monocytogenes* Int. J. Food Micro. 8 (4) 299-316.

PALCAM Broth

L-PALCAM Broth (Liquid, Polymyxin, Acriflavine, Lithium chloride, Ceftazidime, Aesculin, Mannitol)

LAB144

Description

Developed by Van Netten *et al* (1989) L-Palcam is a selective differential medium for the enrichment of *Listeria* spp. in food, environmental and clinical samples. It is unique amongst Listeria enrichment media in that it contains an indicator system (aesculin) which will signal the presence of a possible *Listeria* by a browning/ blackening of the broth; the result being the indication of a potential problem up to 48 hours before growth on plating media can be observed.

Formula	g/litre
Columbia Peptone Mix	23.0
Yeast Extract	5.0
Peptonised Milk	5.0
Sodium chloride	5.0
Mannitol	5.0
Aesculin	0.8
Ferric ammonium citrate	0.5
Phenol red	0.08
Lithium chloride	10.0

Method for Reconstitution

Weigh 54.4 grams of powder and disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix and autoclave at 121˚C for 15 minutes. Cool to 47˚C and add two vials of X144. Mix well and dispense into sterile tubes or bottles.

Appearance: Clear red broth

pH: 7.2 ± 0.2

Minimum Q.C. organisms:
L. monocytogenes NCIMB 50007
E. coli (inhibition) NCIMB 50034

Storage of prepared medium: Capped containers – up to 7 days at 4˚C.

Inoculation: Sample or pre-enriched sample added to the broth in the ratio 1 : 10.

Incubation: 30˚C for 24 hours and 48 hours.

Subculture: Onto Palcam Agar – LAB148. If low numbers of Listeria are present the medium may not produce the brown black colour. All tubes should be subcultured onto selective agar before a sample is scored as negative.

References

Van Netten, P., Perales, I., Curtis, G.D.W., Mossel, D.A.A. (1989) Liquid and solid selective differential media for the enumeration of *L. monocytogenes* Int. J. Food Micro. 8 (4) 299-316.

PEMBA

(Bacillus Cereus Medium)

LAB193

Description

This medium is based on the highly specific and sensitive PEMBA medium. It is used for the isolation and enumeration of *Bacillus cereus*. This formulation specifically enhances egg yolk precipitation and sporulation of *Bacillus cereus.* The bromothymol blue pH indicator gives clear visualisation of alkaline mannitol non-fermenting colonies and egg yolk precipitation indicative of *B. cereus*. The selectivity is provided by the polymyxin B supplement (X193) and provides excellent results for the majority of sample types.

Microscopic examination of presumptive *B. cereus* colony can confirm identity by presence of lipid globules in vegetative cells.

Formula	g/litre
Peptone	1.0
Mannitol	10.0
Sodium chloride	2.0
Magnesium sulphate	0.1
Disodium hydrogen phosphate	2.5
Bromothymol blue	0.12
Sodium pyruvate	10.0
Agar	15.0

Method for Reconstitution

Weigh 41g of powder and disperse in 950ml of deionised water, allow the mixture to soak for 10 minutes, swirl to mix and sterilize by autoclaving for 15 minutes at 121ºC for 15 minutes. Cool to 47ºC and add two vials of X193 and 50ml of Egg Yolk Emulsion (X073) mix well and pour the plates. Dry the agar surface before inoculation.

Appearance: Yellow and opaque.

pH: 7.2 ± 0.2

Minimum Q.C. organisms:
Bacillus cereus NCIMB 50014.
Escherichia coli NCIMB 50034 (inhibition).

Storage of Prepared Medium: up to 7 days at 2-8ºC in the dark.

Inoculation: Surface spreading or streaking for single colonies.

Incubation: 30ºC aerobically for 24-48 hours.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour
B. cereus	3.0-4.0	F.CR.D	Blue white halo
B. subtilis	2.0-3.0	F.CR.D	Yellow
B. coagulans	2.0	F.CR.D	Yellow
B. licheniformis	2.0	F.CR.D	Yellow
Proteus spp.	1.0	F.Rz.D	Blue (swarms)
E. faecalis	0.5	CV.E.G.	Yellow
E. coli	no growth	-	
S. aueus	1.0	CV.E.G.	Yellow white halo

References

Holbrook, R. & Anderson, J.M. (1980). Can. J. Microbiol., 26(7) 753-759.

Donovan, K.O. (1958). J. Appl. Bacteriol., 21(1) 100-103.

Mossel, D.A.A., Koopman, M.J. & Jongerius. E. (1967). J. Appl. Bacteriol. 15(3) 650-653.

Peptone Water

LAB104

Description

A general purpose growth medium that can be used as a base for carbohydrate fermentation studies. The medium has a high level of tryptone making it suitable for use in the indole test.

Formula	g/litre
Peptone	5.0
Tryptone	5.0
Sodium chloride	5.0

Method for reconstitution

Weigh 15 grams of powder, and disperse in 1 litre of deionised water. Allow to dissolve then distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes. If sterile additions are to be made to this medium e.g. carbohydrates, the volume of water for reconstitution must be reduced accordingly. A pH indicator may be added to detect acid production from carbohydrate utilisation.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, colourless

pH: 7.2 ± 0.2

Hazard classification: NR – Not regulated

Minimum Q.C. organisms:
Escherichia coli ATCC 25922 (indole positive)
Salmonella typhimurium ATCC 14028 (indole negative)

Storage of Prepared Medium:

Dehydrated culture media: 10-25°C.

Final medium: capped containers – up to 3 months at 15-20°C in the dark..

Inoculation: A light inoculum from a pure culture.

Incubation: According to organism.

References

Bergey's Manual of Systematic Bacteriology, Vol. 1, (1984). Williams and Wilkins, Baltimore/London.

MacFadden, J.F. (1983). Biochemical Tests for the Identification of Medical Bacteria, 2nd edn. Williams and Wilkins, Baltimore/London.

Perfringens Agar OPSP

LAB109

Description

Oleandomycin, Polymixin, Sulphadiazine, Perfringens (OPSP) agar, has been used as a standard medium for Clostridium perfringens for many years. This medium was developed by Handford in 1974 to overcome some of the problems associated with enumerating Clostridium perfringens in foods. The medium is buffered and utilises sodium metabisulphite and liver extract as sources of H2S with ferric ammonium citrate as the indicator.

The medium is made selective with the addition of X109 Sulphadiazine and X110 Oleandomycin / Polymyxin supplements. Some strains of C. perfringens may demonstrate sensitivity to the sulphadiazine antibiotic (X109) in such cases use of LAB194 TSC Perfringens Agar Base (with X194 D-Cycloserine) should be considered.

Formula	g/litre
Tryptone	15.0
Yeast Extract	5.0
Soy Peptone	5.0
Liver Extract	7.0
Ferric ammonium citrate	1.0
Sodium metabisulphite	1.0
Tris buffer	1.5
Agar No. 2	10.0

Method for reconstitution

Weigh 45.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Allow to cool to 47°C before adding 2 vials each of selective supplements X109 and X110. Mix well before dispensing into Petri dishes.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: pale straw, clear gel

pH: 7.3 ± 0.2

Minimum Q.C. organisms: *C. perfringens* NCTC 8237
E. coli ATCC 25922
(inhibition)

Storage of prepared medium:

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: Use on day of preparation.

Inoculation: Pour plates, 1ml sample plus 9ml medium. When set overlay with sterile medium.

Incubation: Incubate at 37°C anaerobically for 24 hours.

Interpretation: Count large black colonies, presumptively identified as *C. perfringens.*

References

Bowen AB, Braden CR (2006). "Invasive Enterobacter sakazakii disease in infants". Emerging Infect Dis 12 (8): 1185–9.

Handford, P.M. (1974). A new medium for the detection and enumeration of C. perfringens in foods. J. Appl. Bact. 37: 559-570.

Hauschild A.H.W. and Hilsheimer R. (1973). Evaluation and Modifications of Media for Enumeration of Clostridium perfringens. Applied Microbiology 27 p78-82.

Shahidi, S.A. and Ferguson, A.R. (1971). A new quantitative and confirmatory medium for C. perfringens in food. Appl. Microbiol. 21:500-506.

Marshall, R.S., Steenberger, J.F. and McClung, L.S. (1965). A rapid technique for the enumeration of C. perfringens. Appl. Microbiol. 13: 559.

Pharmacopoeia of culture media for food microbiology. (1987). Int. J. Food Microbiol. 5:3:240-241.

Perfringens Agar TSC

(Tryptose Sulphite Cycloserine (TSC) Agar)

LAB194

Description

Perfringens Agar Base is a nutrient medium to which egg yolk emulsion (X073) and cycloserine (X194) are added for the preparation of Tryptose Sulphite Cycloserine (TSC) Agar. Sodium metabisulphite and ferric ammonium citrate are used as an indicator of sulphite reduction by *Clostridium perfringens*. The reduction of sulphite by *Cl. perfringens* produces black colonies and the egg yolk emulsion incorporated into the media detects the lecithinase activity of this bacteria. However not all strains produce lecithinase and therefore black lecithinase positive and black lecithinase negative colonies should be considered as presumptive *Cl. perfringens*.

Formula	g/litre
Tryptose	15.0
Soy Peptone	5.0
Beef extract	5.0
Yeast extract	5.0
Sodium metabisulphite	1.0
Ferric ammonium citrate	1.0
Agar	14.0

Method for reconstitution

Weigh 46.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 10 minutes. Allow the medium to cool to 47°C and supplement with 2 vials of X194 (cycloserine) and 50ml of egg yolk emulsion (X073), mix well and pour into sterile Petri dishes. The egg yolk emulsion is omitted for preparation of Egg Yolk Free TSC Agar and Egg Yolk Free TSC Agar should be used for an overlay medium.

Appearance: Straw, clear gel or pale yellow opaque gel.

pH: 7.6 ± 0.2

Storage of Prepared Medium: Plates can be stored up to 7 days at 2-8°C in the dark.

Minimum Q.C. organisms:
Clostridium perfringens NCIMB 50027
Escherichia coli NCIMB 50034 (inhibition)

Inoculation: For a spread plate inoculate the agar plate with 0.1ml aliquots of an appropriate serial dilution of the homogenised test sample and overlay if required. For a pour plate mix 1ml aliquots of an appropriate serial dilution of the homogenised test sample with approximately 20 ml of TSC plus egg yolk emulsion. For full details refer to appropriate references and standard method protocols.

Incubation: 35°C ± 2°C anaerobically for 18-24 hours.

Interpretation: Count all black colonies with or without a halo as presumptive *C. perfringens*. Further confirmation should be carried out according to standard method protocols e.g. nitrate reduction, lactose fermentation, gelatin liquefaction and absence of motility.

References

Shahidi, S.A. and Furguson, A.R. (1971). Appl. Microbiol. 21. 500-506.

Harmon, S.M., Kauttar, D.A. and Peeler, J.T. (1971). Appl. Microbiol. 22. 688-692.

Hauschild, A. H. W. and Hilsheimar R. (1973). Appl. Microbiol. 27. 78-82.

Hauschild A.H.W. and Hilsheimar, R. (1973). Appl. Microbiol. 27. 521-526.

Hauschild, A.H.W. *et al* (1977). Can. J. Microbiol. 23. 884-892.

Labbe, R. G. and Harmon, S.M. (1992). Compendium of methods for the microbiological examination of foods, 3rd ed 623-635. American Public Health Association, Washington, D.C.

Rhodehamel, E.J. and Harmon, S.M. (1995). Bacteriological Analytical Manual 8th ed. 16.01-16.06 AOAC International, Gaithersberg, MD.

Andrews, W. (1995) Official methods of analysis AOAC International 16th ed. 1-119. AOAC International, Arlington, VA.

Plate Count Agar

LAB149

Description

A medium designed for use with the spiral plating system and other surface inoculation techniques. The formula is equivalent to A.P.H.A. Plate Count Agar and is suitable for the determination of total viable counts in food products by surface count and pour plate methods.

Formula	g/litre
Tryptone	5.0
Yeast Extract	2.5
Glucose	1.0
Agar No. 2	12.0

Method for reconstitution

Weigh 20.5 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C then pour into Petri dishes.

Appearance: Pale straw colour, clear.

pH: 7.0 ± 0.2

Minimum Q.C. organisms: *S. epidermidis* NCIMB 50082
E. coli NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface, or pour plate.

Incubation: 30˚C aerobically for 48 hours for aerobic mesotroph count. 6˚C aerobically for 10 days for aerobic psychrotroph count. 55˚C aerobically for 48 hours for aerobic thermotroph count.

Interpretation: Count all colonies or use spiral plating colony count equipment.

References

Reasoner, D.J., Geldreich, E.E. (1985) A New Medium for the Enumeration and Subculture of Bacteria from potable water. App & Env. Microbiol. Jan. 1985 p.1-7.

American Public Health Association (1985) Standard Methods for the Enumeration of Water and Wastewater. 16th Edition. American Public Health Association Inc. Washington D.C.

Environment Agency: The Microbiology of Drinking Water (2002). Methods for the Examination of Water and Associated Materials.

Plate Count Agar (A.P.H.A.)

(Standard Methods Agar, Tryptone Glucose Yeast Agar)

LAB010

Description

Formulated to A.P.H.A. specifications, this medium is used for establishing total viable counts for aerobes in food, dairy and water bacteriology. The product uses agar of very high gel strength in order that it can be used in pour-plate as well as surface inoculation techniques. The product can be remelted prior to use although it should not be held for a prolonged period in the molten stage.

Formula	g/litre
Tryptone	5.0
Yeast Extract	2.5
Glucose	1.0
Agar No. 1	15.0

Method for reconstitution

Weigh 23.5 grams of powder, disperse in 1 litre of deionised water. Bring to the boil with frequent stirring to dissolve. Dispense into tubes and sterilise by autoclaving at 121˚C for 15 minutes. Cool to 44-46˚C for not more than 3 hours prior to use.

ROLL-TUBES. Add an additional 10g/litre Agar No. 1 prior to reconstitution of the medium.

Appearance: Pale straw coloured, clear gel.

pH: 7.0 ± 0.2

Minimum Q.C. organisms: *S. epidermidis* NCIMB 50082
E. coli NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark. Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation method: Pour plate technique or surface inoculation.

Incubation: 30˚C aerobically for 48 hours for aerobic mesotroph count. 6˚C aerobically for 10 days for aerobic psychrotroph count. 55˚C aerobically for 48 hours for aerobic thermotroph count.

Interpretation: Count all colonies and calculate the number of organisms (or 'colony forming units' c.f.u.) per ml of sample allowing for dilution factors.

References

American Public Health Association (1972). Standard Methods for the Examination of Dairy Products. 13th edn. (ed. Hausler, W.J.) A.P.H.A., Washington.

American Public Health Association (1966). Recommended Methods for the Microbiological Examination of Foods, 2nd edn. (ed. Sharf, J.M.) A.P.H.A., Washington.

American Public Health Association (1976). Standard Methods for the Examination of Water and Waste Water, 14th edn. (ed.. Franson, M.A) A.P.H.A., Washington.

Potato Dextrose Agar

LAB098

Description

Potato Dextrose Agar is recommended by the American Public Health Association for the enumeration of yeasts and moulds in examination of dairy products, soft drinks, dried and frozen foods and other types of product. Depending on whether the medium is to be used as a selective or non-selective agar it can be used with or without acidification.

Formula	g/litre
Potato Extract	4.0
Dextrose	20.0
Agar No. 1	15.0

Method for reconstitution

Weigh 39 grams of powder, disperse in 1 litre of deionised water, then sterilise at 121˚C for 15 minutes. Mix well before pouring into sterile Petri dishes. In certain cases it may be desirable to lower the pH of the medium to 3.5 in order to suppress bacterial growth. This can be done by adding 10ml of sterile 10% Lactic Acid X037, to one litre of Potato Dextrose Agar LAB098. This addition must be after autoclaving and cooling to 47˚C. Once the pH has been lowered the medium may not be heated again without resultant loss of gel strength caused by agar hydrolysis.

Appearance: Translucent white agar.

pH: 5.6 ± 0.2 (3.5-4.0 if X037 is added)

Minimum Q.C. organisms: *Aspergillus* sp. NCIMB 50097
Saccharomyces cerevisiae

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark. Capped containers – up to 1 month at 15-20˚C in the dark.

Inoculum: Pour plate technique.

Incubation: 21˚C aerobically for 5 days.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour
Candida spp.	2.0	C.V.E.D.	White
Candida krusei	2.0	F.Rz.D.	Grey/White
Tryc. mentagrophytes	4.0	Fluffy White	Yellow obverse
Tryc. verrucusum	5.0	Fluffy White	Yellow obverse
Toro. glabrata	3.0	C.V.E.G.	White
Asp. niger	4.0	Black spores centre White surround	Yellow obverse
Pen. notatum	4.0	Green spores centre White surround	Green obverse

References

Association of Official Analytical Chemists (AOAC). Bacteriological Analytical Manual, 5th ed. (1978). Washington D.C. Hausler, W.J. (ed.).

Standard Methods for the Examination of Dairy Prod. 14th edn., Washington D.C.: American Public Health Association, (1976).

Pseudomonas Agar Base

(C.F.C./C.N. Agar)

LAB108

Description

The base medium is a modification of King's medium A which uses magnesium and potassium salts to enhance production of the pigments pyocyanin (green) and fluorescein (detected by U.V./blue light). The medium is made selective for *Pseudomonas aeruginosa* by the addition of X107 C.N. supplement. Alternatively the medium can be made selective for *Pseudomonas* species generally by the addition of X108 C.F.C. supplement. This medium can be made selective for the isolation of *Burkholderia cepacia* by the addition of X140.

Formula	g/litre
Acid Hydrolysed Casein	10.0
Gelatin Peptone	16.0
Potassium sulphate	10.0
Magnesium chloride	1.4
Agar No. 2	11.0

Method for reconstitution

Weigh 48.4 grams of powder and disperse in 1 litre of deionised water. Add 10ml of glycerol. Sterilise by autoclaving at 121˚C for 15 minutes. Allow the medium to cool to 47˚C then add the contents of 2 vials of either X107 C.N. supplement or X108 C.F.C. supplement. Mix well and pour into Petri dishes.

Appearance: Pale straw, opaque.

pH: 7.1 ± 0.2

Minimum Q.C. organisms: *P. aeruginosa* NCIMB 50067
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface, spread 0.1 to 0.5ml of sample over entire surface.

Incubation: 25-30˚C aerobically for 48 hours.

Interpretation: Count all colonies as *Pseudomonas species.* Colonies that exhibit the pyocyanin and fluorescein pigments count as *P. aeruginosa.*

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	fluorescence
Ps. aeruginosa	2.0-3.0	CV.Cr.D.	Green/Blue	yes
P. fluorescens	2.0-3.0	CV.Cr.D.	Yellow	yes
P. fragi	1.0-3.0	CV.Cr.D.	Grey	no
P. maltophila	2.0-3.0	CV.Cr.G.	Grey	no
P. putida	2.0-3.0	F.Cr.D.	Grey	no

References

Burton, M.O., Campbell, J.J.R. and Eagles, B.A. (1948). The mineral requirement for pyocyanin production. Can. J. res. Sect. C. Bot. Sci. 26:15.

King, E.O., Ward, M.K. and Raney, D.E. (1954). Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44: 301.

Goto, S. and Enomoto, S. (1970). Jap. J. Microbiol. 14: 65-72.

Mead, G.C. and Adams, B.W. (1977). Br. Poult. Sci. 18: 661-667.

R2A Broth

LAB203

Description

A medium of low nutritional content for use with membrane methods for the enumeration of bacteria from water samples.

Formulation	g/litre
Yeast Extract	0.5
Meat Peptone	0.5
Casamino acids	0.5
Glucose	0.5
Starch	0.5
Dipotassium hydrogen Phosphate	0.3
Magnesium sulphate	0.05
Sodium pyruvate	0.3

pH: 7.2 ± 0.2

Hazard classification: NR – Not regulated

Appearance:

Powder: fine, free-flowing, homogeneous, buff
Finished medium: clear, pale straw liquid

Method for reconstitution

Disperse 3g of powder in 1 litre of distilled water. Allow to soak for 10 minutes, swirl to mix and dispense into tubes or bottles. Sterilise by autoclaving at 121ºC for 15 minutes.

Minimum QC organisms: *Aeromonas hydrophila* NCTC 8049
Pseudomonas fluorescens NCTC 10038
Escherichia coli ATCC 25922

Storage:

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 1 month at 2-8°C in the dark.

R2A Medium

LAB163

Description

R2A medium was developed to determine the bacterial count in potable waters during treatment and distribution, and has been shown to give significantly higher counts than plate count agar (PCA) or similar high-nutrient media. The standard plate count (SPC) method using PCA provides an enumeration of bacteria which grow best at, or near, body temperature and this estimation at best may correlate to the coliforms present in the sample. However, there will be a population of heterotrophic bacteria which cannot grow at all under the conditions of the SPC method or may grow so slowly that the colonies fail to reach a size detectable to the eye in the 48-h incubation period. In order to enumerate this section of the bacterial population in water, a medium of low nutritional content (R2A) and extended incubation times are required. R2A medium is recommended by the Environment Agency, Methods for the Examination of Waters and Associated Materials, and Standard Methods for the Enumeration of Water and Wastewater.

Formulation	g/litre
Yeast Extract	0.5
Meat Peptone	0.5
Casamino acids	0.5
Glucose	0.5
Starch	0.5
Dipotassium hydrogen Phosphate	0.3
Magnesium sulphate	0.05
Sodium pyruvate	0.3
Agar No.2	15.0

pH: 7.2 ± 0.2

Appearance:

Powder: fine, free-flowing, homogeneous, buff
Finished medium: clear, opalescent gel

Method for reconstitution

Weigh 18 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121ºC. (If required, bring to the boil to dissolve the agar, and pour into smaller volumes before sterilizing.) Cool to 44-46°C for not more than 3 hours before use. Mix well before dispensing into Petri dishes. Dry the agar surface prior to use.

Inoculation: Pour 15ml into a Petri dish containing 1ml of sample, mix well and allow to set. Pour a further 10ml as an overlay and again allow to set. Alternatively it may be used as a spread plate, inoculating 0.1ml onto the plate and spreading over the entire surface of the medium. It can also be used with membrane filters if required.

Incubation: When plates have set, incubate at 22°C for 5-7 days or 30°C for 3 days. Other incubation temperatures between 20°C and 28°C may be used.

Interpretation: Count all colonies and report the number of bacteria in the original sample as the heterotrophic plate count.

Minimum QC organisms: *Pseudomonas fluorescens NCTC 10038*
Aeromonas hydrophila NCTC 8049

Storage:

Dehydrated culture media: 10-25ºC away from direct sunlight.

Prepared media:

Plates - 7 days at 2-8°C in the dark

Capped containers – 3 months at 15-20°C in the dark

References

Reasoner, D.J., Geldreich, E.E. (1985) A New Medium for the Enumeration and Subculture of Bacteria from potable water. App & Env. Microbiol. Jan. 1985 p. 1-7.

American Public Health Association (1985) Standard Methods for the Enumeration of Water and Wastewater. 16th Edition. American Public Health Association Inc. Washington DC.

Environment Agency: The Microbiology of Drinking Water (2002). Methods for the Examination of Water and Associated Materials.

Raka-Ray No.3 Agar

LAB198

Description

Based on the formulation of Saha, Sondag and Middlekauff, Raka-Ray No.3 Agar is for the detection of lactic acid bacteria in beer and for monitoring in-process beer quality. It is recommended for this application by the European Brewing Convention (EBC) and the American Society of Brewing Chemists (ASBC).

Contamination of beer and the beer making process by members of the lactobacilli family results in spoilage, primarily through their production of metabolic products which are detrimental to the flavour of the final product. Detection of these organisms is complicated by their diverse nutritional and environmental requirements.

A number of different formulations have been described for the isolation of lactic acid bacteria in brewing products and processes. Raka-Ray Agar was developed by the addition of various growth promoting compounds to Universal Beer Agar. This work led to the recognition that the addition of sorbitan mono-oleate, liver extract and N-acetylglucosamine produced superior growth when compared to the standard Universal Beer Agar formulation.

Further investigations provided the basis for the final formula of Raka-Ray No. 3 Medium in which fructose is an essential carbohydrate source for *Lactobacillus fructivorans*. Maltose is present to allow the growth of lactobacilli which cannot utilise glucose. The media can be made selective against yeasts by the addition of 7mg/l cycloheximide (Actidione®) and against Gram-negative bacteria by the addition of 3g/l 2-phenylethanol.

Formula	g/litre
Yeast extract	5.0
Tryptone	20.0
Liver concentrate	1.0
Maltose	10.0
Fructose	5.0
Dextrose	5.0
Betaine HCl	2.0
Diammonium hydrogen citrate	2.0
Potassium aspartate	2.5
Potassium glutamate	2.5
Magnesium sulphate $7H_2O$	2.0
Manganese sulphate $4H_2O$	0.66
Potassium phosphate	2.0
N-acetyl glucosamine	0.5
Agar	17.0

Method for reconstitution

Disperse 77.1g of powder in 1 litre of distilled water. Add 10ml Sorbitan mono-oleate and 7mg cycloheximide. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving for 15 minutes at 121°C. Cool to 50°C. If required, aseptically add 3g of 2-phenylethanol. Mix well and pour into sterile Petri dishes.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear to slightly opalescent amber coloured gel

pH: 5.4 ± 0.2

Hazard classification

NR – Not regulated

Minimum Q.C. organisms:
Lactobacillus fermentum ATCC 9338
Pediococcus acidilactici NCTC 6990
Escherichia coli ATCC 25922 (inhibited / suppressed)

Inoculation:

Surface technique

Spread 0.1ml of the sample over the surface of the agar. Alternatively, the sample may be filtered and the membrane placed on the surface of the agar.

Overlay technique

Aseptically dispense 4ml volumes of Raka-Ray No.3 Agar into test tubes and keep molten at 50°C. Mix 1ml of the test sample with 4ml of molten agar and immediately pour the contents into a Petri dish containing 15-20ml Raka-Ray No.3 Agar. Mix to give isolated colonies. As the agar layer is very thin, individual colonies can be picked for further examination.

Incubation: Incubate anaerobically at 25-30°C for 7 days.

Interpretation: Lactobacilli are visible after 48 hours incubation and appear as smooth, cream-coloured, moist colonies approximately 1mm in diameter.

Incubation for 4 days may be sufficient, however slow-growing organisms such as *Pediococcus* may require upto 7 days.

If the number of colonies on the plate exceeds 300, dilute the sample 1:10 in Maximum Recovery Diluent (LAB103) and retest.

References

Coster, E., and White, H.R. (1951). J. Gen. Microbiol. 37:15.

European Brewing Convention, EBC *Analytica Microbiologica: Part II J. institute of Brewing* (1981) 87. 303-321.

Lawrence D. R. and Leedham P. A. (1979) *Journal of the Institute of brewing* 85. 119

Mauld B. and Seidel H. (1971) *Brauwissenschaft* 24, 105

Methods of Analysis of the American Society of Brewing Chemists ASBC (1976) 7th edition, The Society St. Paul. Mn. USA.

Saha R. B., Sondag R. J. AND Middlekauff J. E. (1974). *Proceedings of the American Society of Brewing Chemists*, 9th Congress 1974.

Van Keer C., Van Melkebeke l., Vertrieste W., Hoozee g. and Van Schoonenberghe E. (1983). *Journal of the Institute of brewing* 89. 361 – 363.

Raka-Ray No.3 Agar (Increased Gel Strength)

LAB199

Description

Based on the formulation of Saha, Sondag and Middlekauff, Raka-Ray No.3 Agar is for the detection of lactic acid bacteria in beer and for monitoring in-process beer quality. It is recommended for this application by the European Brewing Convention (EBC) and the American Society of Brewing Chemists (ASBC). LAB199 Raka-Ray No.3 Agar Increased Gel Strength contains the same components as LAB198 Raka-Ray No.3 Agar, however the agar level has been increased to 27g/l.

Contamination of beer and the beer making process by members of the lactobacilli family results in spoilage, primarily through their production of metabolic products which are detrimental to the flavour of the final product. Detection of these organisms is complicated by their diverse nutritional and environmental requirements.

A number of different formulations have been described for the isolation of lactic acid bacteria in brewing products and processes. Raka-Ray Agar was developed by the addition of various growth promoting compounds to Universal Beer Agar. This work led to the recognition that the addition of sorbitan mono-oleate, liver extract and N-acetylglucosamine produced superior growth when compared to the standard Universal Beer Agar formulation.

Further investigations provided the basis for the final formula of Raka-Ray No. 3 Medium in which fructose is an essential carbohydrate source for *Lactobacillus fructivorans*. Maltose is present to allow the growth of lactobacilli which cannot utilise glucose. The media can be made selective against yeasts by the addition of 7mg/l cycloheximide (Actidione®) and against Gram-negative bacteria by the addition of 3g/l 2-phenylethanol.

Due to customer feedback regarding the soft gel strength of the standard formulation, Lab M has developed this modified version of Raka-Ray Agar with a higher gel strength. The increased gel strength is especially useful when performing surface plating techniques.

Formula	g/litre
Yeast extract	5.0
Tryptone	20.0
Liver concentrate	1.0
Maltose	10.0
Fructose	5.0
Dextrose	5.0
Betaine HCl	2.0
Diammonium hydrogen citrate	2.0
Potassium aspartate	2.5
Potassium glutamate	2.5
Magnesium sulphate $7H_2O$	2.0
Manganese sulphate $4H_2O$	0.66
Potassium phosphate	2.0
N-acetyl glucosamine	0.5
Agar	27.0

Method for reconstitution

Disperse 77.1g of powder in 1 litre of distilled water. Add 10ml Sorbitan mono-oleate and 7mg cycloheximide. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving for 15 minutes at 121°C. Cool to 50°C. If required, aseptically add 3g of 2-phenylethanol. Mix well and pour into sterile Petri dishes.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear to slightly opalescent amber coloured gel

pH: 5.4 ± 0.2

Hazard classification

NR – Not regulated

Minimum Q.C. organisms:
Lactobacillus fermentum ATCC 9338
Pediococcus acidilactici NCTC 6990
Escherichia coli ATCC 25922 (inhibited / suppressed)

Inoculation:

Surface technique

Spread 0.1ml of the sample over the surface of the agar. Alternatively, the sample may be filtered and the membrane placed on the surface of the agar.

Overlay technique

Aseptically dispense 4ml volumes of Raka-Ray No.3 Agar into test tubes and keep molten at 50°C. Mix 1ml of the test sample with 4ml of molten agar and immediately pour the contents into a Petri dish containing 15-20ml Raka-Ray No.3 Agar. Mix to give isolated colonies. As the agar layer is very thin, individual colonies can be picked for further examination.

Incubation: Incubate anaerobically at 25-30°C for 7 days.

Interpretation: Lactobacilli are visible after 48 hours incubation and appear as smooth, cream-coloured, moist colonies approximately 1mm in diameter.

Incubation for 4 days may be sufficient, however slow-growing organisms such as *Pediococcus* may require upto 7 days.

If the number of colonies on the plate exceeds 300, dilute the sample 1:10 in Maximum Recovery Diluent (LAB103) and retest.

References

Coster, E., and White, H.R. (1951). J. Gen. Microbiol. 37:15.

European Brewing Convention, EBC *Analytica Microbiologica: Part II J. institute of Brewing* (1981) 87. 303-321.

Lawrence D. R. and Leedham P. A. (1979) *Journal of the Institute of brewing* 85. 119

Mauld B. and Seidel H. (1971) *Brauwissenschaft* 24, 105

Methods of Analysis of the American Society of Brewing Chemists ASBC (1976) 7th edition, The Society St. Paul. Mn. USA.

Saha R. B., Sondag R. J. AND Middlekauff J. E. (1974). *Proceedings of the American Society of Brewing Chemists*, 9th Congress 1974.

Van Keer C., Van Melkebeke l., Vertrieste W., Hoozee g. and Van Schoonenberghe E. (1983). *Journal of the Institute of brewing* 89. 361 – 363.

Rappaport Vassiliadis (R.V.) Medium

LAB086

Introduction

Rappaport Vassiliadis Broth (R10 modification) was born out of a long series of experiments carried out to determine the correct levels of malachite green and magnesium chloride that would allow *Salmonella* to multiply freely yet still inhibit the other enteric organisms.

This formulation has been shown to be superior to Mueller Kauffmann and Selenite Broth for the isolation of *Salmonella* from meat products.

The development work carried out on the formulation shows that it is extremely efficient in detecting small numbers of *Salmonella* in heavily contaminated products. This formulation is very hygroscopic and will produce a slight exothermic reaction when mixed with water.

Formula	g/litre
Soy Peptone	4.5
Sodium chloride	7.2
Potassium dihydrogen phosphate	1.26
Dipotassium hydrogen phosphate	0.18
Magnesium chloride anhydrous	13.58
Malachite green	0.033

Method for reconstitution

Weigh 26.8 grams powder, disperse in 1 litre of deionised water, swirl to mix, when dissolved dispense in 10ml volumes in screw capped bottles and sterilise by autoclaving at 115˚C for 15 minutes.

Appearance: Clear, green fluid.

pH: 5.2 ± 0.2

Minimum Q.C. organisms: *E. coli* (inhibited) NCIMB 50034
S. typhimurium NCIMB 50076

Storage of Prepared Medium: Capped container – 6 months at 2-8˚C

Inoculation: From pre-enrichment broth in the proportions of 1-part inoculum to 99 parts R.V. Broth. Sub-culture onto either XLD Agar, M.L.C.B. Agar or other salmonella selective agars.

Incubation: 41.5 ± 0.5˚C for 24 hours (incubator) or 42 ± 0.1˚C for 24hrs (water bath).

References

Vassiliadis, P., (1983) The Rappaport Vassiliadis (R.V.) Enrichment Medium for the Isolation of salmonellas: An overview J. Appl. Bacteriol. 56 69-76.

Vassiliadis, P., Mavromatti, CH. Efstratiou, M. and Chronas, G. (1985). A note on the stability of Rappaport-Vassiliadis Enrichment Medium J. Appl. Bacteriol. 59 143-145.

Bolton, F.G., Preston, P.H.L. Personal communication.

Int. J. Food Micro. Pharmacopoeia of culture media for Food Microbiology.

Peterz, M., Wiberg, C., and Norberg, P. 1989. The effect of incubation temperature and magnesium chloride concentration on growth of *Salmonella* in home-made and in commercially available dehydrated Rappaport-Vassiliadis broths.

Reinforced Clostridial Agar

LAB023

Description

This is a solidified version of R.C.M. (LAB022) and can be used for the enumeration of anaerobes by pour plate, shake tube or membrane filtration methods. When solidified in tubes or bottles with minimal head space it can be used for anaerobic culture without the need for anaerobic atmosphere.

Formula	g/litre
Yeast Extract	3.0
Beef Extract	10.0
Peptone	10.0
Glucose	5.0
Soluble Starch	1.0
Sodium chloride	5.0
Sodium acetate	3.0
L-Cysteine hydrochloride	0.5
Agar No. 2	12.0

Method for reconstitution

Weigh 49.5 grams of powder, disperse in 1 litre of deionised water, allow to soak for 10 minutes, swirl to mix then sterilise for 15 minutes at 121˚C. Cool to 47˚C. and distribute into sterile dishes or tubes containing decimal dilutions of the sample under test.

Appearance: Pale straw, translucent gel.

pH: 6.8 ± 0.2

Minimum Q.C. organisms: *C. perfringens* NCIMB 50027

Storage of Prepared Medium: Capped container – up to 3 months at 15-20˚C in the dark.

Inoculation: Pour plate technique or tube culture.

Incubation: 30˚C for up to 72 hours. Anaerobic conditions for pour plate. Count as early as possible as prolonged incubation may result in the medium being disrupted due to gas production.

Interpretation: Count all colonies as presumptive clostridia.

References

Miller, N.J., Garrett, O.W. and Prickett, P.S. (1939). Anaerobic technique – a modified deep agar shake. Food Res. 4: 447-451.

Ingram, M. and Barnes, E.M. (1956). A simple modification of the deep shake tube for counting anaerobic bacteria. Lab. Pract. 5: 145.

Reinforced Clostridial Medium

(R.C.M.)

LAB022

Description

This medium was formulated by Hirsch and Grinstead to recover small numbers of *Clostridium* spp. from a variety of sources. Various workers have reported its efficiency with many products and specimens, R.C.M. is rich, non-selective and uses cysteine hydrochloride and glucose as reducing agents. The small amount of agar reduces diffusion of oxygen through the fluid.

Formula	g/litre
Yeast Extract	3.0
Beef Extract	10.0
Peptone	10.0
Soluble Starch	1.0
Glucose	5.0
L-Cysteine hydrochloride	0.5
Sodium chloride	5.0
Sodium acetate	3.0
Agar No. 1	0.75

Method for reconstitution

Weigh 38 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then bring to the boil to dissolve. Distribute 25ml into 1oz Universal containers. Sterilise for 15 minutes at 121˚C.

Appearance: Straw coloured, clear.

pH: 6.8 ± 0.2

Minimum Q.C. organisms: *C. perfringens* NCIMB 50027

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation: Homogenised food sample to a ratio of 1:10 with R.C.M.

Incubation: 30˚C for up to 72 hours.

Growth Indicators

Turbidity, colonies in medium, gas production.

References

Hirsch, A. and Grinstead, E. (1954). Methods for the growth and enumeration of anaerobic spore-formers from cheese. J. Dairy Res. 21: 101-110.

Barnes, E.M. and Goldberg, H.S. (1962). The isolation of anaerobic Gram-negative bacteria from poultry. J. Appl. Bact. 25: 94-106.

Rhamnose MacConkey (VTEC O26) Agar (RMAC)

LAB209

Description

This medium is selective for the isolation of the verocytotoxin producing *Escherichia coli* O26. This strain has been associated with haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS). It is based on the MacConkey formulation No.3, where the fermentable carbohydrate of lactose has been substituted for rhamnose. VTEC O26 colonies are not able to ferment the rhamnose so will remain a translucent colour on the medium. All other non-O26 VTEC colonies present are able to ferment Rhamnose and will appear as pink to red colonies. Selectivity of the medium can be increased by adding X161 Cefixime Tellurite (CT) supplement.

Formula	g/litre
Peptone	15.0
Rhamnose	20.0
Bile salts No.3	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar No.2	12.0

Method for reconstitution

Weigh 53.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C, and add 2 vials of reconstituted X161. Mix well and dispense into sterile Petri dishes.

Appearance:

Powder: fine, free-flowing, homogeneous, buff
Finished medium: red/purple gel

pH: 7.1 ± 0.2

Hazard classification

NR – Not regulated

Minimum Q.C. organisms:
Escherichia coli NCTC 8783
Escherichia coli ATCC 25922
Salmonella typhimurium ATCC 14028
Staphylococcus aureus ATCC 25923
Enterococcus faecalis ATCC 29212

Storage:

Dehydrated culture media: 10-25°C away from direct sunlight
Poured plates: 7 days at 2-8°C in the dark

Inoculation: Surface inoculation as per user's validated method.

Incubation: Incubate at 37°C for 18-24 hours.

References

Hiramatsu R, Matsumoto M, Miwa Y, Saito M, Yatsuyanagi J, Uchimura M, Kobayashi K, Tanaka H, Horikawa K, Mori R, Miyazaki Y. Characterization of enterohemorrhagic Escherichia coli O26 and development of its isolation media. Kansenshogaku Zasshi. 1999 May; 73(5):407-13.

Hiramatsu, R., Matsumoto, M., Miwa, Y., Suzuki, Y., Saito, M., and Miyazaki, Y. Characterization of Shiga Toxin-Producing Escherichia coli O26 Strains and Establishment of Selective Isolation Media for These Strains. J Clin Microbiol. 2002 March; 40(3): 922–925.

Ringer's Solution (1/4 strength) Tablets

LAB100Z

Description

An osmotically controlled solution for the preparation of suspensions of food samples and for use as a diluent in dilution techniques for bacterial enumeration. The solution can also be used in the sampling of food production apparatus by the rinse and swab method.

Formula	g/litre
Sodium chloride	2.25
Potassium chloride	0.105
Calcium chloride	0.12
Sodium bicarbonate	0.05

Method for reconstitution

Dissolve 1 tablet in 500ml deionised water. When completely dissolved dispense into containers as required and sterilise by autoclaving for 15 minutes at 121°C.

Minimum QC organisms: *Escherichia coli ATCC 25922*
Staphylococcus aureus ATCC 25923

Appearance: Tablet: white tablet

Finished medium: colourless, clear liquid

Storage:

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

Rose Bengal Chloramphenicol Agar Base

LAB036

Description

A selective medium for the enumeration of moulds and yeasts in foods. The original formulation of Jarvis (1973) used chlortetracycline, this has been substituted by chloramphenicol because of superior selectivity. The Rose Bengal dye is taken up by the growing colonies making them easier to see and inhibiting their spreading. Rose Bengal becomes increasingly toxic on exposure to light so it is important to store plates in the dark.

Formula	g/litre
Mycological Peptone	5.0
Dextrose	10.0
Dipotassium phosphate	1.0
Magnesium sulphate	0.5
Rose bengal	0.05
Agar No. 2	12.0

Method for reconstitution

Weigh 28.5 grams of powder, disperse in 1 litre of deionised water, allow to soak for 10 minutes, swirl to mix then sterilise for 15 minutes at 121˚C. Allow to cool to 47˚C then add 2 vials of X009 (X209). Chloramphenicol (or 2 vials X089 oxytetracycline). Mix well, then pour into Petri dishes. This medium should be protected from light. Chloramphenicol may be added before autoclaving.

Appearance: Deep purple/red, clear.

pH: 7.2 ± 0.2

Minimum Q.C. organisms: *Aspergillus sp.* NCIMB 50097
Sacchromyces cerevisiae
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark. Capped Container – up to 1 month at 2-8˚C in the dark.

Inoculation: Surface spreading.

Incubation: 25˚C aerobically for 24 hours to 5 days.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
Rhizopus spp.	13.5	Fluffy	White	
Mucor spp.	12.5	Fluffy	White	
Fusarium spp.	10	Fluffy	White	
Aspergillus flavus	8	Flat & hyphae	Yellow/ Green	hyphae
Candida spp.	1.5	CV.E.G.	White	
Sacchromyces spp.	1.0	CV.E.G.	White	
E. coli	no growth			
Ent. faecalis	no growth			
B. subtilis	no growth			
Staphylococcus spp.	no growth			

References

Jarvis, B. (1973). Comparison of an improvised Rose-Bengal Chlortetracycline Agar with other media for the selective isolation and enumeration of moulds and yeasts in food. J. Appl. Bact. 36: 723-727.

Overcast, W.W. and Weakley, D.L. (1969). An aureomycin rose-bengal agar for the enumeration of yeast and mould in cottage cheese. J.Milk and Fd.Tech. 32: 442-445.

Banks, J.G. Board, R.G. (1987). Some factors influencing the recovery of yeasts and moulds from chilled foods. Int. J. Food Microbiol. 4: 197-206.

Sabouraud Dextrose Agar

LAB009

Description

Introduced by Sabouraud in 1910 as a selective medium for fungi and yeasts. The acidic pH (5.6) of this medium inhibits many species of bacteria. The medium can be made more selective by the addition of chloramphenicol supplement (X009) (X209). Diagnostic features, such as sporing structures and pigmentation are well developed on this medium. Because of its low pH this medium is very sensitive to overheating which will soften the agar and caramelise the carbohydrate.

Formula	g/litre
Balanced Peptone No. 1	10.0
Dextrose	40.0
Agar No. 2	12.0

Method for reconstitution

Weigh 62 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving for 15 minutes at 121˚C. NOTE: The gel strength of the medium may diminish if recommended sterilising time or temperature is exceeded.

Cool to 47˚C, mix well then pour plates.

Appearance: Buff opalescent gel.

pH: 5.6 ± 0.2

Minimum Q.C. organisms: *Candida sp.* NCIMB 50010
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark. Capped container – up to 3 months at 15-20˚C in the dark.

Inoculation method: Surface streaking for single colonies or stab method.

Incubation: Aerobically, yeasts 37˚C for 48 hours; fungi 25-30˚C for up to 3 weeks.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
C. albicans	0.5-2.0	CV.E.D.	White	Yeasty smell
C. krusei	1.0-3.0	F.CR.D.	Grey-White	Yeasty smell
T. rubrum	25	White-fluffy	Reverse-shades of red	
T. mentagraphytes	25	White-fluffy	Reverse-yellow-orange	
M. canis	25	White-centre-yellow radial	reverse-yellow-	
E. floccosum	25	White-fluffy	Reverse-tan	

References

Sabouraud, R. (1910). Les Teignes Paris. Pagano. J., Levin, J.D. and Trejo, W. (1957-8). Diagnostic medium for the differentiation of species of Candida. Antibiotics Annual, 137-143.

Sabouraud Liquid Medium U.S.P.

(Fluid Sabouraud Medium)

LAB033

Description

A liquid sterility test medium for the detection of yeasts, moulds and acidophilic bacteria in pharmaceutical products. This medium conforms with the United States Pharmacopeia. It can also be used as a growth medium for the determination of fungistatic activity in pharmaceutical products.

Formula	g/litre
Pancreatic digest of casein	5.0
Peptic digest of fresh meat	5.0
Glucose	20.0

Method for reconstitution

Weigh 30 grams of powder, disperse in 1 litre of deionised water, allow to soak for 10 minutes, swirl to mix. Heat to dissolve, then dispense into final containers before sterilising at 121˚C for 15 minutes.

Appearance: Pale straw clear.

pH: 5.7 ± 0.2

Minimum Q.C. organisms: *Candida* sp. NCIMB 50010
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation: As recommended in the U.S.P.

Incubation: 22-25˚C aerobically for 10 days.

References

The Pharmacopeia of the United States of America. XVII (1965).

Sabouraud Maltose Agar

LAB111

Description

This is a modification of Sabouraud Dextrose Agar, substituting maltose for dextrose, recommended by the American Public Health Association.

Formula	g/litre
Balanced Peptone	10.0
Maltose	40.0
Agar No. 2	12.0

Method for reconstitution

Weigh 62 grams of powder, disperse in 1 litre of deionised water, allow to soak for 10 minutes, swirl to mix then autoclave at 121˚C for 15 minutes. Do not overheat or the agar gel will be softened and the carbohydrate will be caramelised. This medium may be made selective by the addition of 2 ampoules X009 Chloramphenicol selective supplement which may be added either before or after autoclaving. Cool to 47˚C and mix well before pouring plates.

Appearance: Cream/yellow, translucent.

pH: 5.6 ± 0.2

Minimum Q.C. organisms: *Candida* sp. NCIMB 50010
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark. Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation: Surface streaking or stab inoculum.

Incubation: Aerobic; yeasts 37˚C for 48 hours; other fungi 25˚C for up to 3 weeks.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
C. albicans	0.5-2.0	CV.E.D.	White	Yeasty smell
C. krusii	1.0-3.0	F.CR.D.	Grey-White	Yeasty smell
T. rubrum	25mm	White-fluffy	Reverse-shades of Red	
T.mentagraphytes	25mm	White-fluffy	Reverse-yellow-orange	
M. canis	25mm	White-centre yellow radial	Reverse-yellow-orange	
E. floccosum	25mm	White-fluffy	Reverse-tan	

References

Sabouraud, R. (1910). Les Teignes. Paris. Pagano, J., Levin, J. D. and Trejo, W. (1957-8) Diagnostic medium for the differentiation of species of Candida. Antibiotics Annual. 137-143.

Selenite Broth

LAB044A & LAB044B

Description

A medium for the selective enrichment of salmonellae from faeces, food and sewage. First described by Leifson in 1936 the medium is a peptone lactose broth, moderately buffered, which utilises sodium biselenite as a selective agent. This medium can be incubated at various temperatures from 35-43˚C to vary the selectivity. Subcultures should be performed after no more than 24 hours incubation as there is an increasing loss of selectivity if incubation is prolonged.

Formula	g/litre
Selenite Broth Base LAB044A:	
Peptone	5.0
Lactose	4.0
Sodium phosphate	10.0
Sodium biselenite LAB044B:	
Sodium hydrogen selenite	4.0

Method for reconstitution

Dissolve 4 grams of sodium biselenite in 1 litre of cold deionised waer. Add 19 grams of Selenite Broth Base and warm to dissolve. Distribute into tubes or bottles and sterilise for 5-10 minutes in a boiling water bath, or by free steaming. DO NOT AUTOCLAVE.

Appearance: Pale orange/red with slight precipitate (overheating will cause excessive precipitate and loss of selectivity).

pH: 7.1 ± 0.2 (complete medium)

Minimum Q.C. organisms: *Salmonella* sp. NCIMB 50076
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation: Approximately 0.5-1 gram of sample per 10ml tube.

Incubation: Up to 24 hours aerobically at 35-43˚C.

Subculture: Onto two or more selective agars.

References

Leifson, E. (1936). New selenite enrichment media for isolation of typhoid and paratyphoid *(Salmonella)* bacilli. Amer. J.Hyg. 24: 423-432.

MacFaddin, J.F. (1985). Media for the isolation, cultivation, identification of Medical Bacteria Vol 1. Williams and Wilkins, Baltimore.

Selenite Cystine Broth

LAB055A & LAB044B

Description

This formulation is a result of the investigation of North and Bartram in 1953. They examined the effect of varying concentrations of cystine and phosphate on the recovery of salmonellae in egg products using selenite broth. It was found that the addition of 10 micrograms/ml of cystine to Leifson's selenite broth enhanced recovery of salmonellae.

Formula	g/litre
Selenite Cystine Broth Base LAB055A	
Balanced Peptone No. 1	5.0
Lactose	4.0
Sodium phosphate	10.0
L-Cystine	0.01
Sodium biselenite LAB044B	
Sodium hydrogen selenite	4.0

Method for reconstitution

Dissolve 4 grams of Sodium biselenite (LAB044B) in 1 litre of deionised water. Add 19 grams of Selenite Cystine Broth Base and heat to dissolve. Distribute into tubes or bottles, and sterilise for 10 minutes in a boiling water bath, or steamer. DO NOT AUTOCLAVE THIS MEDIUM.

Appearance: Pale straw colour, clear with slight precipitate. (A brick red precipitate indicates overheating).

pH: 7.0 ± 0.2

Minimum Q.C. organisms: *Salmonella* sp. NCIMB 50076
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation: Add sample to broth in the ratio of 1:10. Use a pre-enrichment broth if damaged organisms are to be recovered.

Incubation: 37˚C for 24-48 hours aerobically. Subculture onto *Salmonella* selective media.

References

International Organisation for Standardization. Microbiology (1981). General guidance on methods for the detection of *Salmonella.* ISO, 6579-1981.

International Organization for Standardization. Milk and milk products (1985). Detection of *Salmonella.* ISO 6785-2985 (E).

ICMSF, (1978). Micro-organisms in foods. 1. Their significance and methods of enumeration, 2nd edn., University of Toronto Press, Toronto, Ont.

Leifson, E. (1936). New selenite enrichment media for the isolation of typhoid and paratyphoid *(Salmonella)* bacilli. Am, J. Hyg. 24, 423-432.

North, W.R. and. Bartram, M.T. (1953). The efficiency of selenite broth of different compositions in the isolation of *Salmonella.* Appl. Microbiol. 1, 130, 134.

Speck, M.L. (1984). Compendium of methods for the microbiological examination of foods, 2nd edn., American Public Health Association.

Sensitivity Test Agar

(S.T.A.)

LAB012

Description

A medium formulated for antibiotic susceptibility testing by the Joan Stokes technique. S.T.A. is inhibitor-free, very rich and includes various nucleotides to enable fastidious organisms to be tested. It is necessary to add lysed or 'chocolated' blood for some organisms.

Formula	g/litre
Peptone-Infusion Solids	21.5
Starch	0.6
Sodium chloride	5.0
Disodium citrate	1.0
Adenine sulphate	0.01
Guanine hydrochloride	0.01
Uracil	0.01
Xanthine	0.01
Aneurine hydrochloride	0.01
Uridine	0.1
Agar No. 2	12.0

Method for reconstitution

Weigh 40 grams of powder, disperse in 1 litre of deionised water, allow to soak for 10 minutes, swirl to mix then sterilise by auto-claving for 15 minutes at 121˚C. To prepare blood agar cool to 45˚C and add 7% lysed blood or 6% defibrinated blood according to preference. Mix well then pour plates.

Appearance: Dependent upon the blood additive.

pH: 7.4 ± 0.2

Minimum Q.C. organisms: *S. aureus* NCTC 6571
E. coli NCTC 10418
(antibiotic sensitivity zones)

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation method: Surface, according to technique.

Incubation: 37˚C, atmosphere to suit organisms metabollic requirements.

Interpretation: There are no defined zone sizes as in Mueller Hinton, but all antibiotics should give adequate zone sizes when compared to controls using standard organisms, e.g. *S. aureus* NCTC 6571, *E. coli* NCTC 10418, *Ps. aeruginosa* NCTC 10662.

References

Stokes, E.J. (1968). Clinical Bacteriology 3rd edn. Arnold, London. Committee of the A.C.P. (1965). Report on the Antibiotic Sensitivity test trial organised by the bacteriology committee of the Association of Clinical Pathologists. J.Clin. Pathol., 18: 1-5.

Hanus, F.J. Sands, J.G. and Bennett, E.O. (1967). Antibiotic activity in the presence of agar. Appl. Microbiol., 15: 31-34.

Bechtle, R.M. and Scherr, G.H. (1958). A new agar for in vitro antimicrobial sensitivity testing. Antibiot. Chemother., 8: 599-606.

Simmons Citrate Agar

LAB069

Description

A medium devised by Simmons in 1926 to help in the differentiation of enteric bacteria and in the isolation of fungi. Certain *Enterobacteriacae* have the ability to utilize citrate as the sole source of carbon and utilize inorganic ammonium salts as the sole source of nitrogen resulting in an increase in alkalinity. Bromothymol Blue is used as a pH indicator.

Formula	g/litre
Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	1.0
Dipotassium phosphate	1.0
Sodium citrate	2.0
Sodium chloride	5.0
Bromothymol blue	0.08
Agar No. 2	15.0

Method for reconstitution

Weigh 24 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then heat to dissolve the agar and solids. Dispense into tubes or bottles then sterilise by autoclaving at 121˚C for 15 minutes. Allow to set as slopes.

Appearance: Green, opalescent.

pH: 6.9 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034
C. freundii NCTC 9750

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation: Streak on surface and stab into the butt.

Incubation: 37˚C aerobically for 24-48 hours, with loose caps to allow gaseous exchange.

Interpretation: Utilisation of citrate and ammonium salts results in growth and a change in colour of the medium from green to blue.

Growth Characteristics

organism	growth	colour of medium
(most) *Salmonella* spp	yes	blue
S. typhi	no	green
S. cholerasuis	no	green
S. gallinarum	no	green
S. paratyphi A	no	green
Serratia spp.	yes	blue
Shigella spp.	no	green
Yersinia spp.	no	green
Klebsiella spp.	yes	blue
Proteus mirabilis	yes	blue
P. vulgaris	yes	blue
Providencia rettgeri	no	green
P. providenciae	no	green
E. coli	no	green
C. freundii	yes	blue

References

Simmons, J.S. (1926). A Culture medium for differentiating organisms of typhoid - colon aerogenes groups and for isolation of certain fungi. J.Inf. Dis. 39: 209-215.

Koser, S.A. (1923). Utilisation of the salts of organic Acids by the Colon-aerogenes group. J. Bact. 8: 493-520.

MacFaddin, J.F. (1983). Biochemical Tests for Identification of Medical Bacteria. Williams and Wilkins.

Slanetz and Bartley Medium

(Membrane Enterococcus Agar)

LAB166

Description

This medium was originally described by Slanetz and Bartley for the enumeration of enterococci from water samples using a membrane filtration technique, but it may also be used as a spread plate for the examination of other sample types. Enterococci reduce tetrazolium chloride to the insoluble red dye formazan, producing colonies which are dark red or maroon on the surface of the membrane or agar. This reaction is not exclusive to enterococci, and the count at this stage should be considered presumptive. Colonies may be confirmed as enterococci by demonstrating aesculin hydrolysis using Kanamycin Aesculin Azide Agar LAB106.

Formula	g/litre
Tryptose	20.0
Yeast Extract	5.0
Glucose	2.0
Dipotassium hydrogen phosphate	4.0
Sodium azide	0.4
2,3,4 Tetrazolium chloride	0.1
Agar	12.0

Method for reconstitution

Weigh 43.5 grams of powder and mix with 1 litre of deionised water. Bring to the boil with frequent stirring to dissolve completely. Cool to 47˚C and pour into sterile Petri dishes. DO NOT AUTOCLAVE, OVERHEAT, OR LEAVE FOR GREATER THAN 4hr AT 47˚C.

Appearance: Rose coloured gel.

pH: 7.2 ± 0.2

Mininmum QC organisms:
Enterococcus faecalis NCIMB 50030
Escherichia coli NCIMB 50034 (inhibition)

Storage: Plates – upto 7 days at 2-8˚C. Storage in bottles is not recommended as re-melting the medium will cause damage.

Inoculation

Water: Filter 100ml of the water through a suitable membrane, and place this on the surface of a properly dried Slanetz and Bartley plate.

Other samples: Dilute as necessary and spread 0.5ml over the surface of the plate using a spreader, and allow to soak into the agar.

Incubation

Water: at 37˚C for 48hr if testing potable waters or processed foods. At 37˚C for 4hr then 44˚C for 44hr if testing untreated waters or raw materials.

Interpretation

Count all red and maroon colonies as presumptive enterococci. Confirmation of isolates can be achieved by demonstration of a positive aesculin reaction on KAAA LAB106.

Reference:

Slanetz, L.W., and Bartley, C.H. (1957) J.Bact. **74** 591-595.

Environment Agency: The Microbiology of Drinking Water (2002). Methods for the Examination of Water and Associated Materials.

Sorbitol MacConkey Agar

(SMAC, CT-SMAC)

LAB161

Description

This is a selective differential medium for the isolation of *Escherichia coli* O157:H7, the primary serovar associated with haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS). Pathogenicity of the organism is linked to the production of verocytotoxins (VT1 and VT2), but it should be noted that not all strains of O157:H7 produce verocytotoxins, and that strains from other serovars can be toxin producers (e.g. O26, O111, O113, O145).

O157:H7 has been associated epidemiologically with food poisoning outbreaks involving beefburgers and cold cooked meats. The medium is a modification of MacConkey Agar No. 3 with the substitution of the fermentable carbohydrate from lactose to sorbitol. O157:H7 is sorbitol negative and produces translucent colonies whereas most other *E. coli* strains are sorbitol positive and so produce pink/red colonies. Selectivity of the medium can be increased by adding Cefixime-Tellurite (C.T.) supplement X161.

Formula	g/litre
Peptone	20.0
Sorbitol	10.0
Bile salts no.3	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	12.0

Method for reconstitution

Weigh 48.5 grams of powder and add to 1 litre of de-ionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C, add 2 vials of CT supplement X161, and pour plates. Dry the surface prior to inoculation.

Appearance: Pale red, slight violet tinge.

pH: 7.1 ± 0.2

Minimum QC organisms:
E. coli O157:H7 (non-toxigenic) NCTC 12900 (translucent)
E. coli NCIMB 50034 (Pink/red)
Ent. faecalis NCIMB 50030 (inhibition)

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface streak for single colonies.

Incubation: 37˚C aerobically for 18-24 hr.

Growth Characteristics:

Organism	Size (mm)	Shape	Colour
E. coli O157:H7	2.5 – 4.0	CV.E.G	Translucent
Other *E. coli*	2.5 – 4.0	CV.E.G	Pink/red
Sorbitol +ve organisms	2.5 – 5.0	Any	Pink/red

References

Law, D., Ganguli, L.A., Donohue-Rolfe, A., Acheson, D.W.K. (1992) J. Med. Micro. 36 198-202.

Hitchins, A.D., Hartman, P.A., and Todd, E.C.D. (1992) in "Compendium of methods for the microbiological examination of foods" Ch.24. Published by American Public Health Association.

Varnam, A.H., Evans, M.G., (1991) Foodborne Pathogens an Illustrated Text. Published by Wolfe Publishing Ltd.

Riley, L.W. (1991) Ann. Rev. Micro. 41 383-408.

Riley, L.W. *et al* (1983) New Eng. J. Med 308 681-685.

Salmon, R.L., Farrel, I.D., Hutchinson, J.G.P. (1989) Epid. Inf. 103 249-254.

S.S. Agar
(Salmonella Shigella Agar)

LAB052

Description

This medium is a modification of Leifson's DCA Medium first described in 1941 by Mayfield and Goeber shortly before Hynes described his modification of DCA. The selectivity of the medium was increased by the addition of extra bile salts, sodium citrate and the addition of brilliant green dye. There is also the extra thiosulphate giving good H_2S production which reduces the ferrous ammonium sulphide giving black centred colonies with H_2S positive organisms.

The selectivity of this medium can be such that it was suggested by Taylor *et al* in 1965 to be unsuitable for the isolation of *Shigella* species. Greater understanding of the selection mechanisms involved enable us to adjust the reaction and allow the more delicate *Shigella* to grow without unduly impairing the medium's selective properties.

Formula	g/litre
Beef Extract	5.0
Balanced Peptone No. 1	5.0
Lactose	10.0
Bile Salts No. 3	8.5
Sodium citrate	8.5
Sodium thiosulphate	8.5
Ferric citrate	1.0
Brilliant Green	0.00033
Neutral Red	0.025
Agar No. 2	13.5

Method for reconstitution

Weigh 60 grams of powder, disperse in 1 litre of deionised water, and allow to soak for 10 minutes. Swirl to mix, then bring to the boil, and allow to cool to 47˚C. Mix well then pour plates. Dry the surface before incubation. DO NOT AUTOCLAVE THIS MEDIUM.

Appearance: Pale Pink, clear.

pH: 7.0 ± 0.2

Minimum Q.C. organisms: *Salmonella* sp. NCIMB 50076
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 4˚C in the dark.

Inoculation method: Surface plating, streaking for single colonies.

Incubation: 37˚C aerobically for 18-24 hours.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
E. coli	0.1-2.0	CV.E.D.	Red	Red ppt around colonies (No growth)
K. aerogenes	0.1-3.0	CV.E.G.	Pink-Red	(No growth)
Proteus spp.	1.0-2.0	CV.E.G.	Yellow	(grey centre) (fishy odour)
Ps. aeruginosa	0.1-1.0	CV.CR.D.	Pink Yellow	(Green pigment)
Salmonella spp.	2.0-3.0	CV.E.G.	Yellow	(black centre)
Shigella spp.	0.5-2.0	CV.E.G.	Pink-Yellow	

Gram positive organisms – no growth.

References

Isenberg, H.D., Kominos, S., and Siegel, M. (1969). Isolation of salmonellae and shigellae from an artificial mixture of fecal bacteria. Appl. Microbiol., 18: 4, 656-659.

Leifson, E. (1935). New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Pathol. Bacteriol., 40: 581-589.

Taylor, W.I., Harris, B. (1965) Isolation of shigellae. II. Comparison of plating media and enrichment broths. Am. J. Clin. Pathol. 44: 4, 476-479.

Sugar Free Agar

LAB087

Description

A formula described by the International Dairy Federation for the enumeration of psychrotrophic and mesophilic Gram-negative rods in butter and other dairy products. The Gram-negative rods are able to deaminate proteins as a carbon source, whilst some enterococci are inhibited by this formula. The medium conforms to the formulation of the International Dairy Federation (I.D.F.).

Formula	g/litre
Gelatin Peptone	7.5
Tryptone	7.5
Sodium chloride	5.0
Agar No. 1	14.0

Method for reconstitution

Weigh 34 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, boil to dissolve and disperse into tubes or flasks. Sterilise by autoclaving at 121˚C for 15 minutes.

Appearance: Light straw, clear.

pH: 7.6 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034

Storage of Prepared Medium: Capped container – up to 3 months at 15-20˚C in the dark.

Inoculation: 0.2ml of butter fat in a pour plate technique.

Incubation: 30˚C for 2 days then 20˚C for a further two days – aerobically.

Interpretation: Count colonies.

References

International Dairy Federation (1964). International standard count of contaminating organisms in butter. International Standard FIL-IDF30.

Ritter, P. and Eschmann, K.H. (1966). Alimenta 5(2), 43-45.
Thomas, S. B. (1969). J. Appl. Bact. 32, 269-296.

Mossel, D.A.A., Krol, B. and Moerman, P.C. (1972). Alimenta 11(2), 51-60.

Susceptibility Test 'ISO' Agar

LAB170

Description

Susceptibility Testing 'Iso' Agar is a semi-defined medium for antimicrobial susceptibility (sensitivity) testing (AST), in which the undefined elements are maintained at minimum levels. The antimicrobial susceptibility test is utilised in epidemiological studies and in determining the appropriate usage of antimicrobials in the clinical environment. The response of clinical isolates to antimicrobials, and the detection of microbial resistance, allows for precise and rapid treatment. The AST is performed to detailed standards, the results of which must be reproducible; a major factor is the medium on which it is performed.

The presence of antagonists in the medium e.g. thymidine and metal ions, have a detrimental effect on results obtained. The addition of thymidine for the growth of dependant strains antagonises the antimicrobial action of Trimethoprim and sulphonamides and results in false resistance results. Metal ions can exert known antagonistic effects on a number of antibiotics. Therefore the anion and cation content of the medium must be regulated to prevent adverse effects on performance.

Susceptibility Testing 'Iso' Agar is produced having a stable mineral content, the presence of minimum antagonistic elements, and a constant isotonic pH (preventing the blocking or enhancement of antimicrobials), thereby ensuring production of optimum zones of microbial inhibition.

This medium will support the growth of the majority of pathogens requiring susceptibility testing, without the addition of supplements. However, certain organisms such as some streptococci, staphylococci, *Enterobacteriaceae* and *Neisseria* may require the addition of intrinsic growth factors e.g. lysed horse blood, thymidine, thiamine and menadione. However these supplements can introduce errors as they can affect the activity of certain antibiotics and consequently their affects must assessed before use.

Formula	g/litre
Peptone Mixture	16.0
Glucose	2.0
Starch	1.0
Sodium chloride	2.8
Na_2HPO_4	0.4
Sodium glycerophosphate	0.22
Sodium gluconate	0.1
Sodium acetate	1.0
Uridine	0.3
Defined Chemical Mixture	0.078
Agar	12.0

Method for Reconstitution

Weigh 35.9 grams of powder and disperse in 1 litre of deionised water. Swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and if required add 5-7% sterile lysed horse blood. Pour into sterile petri dishes and allow to set. Cool to 47°C, mix well and dispense into petri dishes.

Appearance: Straw clear gel.

pH: 7.3 ± 0.2

Minimum Q.C. organisms:
(as recommended by the British Society for Antimicrobial Chemotherapy (BSAC))

Escherichia coli NCTC 12241
Staphylococcus aureus NCTC 12981
Pseudomonas aeruginosa NCTC 12934
Enterococcus faecalis NCTC 12697
Haemophilus influenzae NCTC 12699
Streptococcus pneumoniae ATCC 49619
Neisseria gonorrhoeae NCTC 12700.

Storage: Capped containers - up to 3 months at 15-20°C in the dark. Plates - up to 7 days at 2-8°C, in the dark.

Inoculation: Surface, inoculum as described by standard methods.

Incubation: As stipulated in the BSAC methodology.

References

Ericsson, H.M., Sherris, J.C. (1971). Antibiotic Sensitivity Testing. Report of an International Collaborative Study. Acta. Pathol. Microbiol. Scand. Sect B Suppl.; **217**: 1-90.

Amato, R.F., Thornberry, C., (1979). Calcium and Magnesium in Mueller Hinton Agar and their influence on disc diffusion susceptibility results. Current Microbiol. **2**: 135-138.

Hawkey, P.M., Birkenhead, D., Kerr, K.G., Newton, K.E., Hyde, W.A. (1993). Effect of divalent cations in bacteriological media on the susceptibility of Xanthomonas maltophila to imipenem with special reference to zinc ions. J. Antimicrobial Chermother; **31**: 181-183.

Garrod, L.P., Waterworth, P.M. (1969). Effect of medium composition on the apparent sensitivity of pseudomonas aeruginosa to gentimicin. J. Clin. Pathol; **22**: 534-538.

Duncan, I.B.R. (1974). Susceptibility of 1500 isolates of Pseudomonas aeruginosa to gentimicin, carbenecillin, colistin, and polymyxin B. Antimicrobial Agents and Chermother; (Jan) 9-15.

T.C.B.S. Cholera Medium

(Thiosulphate Citrate Bile Salts Sucrose Agar)

LAB096

Description

T.C.B.S. is designed for the selective isolation of *Vibrio* species, particularly *V. cholerae.* The formulation was developed by Kobayashi, Enomoto, Sakazaki and Kuwahara and inhibits most of the *Enterobacteriaceae* for at least 24 hours. Therefore heavy inoculation of the medium is possible.

Formula	g/litre
Yeast Extract	5.5
Peptone Mix	10.0
Sodium thiosulphate	10.0
Sodium citrate	10.0
Bile salts	9.0
Sucrose	17.0
Sodium chloride	10.0
Ferric citrate	1.0
Bromothymol blue	0.04
Thymol blue	0.04
Agar No. 1	15.0

Method of reconstitution

Weigh 88 grams of powder and add to 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then bring to the boil. Cool to 47˚C and pour into Petri dishes. DO NOT AUTOCLAVE OR OVERHEAT THIS MEDIUM.

Appearance: Dark green clear agar.

pH: 8.6 ± 0.2

Minimum Q.C. organisms: *V. cholerae* (type F)
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 4˚C in the dark. Capped containers – up to 1 month at 15-20˚C in the dark.

Inoculation: Surface plating with a heavy inoculum, streak out to single colonies.

Incubation: 37˚C aerobically for 18-24 hours.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
Vibrio. cholerae	2.0-3.0	CV.E.G.	Yellow	may revert to green at R.T.
V. parahaemolyticus	3.0-5.0	CV.E.G.	Blue or Green	
V. alginolyticus	3.0-5.0	CV.E.G.	Yellow	
V. metschnikovii	2.0-4.0	CV.E.G.	Yellow	
V. fluvialis	2.0-3.0	CV.E.G.	Yellow	
V. vulnificus	2.0-3.0	CV.E.G.	Yellow	
V. mimicus	2.0-3.0	CV.E.G.	Green	
Enterococci	1.0	CV.E.G.	Yellow	
Proteus spp.	1.0	F.CR.G.	Green/ Yellow	
Ples. shigelloides	P.P.		Green	

References

Kobayashi, T., Enomoto, S., Sakazaki, R. and Kuwahara, S. (1963). Jap. Bacteriol 18: 10-11, 387-391.

Tetrathionate Broth Base A.P.H.A.

LAB097

Description

A selective enrichment broth for the growth of *Salmonella typhi* and other *Salmonella* spp. from faeces, foods etc. It conforms to the formulation recommended by the American Public Health Association for use in the examination of dairy products and foods for salmonellae. Organisms which reduce tetrathionate, such as salmonellae, proliferate in the medium, whilst most enteric organisms are inhibited. Certain members of the *Proteus* group will also reduce tetrathionate thereby impairing the performance of the medium in some cases. To overcome this, Novobiocin may be added to the medium at a level of 40 microgram/ml before addition of the iodine. Gram-positive organisms are inhibited by the inclusion of bile salts.

Formula	g/litre
Balanced Peptone No. 1	5.0
Bile Salts	1.0
Calcium carbonate	10.0
Sodium thiosulphate	30.0

Method for reconstitution

Weigh 46 grams of powder and add to 1 litre of deionised water. Bring to the boil with frequent swirling to fully dissolve the medium. Cool to 45˚C and add 20ml of iodine solution prepared as indicated below. Mix well before dispensing into bottles and continue swirling whilst dispensing to avoid the calcium carbonate sedimenting. For the best results the medium should be used the same day as prepared.

Iodine solution: Dissolve 5g of potassium iodide and 6g of iodine crystals in 20ml of distilled water.

Appearance: Turbid white.

pH: 8.4 ± 0.2

Minimum Q.C. organisms: *Salmonella* sp. NCIMB 50076
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Capped containers – up to 7 days at 4˚C in the dark (without iodine solution).

Inoculation: Add 1 part of sample suspension or inoculated pre-enrichment medium to 9 parts of Tetrathionate Broth.

Incubation: 12-24 hours at 37˚C.

Subculture: Onto LAB034 Brilliant Green Agar and either LAB032 XLD or LAB110 Hektoen Enteric or other *Salmonella* selective media.

References

Standard methods for the Examination of Dairy products, 10th Edition. APHA, (1953).

Thioglycollate Medium (Brewer)

LAB064

Description

This is the original formula introduced by Brewer in 1940 as a clear medium for the cultivation of anaerobes. It has found applications as a sterility test medium and as a blood culture medium although it has been superseded by Fluid Thioglycollate LAB025 and Fastidious Anaerobe Broth LAB071 for these purposes.

The agar makes the medium viscous slowing down the permeation of oxygen and any convection currents. Sodium thioglycollate acts as a reducing agent and also neutralises the bacteriostatic properties of mercurial compounds. Methylene blue is a redox indicator which is colourless at low Eh but turns green on exposure to oxygen.

Formula	g/litre
Beef Extract	1.0
Yeast Extract	2.0
Balanced Peptone No. 1	5.0
Dextrose	5.0
Sodium chloride	5.0
Sodium thioglycollate	1.1
Methylene blue	0.002
Agar No. 1	1.0

Method for reconstitution

Weigh 20 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes then bring to the boil with gentle agitation to dissolve the solids. Distribute into screw top containers leaving minimal headspace. Sterilise by autoclaving at 121˚C for 15 minutes. Tighten caps as soon as possible after autoclaving.

Appearance: Straw coloured, translucent, viscous liquid which may have a green surface due to contact with oxygen. If the medium has a diffuse green tinge it should not be used until the oxygen has been driven off by holding in a boiling water bath for 5 minutes. Do not reheat more than once.

pH: 7.2 ± 0.2

Minimum Q.C. organisms: *C. perfringens* NCIMB 50027

Storage of Prepared Medium: Capped container – up to 3 months at 15-20˚C in the dark.

Inoculation: Ensure adequate dispersal of the inoculum in the broth.

Incubation: 37˚C for 24-72 hours.

Growth Indicators: A diffuse turbidity or individual colonies.

References

Brewer, J.H. (1940). Clear liquid mediums for the culture of anaerobes. J. Amer. Med. Ass. 115: 598-600.

Todd Hewitt Broth

LAB075

Description

A nutritious broth medium formulated by Todd and Hewitt for the production of antigenic streptococcal haemolysin. Todd Hewitt Broth is also used to cultivate streptococci prior to serological grouping. The use of a fermentable sugar in the formulation leads to the production of acid which would normally inactivate the haemolysin. This is prevented by the inclusion of buffers to maintain the pH of the medium thus preserving the haemolysin, as well as promoting the growth of pneumococci.

Formula	g/litre
Infusion from fat-free minced meat	10.0
Tryptone	20.0
Dextrose	2.0
Sodium bicarbonate	2.0
Sodium chloride	2.0
Disodium phosphate anhydrous	0.4

Method for Reconstitution

Weight 36.4grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and warm to dissolve. Dispense into 10ml volumes in screw capped containers and sterilise by autoclaving at 121˚C for 15 minutes.

Appearance: Pale straw, clear broth.

pH: 7.8 ± 0.2

Inoculation: Pick a well isolated colony for subculture into Todd Hewitt Broth.

Incubation: 37˚C for 18-48hrs, aerobically.

Storage: Capped containers - up to 3 months at 15-20ºC in the dark.

Minimum Q.C. Organisms *Streptococcus pyogenes* ATCC 19615.

Reference:

Todd, E.W., and Hewitt, L.F., (1932) A New Culture Medium for the Production of Antigenic Streptococcal Haemolysin. J. Path. Bact. 35 (1) 973-974.

Updyke, E.,L., and Nickle, M.I. (1954) A Dehydrated Medium for the Preparation of Type Specific Extracts of Group A Streptococci. Appl. Microbiol. 2 117-118.

Triple Sugar Iron Agar

LAB053

Description

This is a modification of the Krumwiede and Kohn medium of 1917 which differentiates some of the *Enterobacteriaceae* on the basis of four reactions; fermentation of lactose, glucose and sucrose and H_2S production. This medium should be used in conjunction with a urease test to eliminate *Proteus* spp. when screening for *Salmonella* spp.

Formula	g/litre
Beef Extract	3.0
Yeast Extract	3.0
Balanced Peptone No. 1	20.0
Sodium chloride	5.0
Lactose	10.0
Sucrose	10.0
Glucose	1.0
Ferric citrate	0.3
Sodium thiosulphate	0.3
Phenol red	0.025
Agar No. 2	12.0

Method for reconstitution

Weigh 65 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then bring to the boil with frequent swirling to dissolve the solids. Distribute into tubes and sterilise at 121˚C for 15 minutes. Allow to set as a slope ensuring that the slant is over a butt approximately 3cm deep.

Appearance: Reddish-orange gel.

pH: 7.4 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034
Ps. aeruginosa NCIMB 50067

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation: A heavy inoculum is streaked over the surface of the slope and stabbed into the butt.

Incubation: 37˚C aerobically for 24 hours.

Interpretation

Slant/butt	Colour	Utilisation
Alkaline/acid	Red/yellow	Glucose only fermented Peptones utilised
Acid/acid	Yellow/yellow	Glucose fermented Lactose + or sucrose fermented
Alkaline/alkaline	Red/Red	Neither glucose, lactose, nor sucrose fermented Peptones utilised

Organism	Butt	Slant	Sulphide production
Shigella dysenteriae		NC	
S. sonnei	Acid	or	-
S. flexneri		Alk.	
Salmonella typhi	Acid	NC	+
S. paratyphi	Acid	NC	-
S. cholerasuis	Gas		
S. typhimurium	Acid		
S. enteritidis	Gas	NC	+
S. pullorum			
S. gallinarum	Acid	NC	+
E. coli			
Enterobacter aerogenes	Acid Gas	Acid	-
E. cloacae			
Proteus mirabilis	Acid Gas	Acid	+
Providencia rettgeri	Acid	NC	-

NC = No Change

References

American Public Health Association (1963). Diagnostic Procedures and Reagents, 4th edn., A.P.H.A., New York.

American Public Health Association (1966). Recommended Methods for the Microbiological Examination of Foods. 2nd edn., A.P.H.A., New York.

Edwards, P.R. and Ewing, W.H. (1962). Identification of *Enterobacteriaceae*. Burgess Publishing Co., Minneapolis.

Tryptone Bile Agar

LAB072

Description

First introduced by Delaney, McCarthy and Grasso in 1962 as a method for detecting faecal coliforms in water supplies based on the production of indole on a bile medium at 44˚C. The idea was applied to foodstuffs by Anderson and Baird-Parker in 1975. The inoculum is placed onto the membrane on a resuscitation agar and incubated at 37˚C for 4 hours. The membrane is then transferred to a Tryptone Bile Agar plate and incubated at 44˚C: after incubation the membrane is flooded with indole reagent. Indole positive colonies produce a red colour on the membrane and are easily counted.

Formula	g/litre
Tryptone	20.0
Bile Salts No. 3	1.5
Agar No. 2	15.0

Method for reconstitution

Weigh 36.5 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C and pour into Petri dishes.

Appearance: Straw coloured, clear gel.

pH: 7.2 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034

Storage of Prepared Medium: Plates – up to 4 days at 2-8˚C in the dark. Capped container – up to 1 month at 2-8˚C in the dark.

Inoculation: 1ml of a 1:10 dilution of homogenised sample onto a membrane. The recommended membranes are 85mm in diameter with a 0.45 micron pore size manufactured from cellulose esters.

Incubation: 4 hours at 37˚C on Nutrient Agar LAB008 or Minerals Modified Glutamate Medium LAB080A and 80B plus agar – then 18-24 hours on Tryptone Bile Agar at 44˚C.

Indole reagent: 5% p-dimethylaminobenzaldehyde in N-HC1 (Vracko & Sherris 1963).

Indole reaction: Pipette 1-2ml of reagent into Petri dish lid, remove membrane with forceps and place on reagent. Allow to stand for 5 minutes for reaction to develop, then dry in sunlight to 'fix' the colour. Count all pink colonies as *E. coli*.

Growth characteristics

organism	colony size (mm)	shape & surface	indole reaction on membrane
E. coli	1.0-3.0	CV.E.G.	positive – pink colour
other *Enterobacteriaceae*	0.5-2.0	CV.E.G.	negative – no colour
Klebsiella spp.	no growth		
Pseudomonas spp.	no growth		
Staphylococcus spp.	no growth		
Bacillus spp.	no growth		

References

Anderson, J.M., Baird-Parker, A.C. (1975). A rapid and direct method for enumerating Eschericia coli biotype I in food. J. Appl. Bact. 39: 111-117.

Delaney, J.E., McCarthy, J.A. & Grasso, R.J. (1962). Measurement of *E. coli* type I by the membrane filter technique Wat. Sewage Wks. 109, 289.

Baird, R.M., Corry, J.E.L., Curtis, G.D.U. (1988). Pharmacopoeia of culture media for food microbiology. Int. J. Food Microbiol. 276-277.

Tryptone Glucose Extract Agar

LAB063

Description

A plate count agar suggested by the American Public Health Association (A.P.H.A.) for estimation of total viable counts in food and dairy products. This medium is also recommended by the Association of Official Analytical Chemists (A.O.A.C.).

Formula	g/litre
Beef Extract	3.0
Tryptone	5.0
Glucose	1.0
Agar No. 1	15.0

Method for reconstitution

Weigh 24 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then boil to dissolve before distributing into tubes or bottles. Sterilise at 121˚C for 15 minutes.

Appearance: Pale straw colour, clear.

pH: 7.0 ± 0.2

Minimum Q.C. organisms: *S. epidermidis* NCIMB 50082
E. coli NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark. Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation: Pour plate technique.

Incubation: 30˚C aerobically for 48 hours for aerobic mesophile count. 6˚C aerobically for 10 days for aerobic psychrotroph count. 55˚C aerobically for 48 hours for aerobic thermophile count.

References

Association of Official Analytical Chemists (AOAC). (1995). Bacteriological Analytical Manual, 8th ed.: Association of Official Analytical Chemists.

Hausler, W.J. (Ed.) (1976). Standard Methods for the Examination of Dairy Products, 14th edn.: American Public Health Association.

Speck, M.J. (Ed.) (1976). Compendium of Methods for the Microbiological Examination of Foods.: American Public Health Association.

Tryptone Soy Agar (U.S.P.)

(Soybean Casein Digest Medium U.S.P.)

LAB011

Description

A general purpose agar which will support the growth of a wide range of micro organisms. The formula conforms with that laid down by the United States Pharmacopeia for sterility testing. The medium can be used for phage typing, colicine typing and for testing the X and V factor requirements of *Haemophilus* spp.

Formula	g/litre
Tryptone (Casein Digest USP)	15.0
Soy Peptone	5.0
Sodium chloride	5.0
Agar No. 2	12.0

Method for reconstitution

Weigh 37 grams of powder, disperse in 1 litre of deionised water, allow to soak for 10 minutes, swirl to mix then sterilise for 15 minutes at 121˚C. Cool to 47˚C, mix well and then pour plates.

Appearance: Pale straw coloured, clear gel.

pH: 7.3 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034
S. epidermidis NCIMB 50082

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark. Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation: Surface plating.

Incubation: Time and temperature to suit organisms, usually aerobic.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
S. aureus	1.0-1.5	CV.E.G.	White-Yellow	
Other *staphylococci*	1.0-1.5	CV.E.G.	White-Yellow	strain-dependent
Strep. pyogenes	PP-0.5	CV.E.G.	Transp.	
S. pneumoniae	PP	CV.E.G.	Transp.	(CO_2)
Ent. faecalis	0.5-1.0	CV.E.G.	Grey-White	
Klebsiella spp.	2.0-3.0	CV.E.G.	White	mucoid
Ps. aeruginosa	0.5-3.0	F.CR.D.	Grey-Green	(marked strain variation)

References

United States Pharmacopeia 21st edition. (1985).

Blair, J.E. and Carr, M. (1953). The bacteriophage typing of staphylococci. J. Infect. Dis. 93: 1-13.

Examination of Dairy Products. A.P.H.A., New York.

Tryptone Soy Broth (U.S.P.)

(Soybean Casein Digest Medium U.S.P.)

LAB004

Description

A general purpose nutritious broth capable of growing a wide range of bacteria and fungi. The medium is recommended by the United States Pharmacopeia for the sterility testing of a wide range of pharmaceutical products. The medium is also widely used for blood cultures although the high carbohydrate level may cause rapid growth and subsequent death of acid-producing organisms.

Formula	g/litre
Tryptone (Casein Digest USP)	17.0
Soy Peptone	3.0
Sodium chloride	5.0
Dipotassium phosphate	2.5
Dextrose	2.5

Method for reconstitution

Weigh 30 grams of powder, disperse in 1 litre of deionised water. Swirl to mix and warm if necessary to dissolve. Dispense into tubes or flasks and sterilise at 121˚C for 15 minutes. Do not exceed temperature.

Appearance: Straw coloured, clear.

pH: 7.3 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034
S. aureus NCIMB 50080

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20˚C in the dark.

Incubation: 20-25˚C aerobically for 14 days, for sterility tests. 37˚C aerobically for 14 days for blood cultures.

Growth indicators: Turbidity or precipitate.

References

United States Pharmacopeia 21st edition (1985).

Tryptone Soy Broth

(without dextrose)

LAB205

Description

A basal media that can be supplemented with carbohydrates and indicators for fermentation studies.

Formula	g/litre
Tryptone (casein digest U.S.P.)	17.0
Soy Peptone	3.0
Sodium chloride	5.0
Dipotassium phosphate	2.5

Method for reconstitution

Weigh 27.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and warm to dissolve if necessary. Distribute into tubes or bottles and sterilise by autoclaving for 15 minutes at 121°C. Do not exceed stated temperature.

Appearance:

Powder: fine, free-flowing, homogeneous, buff
Finished medium: straw, clear liquid

pH: 7.3 ± 0.2

Hazard classification

NR – Not regulated

Minimum Q.C. organisms:
Escherichia coli ATCC 25922
Staphylococcus aureus ATCC 6538
Bacillus subtilis ATCC 6633
Candida albicans ATCC 10231
Aspergillus niger ATCC 16404

Storage:

Dehydrated culture media: 10-25°C away from direct sunlight
Prepared media: capped containers – up to 3 months at 15-20°C in the dark.

Incubation:

Aerobically at 20-25°C for 14 days, for sterility tests.
Aerobically at 37°C for 14 days for blood cultures.

Tryptone Water

LAB129

Description

A substrate for the testing of an organism's ability to produce indole from tryptophan. The indole test is frequently used in the classification of coliform organisms. This product is preferable to peptone water LAB104 because it has a higher content of tryptophan.

Formula	g/litre
Tryptone	10.0
Sodium chloride	5.0

Method for reconstitution

Weigh 15 grams of powder, disperse in 1 litre of deionised water. Heat to dissolve then distribute into screw cap bottles. Sterilise by autoclaving at 121˚C for 15 minutes.

Appearance: Colourless, clear.

pH: 7.5 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation: From pure culture.

Incubation: 37˚C for 24-48 hours.

Interpretation: Indole positive organisms will give a distinct colour change when either Kovac's or Ehrlich's indole reagent is added.

References

American Public Health Association. (1955). American Water Works Association 10th edn. 391-392.

MacFaddin, J. (1983). Biochemical tests for the identification of medical bacteria. 2nd edn. Williams & Wilkins, Baltimore.

Tryptose Phosphate Broth

LAB062

Description

This is a versatile, nutritionally rich buffered glucose broth. The medium is a general purpose broth that has been used as a blood culture medium, and with the addition of sodium azide 0.025% as a selective medium for streptococci in dairy products.

Formula	g/litre
Tryptose	20.0
Dextrose	2.0
Sodium chloride	5.0
Disodium phosphate	2.5

Method for reconstitution

Weigh 29.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, heat to dissolve solids then distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C.

Appearance:

Powder: fine, free-flowing, homogeneous, buff
Finished medium: clear, pale straw liquid

Minimum Q.C. organisms: *E. coli* ATCC 25922
S. aureus ATCC 25923
Strep. pyogenes NCTC 8198

pH: 7.3 ± 0.2

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20˚C in the dark.

Inoculation: For blood culture work dilute sample at least 1:10 in broth.

Incubation: Dependent on application.

References

American Public Health Association (1948). Standard Method for the Examination of Dairy Products, 10th edn. A.P.H.A., New York.

American Public Health Association, (1950). Diagnostic Procedures and Reagents, 3rd edn., A.P.H.A., New York.

T.Y.C. Medium

(Tryptone Yeast Cystine)

LAB035

Description

A medium designed by J. D. de Stoppelaar in 1967 to differentiate *Streptococcus sanguis* (frequently found in dental plaque) from *Streptococcus mutans* (implicated in dental caries). The medium uses a high sucrose content to promote the formation of specific glucans by *S. mutans* thus forming distinctive colonies. It can be made selective by the addition of 0.2 units per ml of Bacitracin.

Formula	g/litre
Tryptone	15.0
Yeast Extract	5.0
L-Cystine	0.2
Sodium sulphite	0.1
Sodium chloride	1.0
Disodium phosphate anhydrous	0.8
Sodium bicarbonate	2.0
Sodium acetate anhydrous	12.0
Sucrose	50.0
Agar No. 2	12.0

Method for reconstitution

Weigh 98 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix, then sterilise for 15 minutes at 121˚C. Cool to 47˚C, mix and pour plates.

Appearance: White, translucent gel.

pH: 7.3 ± 0.2

Minimum Q.C. organisms: *S. mutans* ATCC 25175

Storage of Prepared Medium: Plates – up to 7 days at 4˚C in the dark.

Inoculation: Surface, streaking out for single colonies.

Incubation: 37˚C for 4-5 days in an atmosphere of 90% H_2, 10% CO_2.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
Strep. mutans Type A	1.0-3.0	'heaped' colony granular surface irregular edge	Yellow	Crumbles when touched with wire
Type B	1.0-3.0	As Type A	Grey white	soft consistency, white precip in agar (glistening drop)
S. sanguis	1.0-3.0	Convex glossy crenated	White	very rubbery (glistening drop)

References

de Stoppelaar, J.D. van de Houte, J. and de Moor, C.E. (1967). The presence of dextran forming bacteria resembling *Streptococcus bovis* and *Streptococcus sanguis* in dental plaque. Arch. Oral Biol. 12: 1199-1201.

de Stoppelaar, J.D. (1971). *Streptococcus mutans, Streptococcus sanguis* and dental caries. Thesis, Rijksuniversiteit, Utrecht.

Emilson, C.G., Bratthall, D. (1976). Growth of *Streptococcus mutans* on various selective media. Journal of Clinical Microbiology 4: 95-98.

Gold, O.G., Jordon, H.V., Van Houte, J. (1973). A selective medium for *Streptococcus mutans*. Archives of Oral Biology 19: 1357-1364.

Ikeda, T., Sandham, H.J. (1972). A high-sucrose medium for the identification of *Streptococcus mutans*. Archives of Oral Biol. 4: 781-783.

Wade, W.G., Alldred, M. J., Walker, D.M. (1986). J. Med. Microbiol. 22: 319-323. An improved medium for isolation of *Streptococcus mutans.*

Urea Agar Base

(Christensen)

LAB130

Description

This is a modification of Christensen's urea base for the detection of rapid urease production by *Proteus* spp. Other enterobacteria will split the urea, but this will be delayed. This delay is achieved by the incorporation of glucose and the introduction of a buffering system into the medium. The indicator for ammonia production is phenol red.

Formula	g/litre
Peptone	1.0
Glucose	1.0
Sodium chloride	5.0
Disodium phosphate	1.2
Potassium dihydrogen phosphate	0.8
Phenol red	0.012
Agar No. 1	12.0

Method for reconstitution

Weigh 2.1 grams of powder, disperse in 95ml of deionised water. Allow to soak for 10 minutes, swirl to mix, then sterilise at 121˚C for 15 minutes. Allow to cool to 47˚C, add aseptically 5ml sterile urea solution X130/X135. Distribute into sterile bottles and slopes, allow to set in the sloped position.

Appearance: Yellow/pale pink, translucent.

pH: 6.8 ± 0.2

Minimum Q.C. organisms: *Proteus* spp.
E. coli NCIMB 50034

Storage of Prepared Medium: Capped container – up to 1 month at 2-8˚C in the dark.

Inoculation: Pure culture using straight wire for stab/streak technique.

Incubation: 37˚C for 4-6 hours or overnight, aerobically.

Interpretation: Production of red colour in under 6 hours is positive for rapid urease production.

Organism Growth Characteristics

Proteus spp.	Red colour	4-6 hours
Citrobacter spp.	Red colour	18-24 hours
Klebsiella spp.	Red colour	18-24 hours
Staphylococcus spp.	Red colour	24-48 Hours
Helicobacter pylori	Red colour	30 minutes

References

Christensen, W.B. (1946). Urea decomposition as a means of differentiating Proteus and paracolon cultures from each other and from Salmonella and Shigella types. J. Bacteriol. 52: 461-466.

Urea Broth Base

(Christensen)

LAB131

Description

This is a liquid version of Christensen's medium (LAB130) introduced by Maslen in 1952. This modification allows inoculation by Pasteur pipette, and it is easier to detect contamination in a fluid rather than in a slope. Maslen also claimed that it is easier to detect positive results.

Formula	g/litre
Peptone	1.0
Glucose	1.0
Disodium phosphate	1.2
Potassium dihydrogen phosphate	0.8
Sodium chloride	5.0
Phenol red	0.004

Method for reconstitution

Weigh 0.9 grams of powder, add to 95ml of deionised water. Swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Allow to cool to 47˚C then add aseptically 5ml of X130/X135 sterile urea solution. Distribute into sterile screw cap bijou bottles.

Appearance: Yellow, clear.

pH: 6.8 ± 0.2

Minimum Q.C. organisms: *Proteus* sp.
E. coli NCIMB 50034

Storage of Prepared Medium: Capped container – up to 1 month at 2-8˚C in the dark.

Inoculation: Fluid culture by pasteur pipette or straight wire from pure growth.

Incubation: 37˚C for 4-6 hours – preferably in a water bath for most rapid growth, aerobically.

Interpretation: The production of a red colour in under six hours is a positive result for rapid urease.

Organism Growth Characteristics

Proteus spp.		Red colour	4-6 hours
Corynebacterium hoffmani		Red colour	18-24 hours
C. ulcerans		Red colour	18-24 hours
Some strains	*Citrobacter*	Red colour	18-24 hours
	Klebsiella	,,	,,
	Escherichia	,,	,,
	Yersinia	,,	,,
	Staphylococcus	,,	,,
Pasteurella multocida		Red colour	18-24 hours

References

Maslen L.G.C. (1952). Routine use of liquid urea medium for identifying Salmonella and Shigella organisms. J. Brit. Med. 2: 545-546.

UVM Base

LAB155

Description

UVM (University of Vermont Medium) Base is a two stage selective enrichment broth for the isolation of *Listeria* from meat products and environmental swabs, and forms the basis of the USDA method. The original method has been modified to replace the second stage broth (UVM II) with Fraser broth LAB164 (McClain & Lee 1989).

Formula	g/litre
Tryptone	5.0
Meat Peptone	5.0
Beef Extract	5.0
Yeast Extract	5.0
Sodium chloride	20.0
Disodium hydrogen phosphate	9.6
Potassium dihydrogen phosphate	1.35
Aesculin	1.0

pH: 7.4 ± 0.2

Appearance: Straw opalescent broth

Method for reconstitution

Weigh 52 grams of powder and add to 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise at 121˚C for 15 minutes. Cool to 47˚C and add 2 vials of UVM I supplement (X155) as required. Mix well and distribute into sterile tubes or bottles.

Inoculation: Add 25g sample to 225ml of and homogenise.

Incubation: 30˚C aerobically for 24 hrs. Subculture onto selective agars. .

Minimum QC organism: *Listeria* sp. NCIMB 50007
E. coli (inhibition) NCIMB 50034

References

McClain D., and Lee W.H. (1989) FSIS method for isolation of L.monocytogenes from processed meat and poultry products. Lab.Comm.No.57, Revised May 24, 1989. US Dept of Agric.FSIS, Microbiol. Div.

Warburton D.W. *et al* (1991) A Canadian comparative study of modified versions of the FDA and USDA methods for the detection of L.monocytogenes. J.Food Protection 54 (9) 669-676.

Violet Red Bile Agar

(V.R.B.A.)

LAB031

Description

A medium for the enumeration of coliform organisms in food and dairy products. The selectivity of the medium is due to the presence of bile salts and crystal violet. Lactose fermenters produce red/purple colonies often surrounded by a halo of the same colour. Non lactose fermenters produce pale colonies. Selectivity can be increased by incubation at 42-44°C.

Formula	g/litre
Yeast Extract	3.0
Balanced Peptone No. 1	7.0
Sodium chloride	5.0
Bile Salts No. 3	1.5
Lactose	10.0
Neutral red	0.03
Crystal violet	0.002
Agar No. 2	12.0

Method for reconstitution

Disperse 38.5g of powder in 1 litre of distilled water. Dissolve by bringing to the boil with frequent swirling of the flask to prevent overheating. DO NOT AUTOCLAVE. Cool to 45°C and distribute into bottles or tubes. If held molten in a water bath, use within 3 hours.

Appearance:

Powder: fine, free-flowing, homogeneous, buff
Finished medium: light, purple-violet clear gel

pH: 7.4 ± 0.2

Minimum Q.C. organisms: *E. coli* ATCC 25922
Enterococcus faecalis (inhibition) ATCC 29212

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark. Capped containers – up to 1 month at 15-20˚C in the dark.

Inoculation: Pour plate (with or without overlay) or surface spread.

Incubation: 37˚C for 18-24 hours for 'coliforms'; 4˚C for 10 days for psychrotrophs; 32˚C for 24-48 hours for mesotrophs; 42˚C for 18 hours for thermotrophs.

Interpretation: Count all red/purple colonies > 0.5mm in diameter. Calculate the number of coliforms in original sample.

References

American Public Health Association (1972), Standard Methods for the Examination of Dairy Products. 13th edn. (ed. W.H. Hausler), A.P.H.A., Washington.

American Public Health Association (1966). Recommended Methods for the Microbiological Examination of Foods. 2nd edn. (ed. J.M. Sharf) A.P.H.A., Washington.

Davis, J.G. (1951). Milk Testing Dairy Industries, London.

Mossel, D.A.A., Eelderink, I. and Sutherland, J.P. (1977). Development and use of single, 'polytropic' diagnostic tubes for the approximate taxonomic grouping of bacteria, isolated from foods, water and medicinal preparations. Zbl. Bakt. Hyg. I., Orig., A 278, 66-79.

Mossel, D.A.A., Eelderink, I., Koopmans, M. and van Rossem, F. (1979). Influence of carbon source, bile salts and incubation temperature on the recovery of *Enterobacteriaceae* from foods using MacConkey type agars. J. Food Protec. 42, 470-475.

Mossel, D.A.A., van der Zee, H., Hardon, A.P. and van Netten, P. (1986). The enumeration of thermotropic types amongst the *Enterobacteriaceae* colonizing perishable foods. J. Appl. Bacteriol. 60, 289-295.

Violet Red Bile Agar with MUG

(V.R.B.A. with MUG)

LAB573

Description

Violet Red Bile Agar with MUG (Methylumbelliferyl-β-D-glucuronide) is a medium for the simultaneous enumeration of coliform organisms and *Escherichia coli* in food and dairy products. The selectivity of the medium is due to the presence of bile salts and crystal violet. Lactose fermenters produce red/purple colonies often surrounded by a halo of bile precipitate. *Escherichia coli* produce red/purple fluorescent colonies due to the fermentation of lactose and production of the enzyme glucuronidase, which hydrolyses MUG to yield the fluorescent compound methylumbelliferone, detectable by long-wave UV light. Non-lactose fermenters produce pale colonies.

Standard Methods procedures specify VRBA with MUG for detecting *E. coli* in food and dairy products by fluorescence.

Formula	g/litre
Yeast extract	3.0
Balanced peptone No.1	7.0
Sodium chloride	5.0
Bile Salts No. 3	1.5
Lactose	10.0
Neutral red	0.03
Crystal violet	0.002
Agar No. 2	12.0
MUG, 4 methylumbelliferyl-ß-D-glucuronide	0.1

Method for reconstitution

Weigh 38.6 grams of powder, disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and then sterilise the medium, with frequent mixing, by bringing to the boil. DO NOT AUTOCLAVE. Cool to 47°C and distribute into bottles or tubes. If held molten in a water bath, use within 3 hours.

Appearance: Light purple-violet clear gel.

pH: 7.4 ± 0.2

Minimum Q.C. organisms:
Escherichia coli NCIMB 50034
Enterobacter aerogenes NCIMB 50029
Staphylococcus aureus NCIMB 50080

Storage of Prepared Medium: Plates - up to 7 days at 2 - 8°C in the dark. Capped containers up to 1 month at 15-20°C in the dark.

Inoculation: Pour plate method (with or without overlay) or surface spread.

Incubation: 37°C for 18-24 hours for 'coliforms'; 4°C for 10 days for psychrotrophs; 32°C for 24-48 hours for mesotrophs; 42°C for 18 hours for thermotrophs.

Interpretation: Examine plates for growth and fluorescence. Count all red/purple colonies > 0.5mm in diameter as coliforms and all fluorescent colonies as presumptive *E. coli*. Calculate the number of coliforms and *E. coli* in the original sample.

References

Christen, G.L., Davidson, P.M., McAllistair, J.S. and Roth, L.A. (1993). Coliform and other indicator bacteria. 247-269. R.T. Marshall (ed.) Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

Hitchens, A.D., Hartman, P.A. and Todd, E.C.D. (1992) Coliforms-*Escherichia coli* and its toxins. C. Vanderzant and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Hitchens, A.D., Peng, P., Watkins, W.D., Rippey, S.R. and Chandler, L.A. (1995). *Escherichia coli* and the coliform bacteria. 4.01-4.29. Bacteriological Analytical Manual, 8th ed. AOAC International, Gaithersburg, MD.

Feng, P.C.S. and Hartman, P.A. (1982). Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl. Environ. Microbiol. 43:1320-1329.

Chang, G.W., Brill, J. and Lum, R. (1989). Proportion of β-D-glucuronidase negative *Escherichia coli* in human fecal samples. Appl. Environ. Microbiol. 55:335-339.

Hansen, W. and Yourassowsky, E. (1984). Detection of β-D-glucuronidase in lactose fermenting members of the family Enterobacteriaceae and its presence in bacterial urine cultures. J. Clinical Microbiol. 20:1177-1179.

Kilian, M. and Bulow, P. (1976). Rapid diagnosis of Enterobacteriaceae. Acta. Pathol. Microbiol. Scand. Sect. B. 84:245-251.

Mates, A. and Shaffer, M. (1989). Membrane filtration differentiation of *E. coli* from coliforms in the examination of water. J. Appl. Microbiol. 67:343-346.

Damare, J.M., Campbell, D.F. and Johnston, R.W. (1985). Simplified direct plating method for enhanced recovery of *Escherichia coli* in food. Journal of Food Science. 50:1736-1746.

Violet Red Bile Glucose Agar

(V.R.B.G.A.)

LAB088

Description

A modification of Violet Red Bile Agar LAB031 introduced by Mossel in 1978. V.R.B.A. LAB031 contains lactose which is fermented by members of the coli/aerogenes group, this medium gives a 'coliform' count. V.R.B.G.A. LAB088 has substituted lactose with glucose. Glucose is fermented by all members of the *Enterobacteriaceae* thus V.R.B.G.A. gives a presumptive *Enterobacteriaceae* count. Bile salts and crystal violet are used to inhibit Gram positive and non-enteric organisms. The overlay procedure ensures anaerobic conditions and suppresses the growth of non-fermentative Gram negative bacteria.

Formula	g/litre
Yeast Extract	3.0
Balanced Peptone No. 1	7.0
Sodium chloride	5.0
Bile Salts No. 3	1.5
Glucose	10.0
Neutral red	0.03
Crystal violet	0.002
Agar No. 2	12.0

Method for reconstitution

Weigh 38.5 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes then swirl to mix. Bring to the boil with frequent swirling to prevent overheating. Further sterilisation is not required. Cool to 45˚C, mix well and dispense into tubes or bottles. If held molten in a water bath, use within 3 hours.

Appearance: Light purple-violet, clear.

pH: 7.4 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034
S. epidermidis (inhibition) NCIMB 50082

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark. Capped containers – up to 1 month at 15-20˚C in the dark.

Inoculation: Pour plate method with overlay.

Incubation: 37˚C aerobically for 18-24 hours.

Interpretation: Count all red/purple colonies > 0.5mm in diameter. Calculate the number of *Enterobacteriaceae* in original sample.

References

Pharmacopoeia of Culture Medium for Food Microbiology (1987). Int. J. Food Microbiol. 5: 3: 280-81.

Mossel, D.A.A., Mengerink, W.H.J. and Scholts, H.H. (1962). Use of a modified MacConkey agar medium for the selective growth and enumeration of *Enterobacteriacaea.* J. Bacteriol. 84: 381.

Water Plate Count Agar (ISO)

LAB197

Description

A nutritious non-selective medium which conforms to **ISO 6222:1999(E)** *Water quality - Enumeration of culturable micro-organisms - Colony count by inoculation in a nutrient agar culture medium.* The estimation of overall numbers of microorganisms can be used for the assessment and surveillance of water quality. Colony counts are useful for assessment of ground water integrity and the efficiency of water treatment processes. They also give an indication of the cleanliness and integrity of the distribution system. They can be used to assess the suitability of a water supply for the preparation of food and drink, thus avoiding contamination of the product with spoilage organisms. The main value of colony counts lies in the detection of changes in water supply quality from those expected, based on frequent long term monitoring. A sudden increase in the numbers can be a warning of pollution and can call for immediate remedial action

Formula	g/litre
Tryptone	6.0
Yeast Extract	3.0
Agar	15.0

Method for reconstitution

Weigh 24.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix then sterilise at 121°C for 15 minutes. Cool to 47°C before use.

Appearance: Pale straw coloured, clear gel.

pH: 7.2 ± 0.2

Minimum Q.C. organisms:
Staphylococcus epidermidis NCIMB 50082
Escherichia coli NCIMB 50034

Storage of Prepared Medium: Plates can be stored up to 7 days at 2-8°C in the dark.

Inoculation: Pour plate technique or surface inoculation.

Incubation: Aerobically at 36°C ± 2°C for 44 ± 4 hours and 22°C ± 2°C for 68 ± 4 hours.

Interpretation: Count all colonies and calculate the number of organisms (or 'colony forming units' c.f.u.) per ml of sample allowing for dilution factors.

References

ISO 6222 (1999) Water quality - Enumeration of culturable microorganisms - Colony count by inoculation in a nutrient agar medium.

W. L. Nutrient Agar

(Wallerstein Laboratory)

LAB079

Description

This medium was developed by Green and Gray in 1950 for the isolation and enumeration of yeasts, moulds and bacteria in the brewing process. The medium has a pH of 5.5 which is optimum for Brewers yeast and will allow the growth of a wide range of organisms including *Enterobacteriaceae*, *Flavobacterium*, *Lactobacillus* and *Pediococcus spp.* as well as yeasts and moulds. If a process involving bakers or distillers yeast is under examination the pH should be adjusted to 6.5. The medium may be adapted to detect bacteria only by the addition of 0.004 g/litre of Actidione to suppress the yeasts.

Formula	g/litre
Yeast Extract	4.0
Tryptone	5.0
Dextrose	50.0
Potassium dihydrogen phosphate	0.55
Potassium chloride	0.425
Calcium chloride	0.125
Magnesium sulphate	0.125
Ferric chloride	0.0025
Manganese sulphate	0.0025
Bromocresol green	0.022
Agar No. 2	15.0

Method for reconstitution

Weigh 75 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. If adjustment of pH to 6.5 is required used 1% sodium bicarbonate. Cool to 47°C, mix well and dispense into Petri dishes.

Appearance:

Powder: fine, free-flowing, homogeneous, buff
Finished medium: Green-blue, clear gel

pH: 5.5 ± 0.2

Minimum Q.C. organisms: *S. cerevisiae.*

Storage:

Dehydrated culture media: 10-25°C

Final medium: up to 7 days (plates) or up to 1 month (capped containers) at 2-8°C in the dark

Inoculation: Surface plating or pour plate.

Incubation: 30˚C aerobically for 48 hours (bacteria); 20˚C aerobically for 48 hours (yeasts).

Interpretation: Count all colonies. Calculate organisms per ml in original sample.

References

Green, S.R. and Gray, P.P. (1950). Differential Procedure Applicable to Investigation in Brewing. Wallerstein Lab. Comm. 13,357.

Hall, J.F. (1971). Detection of Wild Yeasts in the Brewery. J. Inst. Brewing, 77: 513-516.

Wort Agar

LAB038

Description

A medium for the enumeration of yeasts and moulds in butter, developed by Parfitt in 1933. The medium can be modified to enable it to isolate osmophilic yeasts from soft drinks and sugar products by the addition of high concentrations of sucrose and glucose.

Formula	g/litre
Malt Extract	15.0
Peptone	0.78
Maltose	12.75
Dextrin	2.75
Dipotassium phosphate	1.0
Ammonium chloride	1.0
Agar No. 2	15.0

Method for reconstitution

Weigh 48.3 grams of powder and disperse in 1 litre of deionised water. Add 2.35ml of glycerol. Allow to soak for 10 minutes, swirl to mix then sterilise for 15 minutes at 121°C. Use 60 grams per litre if required for inoculation by plate streaking with a wire loop. Do not exceed time or temperature of sterilisation. If osmophilic modification is required add 48.3 grams of powder to 1 litre of a solution containing 35% w/v sucrose and 10% w/v glucose then sterilise at 108˚C (5 p.s.i.) for 20 minutes.

Appearance: Light Brown, translucent.

pH: 5.0 ± 0.2

Minimum Q.C. organisms: *S. cerevisiae.*

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark. Capped container – up to 1 month at 15-20˚C in the dark.

Inoculation: Pour plate or surface spread.

Incubation: 25˚C aerobically for 5 days.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
Candida spp.	4.0	CV.E.G	White	
Fungi	Varies with species			
S. cerevisiae	2.0-3.0	Varies with strain	Cream	

References

Parfitt, E.H. (1933). The influence of media upon the yeast and mould count of butter. J. Dairy Sci. 16: 141-147.

Scarr, M.P. (1959). Selective media used in the microbiological examination of sugar products. J. Sci. Fd. Agric. 10: 678-681.

Wort Broth

LAB099

Description

A broth version of the medium LAB038 Wort Agar developed by Parfitt for the enumeration of yeasts and moulds, in butter. The medium can be modified for the isolation of osmophilic yeasts from soft drinks and sugar products by the addition of high concentrations of sucrose and glucose.

Formula	g/litre
Malt Extract	15.0
Peptone	0.78
Maltose	12.75
Dextrin	2.75
Dipotassium phosphate	1.0
Ammonium chloride	1.0

Method for reconstitution

Weigh 33.3 grams of powder and disperse in 1 litre of deionised water, add 2.35ml of glycerol. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. If osmophilic version is required disperse 33.3 grams of powder in 1 litre of a solution of 35% w/v sucrose and 10% w/v glucose then sterilise at 108˚C (5 p.s.i.) for 20 minutes.

Appearance: Light Brown, translucent.

pH: 4.8 ± 0.2

Minimum Q.C. organisms: *S. cerevisiae.*

Storage of Prepared Medium: Capped container – up to 1 month at 15-20˚C in the dark.

Incubation: 25˚C aerobically for 5 days.

X.L.D. Agar

(Xylose Lysine Decarboxylase Agar)

LAB032

Description

This medium was introduced by Taylor in 1965 to improve the recovery and recognition of *Shigella* spp, and has proved to be an excellent medium for *Salmonella* spp. The medium is low in nutrients and relies on a small amount of sodium desoxycholate for selectivity. The indicator system is novel and complex. Most enteric organisms except *Shigella,* will ferment xylose to produce acid. However the salmonellae will also decarboxylate the lysine to keep the pH neutral. At near neutral pH *Salmononella* can produce H_2S from the reduction of thiosulphate producing black or black centred colonies. *Citrobacter* spp. can also decarboxylate lysine, however, the acid produced by fermentation of both lactose and sucrose will keep the pH too acid for H_2S to be produced.

Formula	g/litre
Xylose	3.75
L-Lysine	5.0
Lactose	7.5
Sucrose	7.5
Sodium chloride	5.0
Yeast Extract	3.0
Phenol red	0.08
Agar No. 2	13.0
Sodium desoxycholate	1.0
Sodium thiosulphate	6.8
Ferric ammonium citrate	0.8

Method for reconstitution

Weigh 53.5 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix. Bring rapidly to the boil with frequent stirring, and transfer immediately to a 47˚C water bath. Pour into plates as soon as the medium has cooled. Protracted boiling or prolonged holding at elevated temperature induces precipitation.

Appearance: Light rose, clear gel.

pH: 7.4 ± 0.2

Minimum Q.C. organisms: *Salmonella* sp. NCIMB 50076
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface, streaking out for single colonies.

Incubation: 37˚C for 18-24 hours aerobically.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
Salmonella spp.	1.0-2.5	CV.E.G.	Trans. black centre	(clearing of acid ppt of coliforms)
Shigella sonnei	1.5-2.5	CV.E.G.	Pink	
S. flexneri	1.0-2.0	CV.E.G.	Pink	
S. dysenteriae	0.5-1.5	CV.E.G.	Pink	
E. coli	0.5-1.5	CV.E.G.(D)	Yellow	inhibited (ppt around colony)
Citrobacter spp.	1.0-1.5	CV.E.G.(D)	Yellow	(black centre)
Proteus spp	1.0-2.5	CV.E.G.	Trans. Pink (black centre)	fishy odour

References

Taylor, W.I. (1965). Isolation of shigellae. I. Xylose Lysine Agars: New media for the isolation of enteric pathogens. Am. J. Clin. Pathol., 44: 471-475.

Taylor, W.I., and Harris, B. (1965). Isolation of shigellae. II. Comparison of plating media and enrichment broths. Am. J. Clin. Pathol., 44(4), 476-479.

Taylor, W.I., and Harris, B. (1967). Isolation of shigellae. III. Comparison of new and traditional media with stool specimens. Am. J. Clin. Pathol., 48: 350-355.

Taylor, W.I., and Schelhart, D. (1967). Isolation of shigellae. IV. Comparison of plating media with stools. Am. J. Clin. Pathol., 48: 356-362.

XLT4 Agar

Xylose Lysine Tergitol-4 Agar

NEW **LAB221**

Description

XLT4 Agar is a selective differential isolation medium for the specific detection of *Salmonella* spp. from environmental, food and clinical samples. Due to its highly selective nature, XLT4 Agar is particularly effective when used with samples where overgrowth of contaminating flora is expected, for example, faecally-contaminated agricultural samples.

Developed by Miller & Tate in 1990, this medium was found to improve the recovery of non-typhi *Salmonella* from chicken and farm environmental samples. Dusch & Altwegg further established the application of XLT4 Agar to salmonellae detection in clinical samples, with the notable exceptions of *Salmonella* Typhi and *Salmonella* Paratyphi. The presence of peptone and yeast extract provides sufficient nutrients to allow the optimal growth of *Salmonella* spp.

Selectivity is provided by the anionic surfactant Niaproof® 4 (formerly known as Tergitol-4 / sodium tetradecylsulfate). This compound acts as an effective selective agent which is active against Gram-positive and many Gram-negative organisms, including *Proteus* spp.

Differentiation is based on fermentation of the sugars xylose, lactose and sucrose in addition to the decarboxylation of lysine. The inclusion of the pH indicator, phenol red, provides visual evidence of a pH decrease (yellow) or increase (red) in the medium. Ammonium iron (III) citrate is present to distinguish hydrogen-sulphide (H_2S) producing from non-H_2S producing organisms.

Most enteric organisms, except *Shigella*, will ferment xylose to produce acid. However the salmonellae will also decarboxylate the lysine to keep the pH neutral to alkali, thus maintaining red colouration. At near-neutral pH *Salmonella* can produce H_2S from the reduction of ammonium iron (III) citrate and thiosulphate ions producing black or black-centred colonies. Non H_2S-producing salmonellae will be red without a black centre.

Other Enterobacteriaceae (non-salmonellae) which are not inhibited by Niaproof-4, will ferment xylose, lactose and/or sucrose but will not decarboxylate lysine. This fermentation activity causes a decrease in pH, resulting in a colour change within the colonies from red to yellow.

Formula	g/litre
Proteose peptone	1.6
Yeast extract	3.0
L-lysine	5.0
Xylose	3.75
Lactose	7.5
Sucrose	7.5
Ammonium iron (III) citrate	0.8
Sodium thiosulphate	6.8
Sodium chloride	5.0
Phenol red	0.08
Agar	18.0

Method for reconstitution

Weigh 59 grams of powder and disperse in 1 litre of deionised water. Add 4.6ml of Niaproof-4 supplement (Sigma Niaproof® 4, product code N1404). Allow to soak for 10 minutes, swirl to mix. Heat the medium with frequent agitation and boil for 1 minute. DO NOT OVERHEAT OR AUTOCLAVE THIS MEDIUM. Cool to 48-50°C, mix well and dispense into sterile Petri dishes.

Appearance:

Powder: fine, free-flowing, homogeneous, buff (may have a slight pink colouration)

Finished medium: clear, red gel

pH: 7.4 ± 0.2

Hazard classification

NR – Not regulated

Minimum Q.C. organisms:

Salmonella Typhimurium ATCC 14028
Salmonella Enteritidis ATCC 13076
Escherichia coli ATCC 25922
Enterococcus faecalis ATCC 29212

Storage:

Dehydrated culture media: 10-25°C.
Final medium: 7 days at 2-8°C in the dark

Inoculation: Surface inoculation, streaking out / spreading to achieve single colonies.

Incubation: Incubate plates at 37°C. Examine plates for growth at 24 hours and 48 hours.

Interpretation: Typical *Salmonella* (lactose-negative, H_2S positive) appear as colourless or red colonies with a black centre, giving the traditional "fish-eye" appearance. All isolates with this appearance should be regarded as presumptive salmonellae.

Lactose-positive, H_2S positive salmonellae, e.g. *Salmonella* Arizonae will appear yellow-red with black centre.

Lactose-negative, H_2S negative salmonellae, e.g. *Salmonella* New Brunswick will appear yellow-red without a black centre.

Some strains of *Salmonella* Poona may demonstrate sensitivity to Niaproof® 4.

Interpretation

organism	growth	colony size (mm)	shape & surface	H_2S	colour
Salmonella Typhimurium	Good >50% recovery	1.2 - 2.5	CV, E, G	+	Clear/red, black centre
Salmonella Enteritidis	Good >50% recovery	1.5 - 2.5	CV, E, G	+	Clear/red, black centre
Citrobacter freundii	Good	1.5 - 2.5	CV/DR, E, D	+/-	Yellow
Escherichia coli	Suppressed				
Proteus mirabilis	Inhibited				
Enterococcus faecalis	Inhibited				
Staphylococcus aureus	Inhibited				

KEY CV = Convex E = Entire D = Dull G = Glossy DR = Draughtsman

References

Dusch, H. and Altwegg, M. (1995). Evaluation of five new plating media for the isolation of *Salmonella* species. *Journal of Clinical Microbiology*. **33**. No.4. 802-804.

Miller, R.G. and Tate, C.R. (1990). A highly selective plating medium for the isolation of *Salmonella*. *The Maryland Poultryman*, April: 2-7.

Miller, R.G., Tate, C.R., Mallinson, E.T. and Scherrer, J.A. (1991). Xylose-Lysine-Tergitol 4: An improved selective agar for the isolation of *Salmonella*. *Poultry Science* **70**. 2429-2432.

Miller, R.G., Tate, C.R., Mallinson, E.T. and Scherrer, J.A. (1992). *Erratum*. Xylose-Lysine-Tergitol 4: An improved selective agar for the isolation of *Salmonella*. *Poultry Science* **71**. 398.

Tate, C.R., Miller, R.G. and Mallinson, E.T. (1992). Evaluation of two isolation and non-isolation methods for detecting naturally occurring salmonellae from broiler flock environmental drag-swab samples. *J. Food Prot.* **55**. 964-967.

Yeast Extract Agar

(Yeastrel Milk Agar)

LAB018

Description

A nutrient agar corresponding to the Standard Formulation for the plate count of micro-organisms in water and dairy products. This medium is also useful for teaching and demonstration purposes using non-fastidious organisms.

Formula	g/litre
Yeast Extract	3.0
Balanced Peptone No. 1	5.0
Agar No. 1	15.0

Method for reconstitution

Weigh 23 grams of powder, disperse in 1 litre of deionised water. Free steam or boil to dissolve. Mix well, and dispense into containers. Sterilise for 15 minutes at 121˚C.

To prepare Yeastrel Milk Agar add 10ml of fresh milk before autoclaving.

Appearance: Pale straw, clear gel.

pH: 7.2 ± 0.2

Minimum Q.C. organisms: *E. coli* NCIMB 50034
S. epidermidis NCIMB 50082

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark. Capped container – up to 3 months at 15-20˚C in the dark.

Inoculation: Pour plate technique or surface spreading.

Incubation: 30˚C aerobically for 48 hours for aerobic mesophile count. 6˚C aerobically for 10 days for aerobic psychrotroph count. 55˚C aerobically for 48 hours for aerobic thermophile count.

References

Environment Agency: The Microbiology of Drinking Water (2002). Methods for the Examination of Water and Associated Materials.

British Standard 4285: Methods of Microbiological Examination for Dairy Purposes.

Yeast Extract Dextrose Chloramphenicol Agar

LAB119

Description

A selective medium for the enumeration of yeasts and moulds in milk and other dairy products. The formulation meets the requirements of the International Milk Union (1980), the International Organisation for Standardisation (I.S.O.) and the British Standards Institute (B.S.I.). The medium is said to have superior storage properties to O.G.Y.E. and also has the advantage of incorporating an autoclavable supplement.

Formula	g/litre
Yeast Extract	5.0
Dextrose	20.0
Agar No. 1	15.0

Method for reconstitution

Weigh 40 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then bring to boil. Add 2 vials of X009 (X209) which have been dissolved in ethanol and autoclave at 121˚C for 10 minutes. Allow to cool to 45˚C before using with poured plate technique. THIS MEDIUM MUST NOT BE RE-AUTOCLAVED.

Appearance: Pale yellow, clear.

pH: 6.6 ± 0.2

Minimum Q.C. organisms:
Aspergillus sp. NCIMB 50097
S. cerevisiae
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Incubation: 25˚C for 5 days, aerobically.

Inoculation: Pour plate technique.

Interpretation: Count all colonies.

References

Engel, G. (1982). Verglich verschieden Nährböden zum quantitativen Nachweis von Hefen und Schimmelpilzen in Milch und Milchprodukten. Milchwiss. 37: 727-730.

International Organisation for Standardization (ISO): Milk and milk products – enumeration of yeasts and moulds – colony count technique at 25˚C – standard method ISO/DIS 6611.

International Milchwirtschaftsverband: Milch und Milchprodukten – Zählung von Hefen und Schimmelpilzen (Kolonieählung bel 25 C). – International IMV Standard 94: (1980) in Milchwiss. 36: 220-222.

Normenausschufl Lebensmittel und landwirtshaft. Produkte in DIN Deutsches Institut für Normung e.V. Mikrobiologische Milchuntersuchung. Bestimmung der Anzahl von Hefen und Schimmelpilzen. Reference method DIN 10186.

British Standards Institute. B.S. 4285. Section 3.6: (1986).

Yeast & Mould Agar

LAB200

Description

A medium recommended for the isolation and maintenance of yeasts and moulds. This medium cab also be used for the detection of wild yeasts in beer.

Formula	g/litre
Yeast extract	3.0
Malt extract	3.0
Peptone	5.0
Dextrose	10.0
Agar	20.0

Method for reconstitution

Weigh 41.0 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C, mix well and dispense into sterile Petri dishes.

Increased selectivity of the medium can be achieved by the addition of 12-15ml of sterile lactic acid (X037) to reduce the pH to 4.0. For the detection of wild yeasts, supplement the medium to the desired level with copper sulphate prior to sterilisation.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear straw gel

pH: 6.2 ± 0.2

Hazard classification

NR – Not regulated

Minimum Q.C. organisms:
Escherichia coli ATCC 25922
Candida albicans ATCC 10231
Saccharomyces pastorianus NCYC 185
Lactobacillus fermetum ATCC 9338

Storage:

Dehydrated culture media: 10-25°C away from direct sunlight
Poured plates: 7 days at 2-8°C in the dark

Yersinia Selective Agar

(Schiemann's C.I.N. Agar)

LAB120

Description

This medium is based on the work of Schiemann. It is used for the isolation and enumeration of *Yersinia* spp. from clinical samples and from food. The selective components are sodium desoxycholate, crystal violet, cefsulodin, irgasan and novobiocin. Yersiniae ferment mannitol with an intense, localised, acid production in the centre of the colony which produces a red 'bull's eye' appearance. The ratio of transparent border to red centre varies with serotype and environmental strains may appear rough with an irregular edge. Most other enteric bacteria, if they grow, produce a larger colony with a diffuse pinkish centre and opaque outer zone.

Formula	g/litre
Peptone Mixture	22.5
Mannitol	20.0
Sodium chloride	1.0
Magnesium sulphate	0.01
Sodium pyruvate	2.0
Sodium desoxycholate	0.5
Neutral red	0.03
Crystal violet	0.001
Agar No. 2	12.0

Method for reconstitution

Weigh 58 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, then bring to the boil for 1 minute only. DO NOT AUTOCLAVE. Allow to cool to 47˚C add 2 ampoules C.I.N. supplement X120. Mix well, pour plates.

Appearance: Red, clear.

pH: 7.4 ± 0.2

Minimum Q.C. organisms: *Y. enterocolitica*
E. coli (inhibition) NCIMB 50034

Storage of Prepared Medium: Plates – up to 7 days at 2-8˚C in the dark.

Inoculation: Surface, streaking out for single colonies.

Incubation: 30˚C aerobically for 24 hours.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
Y. enterocolitica	1.0-2.5	CV.E.G.	Red centre	Colony varies with strain, may be rough & irregular
Citrobacter spp.	2.5-3.0	CV.E.G.	Pale pink colony	(may not grow)
Gram +ve organisms	no growth			

References

Schiemann, D.A. (1979). Synthesis of a selective agar medium for *Yersinia enterocolitica.* Can. J. Microbiol. 25: 1298-1304.

Schiemann, D.A. (1982). Development of a two step enrichment procedure for recovery of *Yersinia enterocolitica* from food. Appl. Eniviron. Microbiol. 43: 14-27.

Mossel, D.A.A. (1987). Cefsulodin Irgasan Novobiocin (C.I.N.) agar. Int. J. Food. Microbiol. 5: 208, 209.

2. Harlequin™ Chromogenic Media

The Harlequin™ chromogenic microbiological culture media range has been developed to improve the isolation and identification of a range of microorganisms. Traditional culture media generally rely on the fermentation of sugars or other biochemical reactions for presumptive identification of bacteria. Harlequin™ chromogenic media provide a more specific identification by detection of specific enzymes produced by certain groups of bacteria. The advantage of this type of media is that it can eliminate or reduce the need for subculture and the performance of confirmatory biochemical tests to determine the identity of some microorganisms.

Chromogenic substrates act as the substrate for specific enzymes and change colour due to the action of the enzyme. Lab M have their own patented chromogenic compounds called the novel CHE (cyclohexenoesculetin) substrates which give the bacterial colony a black non-diffusing colouration when hydrolysed by the enzyme involved in the presence of iron salts. Some of the CHE derivatives are used in Harlequin™ media along with indolyl derivatives (5-bromo-4-choro-3-indolyl), which give a blue-green colour on cleavage.

Chromogenic media are most rapidly gaining acceptance as an indicator for *Escherichia coli*. The β-D-glucuronide enzyme is present in approximately 95% of *E. coli* and is uncommon in the other *Enterobacteriaceae*. This type of test is now used widely for water and food microbiology. There is also an increase in interest for chromogenic *Salmonella* media as traditional tests have a very poor specificity resulting in many false positive results. The use of chromogenic media simplifies *Salmonella* testing and saves much time in unnecessary confirmation tests.

Harlequin™ Salmonella ABC

(Freeman Formulation)

HAL001

Description

Salmonella spp. can be differentiated from other members of the family *Enterobacteriaceae* by their ability to produce α-galactosidase in the absence of β-galactosidase. This medium, developed for the isolation of *Salmonella* spp. from food and clinical samples, utilises a dual chromogen system to visualise these enzyme activities. This medium will also detect *Salmonella typhi* and *paratyphi.*

The first substrate, CHE-β-Gal, is enzymatically cleaved by β-galactosidase producing organisms giving black colonies in the presence of iron. Most *Enterobacteriaceae* are β-galactosidase positive and these produce black colonies on *Salmonella* ABC. The second substrate, X-α-Gal, is hydrolysed by *Salmonella* spp. producing green colonies that are easily distinguished from the black or colourless colonies of other organisms. The medium is based on D.C.A Hynes and hence utilises sodium desoxycholate and sodium citrate as inhibitors. Isolation of *Salmonella* spp. by culture remains the most reliable method of detection. However, most media are highly non-specific and consequently place a heavy burden on the laboratory in terms of biochemical and serological confirmation of suspect colonies. With improved specificity, the ABC medium dramatically reduces the need for 'false positive' screening, saving labour and reducing consumable costs.

Formula	g/litre
Beef Extract	5.0
Peptone	5.0
Sodium citrate	8.5
Sodium desoxycholate	5.0
Agar	12.0
X-α-Gal	0.08
CHE-β-Gal	0.3
Ferric ammonium citrate	0.5
IPTG	0.03

Method for reconstitution

Weigh 36.5 grams of powder, disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and then sterilise the medium by bringing to the boil. Cool to 47°C, mix well and dispense into Petri dishes.

DO NOT REMELT OR AUTOCLAVE THIS MEDIUM.

Appearance: Translucent straw gel.

pH: 7.2 ± 0.2

Minimum QC organisms:
Salmonella typhimurium NCIMB 50076
Escherichia coli NCTC 9111

Storage of Prepared Medium: Plates - up to 7 days at 2 - 8°C in the dark.

Inoculation:
Clinical: Streak for single colonies after selective enrichment in Selenite Broth.
Food: Streak for single colonies after selective enrichment.

Incubation: 37°C aerobically for 18 - 24 hours

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
Salmonella spp.	1.0 - 2.0	CV.E.G.	Green	(Black if β-galactosidase +ve)
Shigella spp.	1.0 - 2.0	CV.E.G.	Colourless	(Black if β-galactosidase +ve)
E.coli	PP - 1.5	CV.E.G.	Black	(No Growth)
K. aerogenes	1.0 - 2.5	CV.E.G.	Black.	(Mucoid)
Proteus spp.	0.5-2.0	CV.E.G.	Colourless	(Fishy Odour)
P.aeruginosa spp.	0.5-1.0	CV.CR.D	Colourless	(Green)

References

Perry, J.D., Ford, M., Taylor, J., Jones, A., Freeman, R., Gould, F.K., (1999). ABC Medium, a New Chromogenic Agar for Selective Isolation of *Salmonella* spp. J. Clin. Micro. 37: 766-768.

Harlequin™ TBGA (TBX)

(Tryptone Bile Glucuronide Agar)

HAL003

Description

A medium developed for the simple enumeration of *E. coli* without the need for membranes, or pre-incubation on Minerals Modified Glutamate Medium. It is based upon the formulation of Tryptone Bile Agar, LAB072, the medium has been modified by the addition of a chromogenic substrate to detect the ß-glucuronidase enzyme, which is highly specific for *E. coli**, and is detected by the MUG reagent in other formulations. The advantage of the chromogenic substrate is that it requires no UV lamp to visualise the reaction, and it is concentrated within the colony, facilitating easier enumeration in the presence of other organisms, or when large numbers are present on the plate.

Formula	g/litre
Tryptone	20.0
Bile Salts No.3	1.5
X-glucuronide	0.075
Agar	15.0

Method for reconstitution

Weigh 36.5 grams of powder, disperse in 1 litre of deionised water and allow the mixture to soak for 10 minutes. Swirl to mix and sterilise at 121°C for 15 minutes. Cool to 47°C and pour in to Petri dishes. Dry the surface prior to inoculation.

Appearance: Straw, clear gel.

pH: 7.2 ± 0.2

Minimum QC organisms: *Escherichia coli* NCIMB 50034 (blue/green)
Enterobacter aerogenes NCIMB 50029 (cream)

Inoculation: Inoculate 0.5 ml of a 1:10 dilution of the sample and spread over the entire surface of the plate. Further dilution may be necessary if large numbers of *E. coli* are present, to ensure colonies can be easily counted.

Incubation: 30°C for 4 hours, followed by 18 hours at 44°C.

Interpretation: Count all blue/green colonies as presumptive *E. coli*, calculate the cfu/g in the original material. A simple indole test can be performed by placing one drop of Kovac's reagent onto a colony and if positive, a red halo will appear in the medium around the colony. If negative, then the halo will be white.

*96-97% of *E. coli* strains positive. A notable exception is *E. coli* 0157:H7.

References

Dibb, W.L. and Bottolfsen, K.L. (1984). Evaluation of Rosco Diagnostic ß-glucuronidase Tablets in the Identification of Urinary Isolates of *Escherichia coli*. Acta Path.Microbiol. Immunol. Scand. Sect. B 92 261-264.

Hansen, W. and Yourassowsky, E. (1984). Detection of ß-glucuronidase in Lactose Fermenting Members of the Family Enterobacteriaceae and its Presence in Bacterial Urine Cultures. J. Clin. Micro.20 (6) 1177-1179.

Robinson, B.J. (1984). Evaluation of a Fluorogenic Assay for Detection of *E. coli.* App & Env. Microbiol.48 (2) 285-288.

Perez, J.L., Berrocal, C.I. and Berrocal, L. (1986). Evaluation of a Commercial ß-glucuronidase Test for the Rapid and Economical Identification of *Escherichia coli*. J.App.Bacteriol. 61 541-545.

Raghubeer, E. and Matches, J.R. (1990). Temperature Range for Growth of *Escherichia coli* Serotype 0157:H7 and Selected Coliforms in *E. coli* Medium. J.Clin. Micro. 28 (4) 803-805.

Bolton, F.J. (1995) Personal Communication

LB Agar

HAL004

Refer to the Biomolecular Section

LB Top Agar

HAL005

Refer to the Biomolecular Section

Harlequin™ SMAC-BCIG

(Sorbitol MacConkey Agar with BCIG)

HAL006

Description

This is a specific substrate medium for the isolation of *Escherichia coli* O157:H7, the primary serovar associated with haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS). Pathogenicity of the organism is linked to the production of verocytotoxins (VT1 and VT2), but it should be noted that not all strains of O157 produce verocytotoxins, and that strains from other serovars can be toxin producers (e.g. O26, O103, O111, O113, O145). *E. coli* O157 has been associated epidemiologically with food poisoning outbreaks involving beef burgers and cold cooked meats.

This medium is a modification of Sorbitol MacConkey Agar (SMAC). The addition of the chromogenic substrate BCIG (5-bromo-4-chloro-3-indoxyl-β-D-glucuronide) improves the specificty of the medium. *E. coli* O157:H7 is typically sorbitol negative and β-glucuronidase negative producing pale translucent colonies on this medium. The majority of other *E. coli* strains are β-glucuronidase positive and sorbitol positive (pink/red colonies). A small percentage of *E. coli* are β-glucuronidase positive and sorbitol negative and thus appear as blue/green colonies on this medium. Consequently this medium can distinguish between non-O157 sorbitol negative *E. coli* and the genuine toxigenic *E. coli* O157:H7. This reduces the number of unnecessary confirmation tests that are performed. The medium can be made more selective by the addition of Cefixime Tellurite supplement X161 to prepare CT-SMAC. Most workers recommend the use of CT-supplemented medium alongside unsupplemented medium to ensure maximum isolation of *E. coli* O157. This medium can also be useful for the detection of other VTEC producing *E. coli* in conjunction with specifically targetted IMS particles (***Captivate™***).

Formula	g/litre
Peptone	20.0
Sorbitol	10.0
Bile Salts No. 3	1.5
Sodium Chloride	5.0
BCIG	0.1
Neutral Red	0.03
Crystal Violet	0.001
Agar	12.0

Method for reconstitution

Weigh 48.6 grams of powder and add to 1 litre of de-ionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C, add 2 vials of X161 CT supplement and pour plates. Dry the surface prior to inoculation.

Appearance: Pale red, light violet tinge.

pH: 7.1 ± 0.2

Minimum QC organisms:
Escherichia coli O157:H7 NCTC 12900 (non-toxigenic)
Escherichia coli NCIMB 50034
Enterococcus faecalis NCIMB 50030 (inhibition)

Inoculation: From O157 Broth LAB 165, surface streak for single colonies.

Incubation: 37°C aerobically for 18-24 hr. Examine plates for sorbitol negative, β-glucuronide negative colonies. Confirm as O157:H7 by serology, (commercial kits or antiserum available).

Growth Characteristics

organism	colony size (mm)	shape & surface	colour
E. coli O157:H7 sorbitol -ve β-glucuronide -ve	2.5 - 4.0	CV.E.G.	Translucent
E. coli sorbitol +ve β-glucuronide +ve	2.5 - 4.0	CV.E.G.	Pink/red or purple centre
E. coli sorbitol -ve β-glucuronide +ve	2.5 - 5.0	CV.E.G.	Green or translucent with green centre

Note: Sorbitol positive toxigenic *E. coli* O157:H7 have been isolated and appear as sorbitol positive and β-glucuronide positive on this medium. To ourknowledge these isolates are limited to a small geographical area in Germany.

References

1) Okrend, A.J.G., Rose, B.E., and Lattuada, C.P. (1990) Use of 5-Bromo-4-Chloro-3-Indoxyl-*β*-D-Glucuronide in MacConkey Sorbitol Agar to Aid in the Isolation of *Escherichia coli* O157:H7 from Ground Beef. J.Food Protection **53** (11) 941-943

Harlequin™ E. coli/Coliform Medium

HAL008

Description

This dual chromogenic substrate medium has been developed for the simultaneous enumeration of *Escherichia coli* and coliforms in food and environmental samples. The different colony types are simple to distinguish allowing rapid counting of both *E. coli* and coliforms on a single medium.

Based upon the formulation of Tryptone Bile Agar LAB072, the medium has been modified by the addition of two chromogenic substrates, one to detect the ß-glucuronidase enzyme (X-glucuronide) and another to detect the ß-galactosidase enzyme (magenta-ß-gal). Typical *E. coli* strains possess both enzymes but only cleave the X-glucuronide substrate, thereby producing blue-green colonies. Typical coliforms, however, possess only the ß-galactosidase enzyme and produce rose-pink colonies.

The colony types are easily distinguishable, even in the presence of other organisms, or when large numbers are observed, making simultaneous enumeration of *E. coli* and coliforms a quick and simple procedure.

N.B. This product is not available for sale in the USA.

Formula	g/litre
Tryptone	20.0
Bile Salts No.3	1.5
X-glucuronide	0.075
Magenta-β-galactoside	0.1
Agar	15.0

Method for reconstitution

Weigh 36.6 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C and mix well before dispensing into Petri dishes. Dry the agar surface prior to use.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, straw gel.

pH: 7.2 ± 0.2

Minimum Q.C. organisms:
Escherichia coli ATCC 25922
Enterobacter aerogenes ATCC 13048
Staphylococcus aureus ATCC 25923 (Inhibition)

Storage of Prepared Medium: Plates - up to 7 days at 2 - 8°C in the dark.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
Escherichia coli	1.0 - 2.0	CV.E.G.	Blue-Green	
Enterobacter aerogenes	1.5 - 2.5	CV.E.G.	Rose-Pink	
Pseudomonas aeruginosa	0.5 - 1.0	F.CR.D/ CV.E.G.	Buff	
Enterococcus faecalis				No growth
Staphylococcus aureus				No growth

Inoculation: Inoculate 0.5 ml of a 1:10 dilution of the sample and spread over the entire surface of the plate. Further dilution may be necessary if large numbers of *E. coli* and/or coliforms are present, to ensure colonies can be easily counted.

Incubation: 18 - 24 hours at 37° C

Interpretation: Count all blue-green colonies as presumptive *E. coli*, and calculate the cfu/g. Count all rose-pink colonies as presumptive coliforms, and calculate cfu/g.

References

1) Baylis, C.L., Patrick, M. (1999). Comparison of a range of Chromogenic media for enumeration of total Coliforms and *Escherichia coli* in foods. Leatherhead International Technical Notes. No.135: 99.

Harlequin™ mLGA

(Membrane Lactose Glucuronide Agar)

HAL009

Description

Traditionally, membrane Lauryl Sulphate Broth (mLSB) has been used as the standard media for isolating coliforms (including *E. coli*) from water potentially contaminated with sewage. Harlequin™ membrane Lactose Glucuronide Agar (mLGA) is a modification of mLSB aimed at reducing costs by reducing the number of filters used per test sample and aiding in the recovery and identification of coliforms and *E. coli*. The medium has been modified from the mLSB formulation by the incorporation of X-glucuronide, sodium pyruvate and agar. X-glucuronide is incorporated to allow for the presumptive isolation of *E. coli*, sodium pyruvate aids the recovery of chlorine stressed organisms and agar is incorporated to remove the need for absorbent pads. This medium is recommended for the enumeration of coliform bacteria and *E. coli* by a single membrane filtration technique in The Microbiology of Drinking Water 2002 (previously Report 71).

Formula	g/litre
Peptone	39.0
Yeast Extract	6.0
Lactose	30.0
Phenol Red	0.2
Sodium Lauryl Sulphate	1.0
Sodium Pyruvate	0.5
X-Glucuronide	0.2
Agar	10.0

Method for reconstitution

Weigh 87.0 grams of powder, disperse in 1 litre of deionised water and allow the mixture to soak for 10 minutes. Swirl to mix and sterilise at 121°C for 15 minutes. Cool to 47°C and pour into sterile Petri dishes.

Appearance: Red, clear gel.

pH: 7.4 ± 0.2

Minimum Q.C. organisms:
Escherichia coli NCIMB 50034
Enterobacter aerogenes NCIMB 50029
Staphylococcus aureus NCIMB 50080 (inhibition)

Storage of Prepared Medium: Plates - up to 7 days at 2-8°C in the dark.

Inoculation: *E. coli* and coliform counts can be performed on the same sample of water. The volume and dilution of test sample should be chosen so as the number of colonies on the membrane lies between 20 and 80. With waters expected to contain low numbers of coliforms, a sample of 100ml should be filtered. For full methodology refer to The Microbiology of Drinking Water 2002 section 4 B - The enumeration of coliform bacteria and *E. coli* by a single membrane filtration technique.

Incubation: 4 hours at 30 °C followed by 14 hours at 37 °C

Interpretation: Count all green-blue colonies as presumptive *E. coli*, and all green-blue and yellow colonies as presumptive coliforms.

Growth Characteristics

organism	colony size (mm)	shape & surface	colour	other
*Escherichia coli**	0.5 - 1.5	CV.E.G.	Green	Yellow if glucuronidase -ve
Lactose fermenters	0.5 - 1.5	CV.E.G.	Yellow	
Non-lactose fermenters	0.5 - 1.5	CV.E.G.	Red	
Staphylococcus aureus				No growth (suppressed)

*96-97% of E. coli strains positive. A notable exception is E. coli O157:H7

References

Sartory, D.P. & Howard, L. (1992). A medium detecting B-glucuronidase for the simultaneous membrane filtration enumeration of *Escherichia coli* and coliforms from drinking water. *Letters in Applied Microbiology* **15**, 273-276.

Calabrese, J.P. & Bisssonette, G.K. (1990). Improved membrane filtration method incorporating catalase and sodium pyruvate for detection of chlorine stressed coliform bacteria. *Applied and Environmental Microbiology* **56**, 3558-3564.

Microbiology of Drinking Water 2002 section 4 B - Environment Agency. The enumeration of coliform bacteria and *E. coli* by a single membrane filtration technique.

Harlequin™ Listeria Chromogenic Agar (ISO)

HAL010

Description

Listeria Chromogenic Agar (according to the formulation of Ottaviani and Agosti) is a selective medium for the isolation and presumptive identification of *Listeria monocytogenes* from foodstuffs and related materials as described in ISO 11290-1:1997.

Lithium chloride in the base medium and supplementary antimicrobial compounds Ceftazidime, Polymyxin, Nalidixic acid and Cycloheximide provide the medium's selectivity. Chromogenic activity is as a result of a chromogenic substrate for the detection of the β-glucosidase enzyme, common to all *Listeria* spp. and to a few strains of Enterococci and Bacilli.

The specific differential activity of this agar is obtained with a proprietary lecithin substrate for the detection of the phospholipase enzyme that will only be present in the *L. monocytogenes* colonies growing on this media. This enzyme activity will result in a halo of precipitation surrounding the target colonies.

With the combination of both the chromogenic and phospholipase enzyme reactions, it is possible to differentiate *Listeria monocytogenes* (blue colonies surrounded by an opaque halo) from other *Listeria spp* (blue colonies without an opaque halo).

Formula	g/litre
Meat Peptone	18.0
Tryptone	6.0
Yeast extract	10.0
Lithium chloride	10.0
Sodium chloride	5.0
Disodium hydrogen orthophosphate anhydrous	2.5
Sodium pyruvate	2.0
Glucose	2.0
Glycerophosphate	1.0
Magnesium sulphate	0.5
5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside	0.05
Agar	13.5

Method for reconstitution

Weigh 70.5 grams of powder and disperse in 950mL of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 48-50°C, and add 2 vials of reconstituted X072 supplement. Swirl to mix. Add 2 vials of X010 supplement (**pre-heated to 48-50°C**). Mix well with gentle end-over-end mixing and dispense into Petri dishes. Dry the agar surface prior to use.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: opaque, cream-yellow gel

pH: 7.4 ± 0.2

Minimum Q.C. organisms:
Listeria monocytogenes NCTC 11994
Listeria monocytogenes NCTC 10527
Escherichia coli ATCC 25922 (Inhibited)
Enterococcus faecalis ATCC 29212 (Inhibited)

Storage:

Dehydrated culture media: 10-25°C

Final medium: 7 days at 2-8°C in the dark

Inoculation: Surface inoculation - streak out to single colonies. This medium is highly selective and a heavy inoculum can be used.

Incubation: 37°C aerobically for 48 hours.

Interpretation

organism	colony size (mm)	shape & surface	colour
Listeria monocytogenes	1 - 2	Round, Regular	Blue to blue-green, surrounded by opaque halo
Listeria spp.	1 - 2	Round, Regular	Blue to blue-green, without opaque halo

Isolates presumptively identified as *Listeria* spp. and *Listeria monocytogenes* must be subjected to further biochemical tests to confirm their identity. Some strains of *Listeria ivanovii* may demonstrate lecithinase activity.

References

ISO 11290-1:1997 Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Listeria monocytogenes* - Part 1: Detection method. Incorporating Amendment 1.

ISO/TS 11133-2:2003. Microbiology of food and animal feed stuffs-Guidelines on preparation and production of culture media – Part 2: Practical guidelines on performance testing of culture media.

Harlequin™ CSIM (ISO)

Harlequin™ Cronobacter sakazakii Isolation Medium (ISO)

HAL012

Description

Cronobacter sakazakii (formerly *Enterobacter sakazakii*) is a member of the *Enterobacteriaceae* family and has been associated with serious outbreak infections in neonates (premature infants) which have been fed on infant formula milk. Although rarely causing infections in immunocompetent adults, *C. sakazakii* has been implicated in sepsis, meningitis and necrotising enterocolitis with a high death rate in neonates. This opportunistic pathogen is common in the environment and its ability to survive desiccation presents a significant risk for post pasteurisation contamination and survival in spray dried milk products.

C. sakazakii appears to constitutively express high levels of α-glucosidase. This enzyme hydrolyses the chromogenic substrate 5-bromo-4-chloro-3-indolyl-α-D-glucopyranoside present in the medium, producing green to blue-green coloured colonies. Other *Enterobacteriaceae* such as *E. coli* do not express strong α-glucosidase activity and appear colourless or purple due to the uptake of crystal violet.

The combination of sodium desoxycholate, crystal violet and elevated incubation temperature produce a very selective and specific medium. Non-*Enterobacteriaceae* may appear colourless or violet coloured (due to their inability to hydrolyse the chromogenic substrate) or are inhibited by the selective components and incubation temperature.
This media formulation is currently recommended as part of the isolation protocol under ISO/TS 22964:2006(E) for the isolation of *Enterobacter sakazakii* from milk and milk products.

Formula	g/litre
Pancreatic peptone of casein	7.0
Yeast extract	3.0
Sodium chloride	5.0
Sodium desoxycholate	0.6
5-bromo-4-chloro-3-indolyl-α-D-Glucoside	0.15
Crystal violet	0.002
Agar	14.0

Method for reconstitution

Weigh 29.75 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C and mix well before dispensing into Petri dishes. Dry the agar surface prior to use.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, purple gel

pH: 7.0 ± 0.2

Minimum Q.C. organisms:
Cronobacter sakazakii ATCC 12868
Enterobacter aerogenes ATCC 13048
Bacillus cereus ACTC 11778
Staphylococcus aureus ATCC 25923

Hazard classification: NR – Not regulated

Storage:

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

Inoculation: Following selective enrichment in Modified Lauryl Sulphate Tryptose Broth Vancomycin Medium, streak onto HAL012 Harlequin™ *Cronobacter sakazakii* Isolation Medium (ISO).

Incubation: Incubate at 44°C + 0.5°C for 24 hours + 2 hours.

Interpretation: After incubation the plate should be assessed for typical colonies of *C. sakazakii*. Typical colonies are 1-3mm and are green to blue-green.

References

Bowen AB, Braden CR (2006). "Invasive *Enterobacter sakazakii* disease in infants". *Emerging Infect Dis* **12** (8): 1185–9.

Caubilla-Barron J & Forsythe S (2007). "Dry stress and survival time of *Enterobacter sakazakii* and other Enterobacteriaceae in dehydrated infant formula". *Journal Food Protection* **13**: 467-472.

"*Enterobacter sakazakii* infections associated with the use of powdered infant formula--Tennessee, 2001" (2002). *MMWR Morb Mortal Wkly Rep* **51** (14): 297–300.

Farmer JJ III, Asbury MA, Hickman FW, Brenner DJ, the Enterobacteriaceae Study Group (USA) (1980). "*Enterobacter sakazakii*: a new species of "Enterobacteriaceae" isolated from clinical specimens". *Int J Syst Bacteriol* **30**: 569–84.

ISO/TS 22964:2006(E) Milk and milk products – Detection of *Enterobacter sakazakii*.

Iversen C, Lehner A, Mullane N, *et al* (2007). "The taxonomy of *Enterobacter sakazakii*: proposal of a new genus *Cronobacter* gen. nov. and descriptions of *Cronobacter sakazakii* comb. nov. *Cronobacter sakazakii* subsp. *sakazakii*, comb. nov., *Cronobacter sakazakii* subsp. *malonaticus* subsp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov. and *Cronobacter genomospecies* 1". BMC Evol Biol **7**: 64.

Iversen C, Mullane N, Barbara McCardell, *et al* (2008). "Cronobacter gen. nov., a new genus to accommodate the biogroups of *Enterobacter sakazakii*, and proposal of *Cronobacter sakazakii* gen. nov. comb. nov., *C. malonaticus* sp. nov., *C. turicensis* sp. nov., *C. muytjensii* sp. nov., *C. dublinensis* sp. nov., *Cronobacter genomospecies* 1, and of three subspecies, *C. dublinensis* sp. nov. subsp. *dublinensis* subsp. nov., *C. dublinensis* sp. nov. subsp. *lausannensis* subsp. nov., and *C. dublinensis* sp. nov. subsp. *lactaridi* subsp. nov.". IJSEM.

Lai KK (2001). "*Enterobacter sakazakii* infections among neonates, infants, children, and adults. Case reports and a review of the literature". *Medicine (Baltimore)* **80** (2): 113–22.

Harlequin™ Cronobacter sakazakii Agar – DFI Formulation

(Druggan, Forsythe & Iversen)

HAL013

Description

Cronobacter sakazakii (formerly *Enterobacter sakazakii*) is a member of the *Enterobacteriaceae* family and has been associated with serious outbreak infections in neonates (premature infants) which have been fed on infant formula milk. Although rarely causing infections in immunocompetent adults, *C. sakazakii* has been implicated in sepsis, meningitis and necrotising enterocolitis with a high death rate in neonates. This opportunistic pathogen is common in the environment and its ability to survive desiccation presents a significant risk for post pasteurisation contamination and survival in spray dried milk products.

Based on the formulation described by Druggan, Forsythe and Iversen, Harlequin™ *Cronobacter sakazakii* Agar is a medium on which *Cronobacter sakazakii* appears to constitutively express high levels of α-glucosidase. This enzyme hydrolyses the chromogenic substrate 5-bromo-4-chloro-3-indolyl-α-D-glucopyranoside present in the medium, producing green coloured colonies. Other *Enterobacteriaceae* such as *E. coli* do not express strong α-glucosidase activity and appear colourless. Hydrogen sulphide producing organisms, such as Salmonella, and *Proteus* spp. appear grey, brown or black on this formulation due to the production of precipitated ferrous sulphate, which results from the hydrogen sulphide produced by these organisms interacting with ferric ions in the medium. This reaction prevents the weakly α-glucosidase positive *Proteus vulgaris* from appearing as green on the medium.

Selectivity is achieved from the inclusion of sodium desoxycholate which serves to inhibit the growth of most Gram-positive organisms.

Formula	g/litre
Tryptone	15.0
Soya peptone	5.0
Sodium chloride	5.0
Ferric ammonium citrate	1.0
Sodium desoxycholate	1.0
Sodium thiosulphate	1.0
X-α-D-glucopyranoside	0.1
Agar	15.0

Method for reconstitution

Weigh 43.1 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C and mix well before dispensing into Petri dishes. Dry the agar surface prior to use.

Appearance:

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, straw-coloured gel

pH: 7.3 ± 0.2

Hazard classification: NR – Not regulated

Storage:

Dehydrated culture media: 10-25°C

Final medium: 7 days at 2-8°C in the dark

Incubation: Incubate plates at 37+1°C for 24 hours.

Interpretation: *Cronobacter sakazakii* appear as green or pale green with a green 'bullseye' centre and 1-2.5mm in size. Other organisms generally appear black (if hydrogen sulphide producers) or colourless.

References

Bowen AB, Braden CR (2006). "Invasive Enterobacter sakazakii disease in infants". *Emerging Infect Dis* **12** (8): 1185–9.

Caubilla-Barron J & Forsythe S (2007). "Dry stress and survival time of *Enterobacter sakazakii* and other *Enterobacteriaceae* in dehydrated infant formula". *Journal Food Protection* **13**: 467-472.

"Enterobacter sakazakii infections associated with the use of powdered infant formula--Tennessee, 2001" (2002). *MMWR Morb Mortal Wkly Rep* **51** (14): 297–300.

Farmer JJ III, Asbury MA, Hickman FW, Brenner DJ, the Enterobacteriaceae Study Group (USA) (1980). "*Enterobacter sakazakii*: a new species of "*Enterobacteriaceae*" isolated from clinical specimens". *Int J Syst Bacteriol* **30**: 569–84.

Iversen C, Druggan P & Forsythe S (2004). "A selective differential medium for *Enterobacter sakazakii*, a preliminary study". *International Journal of Food Microbiology* **96** (2): 133-139.

Iversen C, Lehner A, Mullane N, et al (2007). "The taxonomy of *Enterobacter sakazakii*: proposal of a new genus *Cronobacter* gen. nov. and descriptions of *Cronobacter sakazakii* comb. nov. *Cronobacter sakazakii* subsp. *sakazakii*, comb. nov., *Cronobacter sakazakii* subsp. *malonaticus* subsp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov. and *Cronobacter genomospecies* 1". BMC Evol Biol 7: 64.

Iversen C, Mullane N, Barbara McCardell, *et al* (2008). "Cronobacter gen. nov., a new genus to accommodate the biogroups of Enterobacter sakazakii, and proposal of Cronobacter sakazakii gen. nov. comb. nov., C. malonaticus sp. nov., C. turicensis sp. nov., C. muytjensii sp. nov., C. dublinensis sp. nov., Cronobacter genomospecies 1, and of three subspecies, C. dublinensis sp. nov. subsp. dublinensis subsp. nov., C. dublinensis sp. nov. subsp. lausannensis subsp. nov., and C. dublinensis sp. nov. subsp. lactaridi subsp. nov.". *IJSEM*.

Lai KK (2001). "*Enterobacter sakazakii* infections among neonates, infants, children, and adults. Case reports and a review of the literature". *Medicine (Baltimore)* **80** (2): 113–22.

3. Biomolecular Products

LB Agar	**YPD Agar**
LB Broth	**YPD Broth**
Harlequin™ LB agar*	**2 x YT Agar**
Harlequin™ LB Top*	**2 x YT Broth**
LB Agar (Lennox)	**NZY Broth**
LB Broth (Lennox)	**NZCYM Broth**
Terrific Broth	**Luria Bertani Agar (Hi-Salt)**

*Available from Lab M in the UK only. Outside the UK Harlequin LB agar is available from Sigma-Aldrich as S-Gal LB agar (C4478).

Lab M's Biomolecular products form the basis of gene reporter assays that employ enzyme substrates, such as X-gal and our patented CHE-gal, to indicate inactivation of α-complementation. These products are formulated to promote the growth of the recipient and donor cells used in DNA insertion technology. More importantly, they are formulated to provide optimum conditions for plasmid retention or bacteriophage reproduction and survival.

As different applications have varied requirements of the culture medium used, Lab M offer a range of media types. Some are of standard formulation whilst others are modified to enhance the performance of specific applications. This variety allows the researcher to choose the appropriate medium for the application being used.

Lab M have formulated unique versions of LB Agar and LB Top Agar which incorporate the patented water soluble chromogen CHE-β-gal into the complete medium. This improves colour definition of α-complemented colonies compared to the standard X-β-gal plate and removes the need for hazardous chemicals in the preparation of the medium. Therefore we have produced a safe, fast and easy way to differentiate between $lac^+$and lac^- colonies. Simply add water to the powder and autoclave. All products are available directly from Lab M in the UK.

Harlequin™ LB Agar

HAL004

Description
A nutritious molecular biology medium containing the novel chromogen CHE-galactoside to enable rapid, safe and unambiguous detection of plasmid transformed bacteria. The CHE-galactoside replaces the traditional X-gal substrate, simplifying the technique as there is no preparation of stock solutions in dimethyl formamide or dimethyl sulphoxide and surface application of the chromogen to the medium. The intense black colour of the colonies gives a sharper contrast between lac and lac colonies, giving improved colony detection compared to blue X-gal stained colonies.

Formula	g/litre
Tryptone	10.0
Yeast Extract	5.0
Sodium chloride	10.0
CHE-galactoside	0.3
IPTG	0.03
Ferric ammonium citrate	0.5
Agar	12.0

Method for reconstitution
Weigh 37.8 grams of powder and disperse in 1 litre of deionised water. Swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and add appropriate filter sterilised antibiotic if required. Pour into sterile Petri dishes, allow the medium to set and dry the surface prior to inoculation.

Appearance: Straw, clear gel.

pH: 7.0 ± 0.2

Minimum QC organisms - ß-gal reaction
Escherichia coli DH5a (ATCC®53868) Lac Z+ve (black)
Escherichia coli DH5a, Lac Z -ve (remains cream)

Storage of Prepared Medium: Plates – up to 7 days at 2-8°C in the dark.

Inoculation: Typically surface spread over plate to detect cream colonies indicating disruption of β-complementation. Alternatively, spread for single colonies if required.

Incubation: 37°C aerobically, for 16-18 hours. The colour of the colonies will substantially increase with prolonged incubation (up to 24 hours).

Interpretation: Examine for the presence of cream colonies, which indicates a successful insertion of the target DNA.

References
Miller, J.H. (1972). Experiments in Molecular Genetics. Cold Spring Harbour Laboratory. Cold Spring Harbour New ,York.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbour Laboratory. Cold Spring Harbour New, York.

Harlequin™ LB Top Agar

HAL005

Description
A nutritious molecular biology medium containing the novel chromogen CHE-galactoside to enable rapid, safe and unambiguous detection of phage transformed bacteria.

Formula	g/litre
Tryptone	10.0
Yeast Extract	5.0
Sodium chloride	10.0
CHE-galactoside	1.0
IPTG	0.03
Ferric ammonium citrate	2.0
Agar	4.0

Method for reconstitution
Weigh 32 grams of powder and disperse in 1 litre of deionised water. Swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C before seeding the medium with required strains.

Appearance: Straw, clear gel.

pH: 7.0 ± 0.2

Storage of Prepared Medium: Storage of capped medium in bottles for up to 1 month at room temperature in the dark.

References
Miller, J.H. (1972). Experiments in Molecular Genetics. Cold Spring Harbour Laboratory. Cold Spring Harbour New,York.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbour Laboratory. Cold Spring Harbour New, York.

LB Agar

LAB168

Description

A nutritious medium designed for rapid bacterial growth, typically used in molecular biology procedures e.g. in the detection of phage or plasmid transformed bacteria and the maintenance of recombinant strains. This agar contains the required concentration of sodium chloride to promote replication of plasmids.

Formula	g/litre
Tryptone	10.0
Yeast Extract	5.0
Sodium chloride	10.0
Agar	15.0

Method for reconstitution

Weigh 40.0 grams of powder and disperse in 1 litre of deionised water. Swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and add filter sterilised antibiotic if required. Pour into sterile Petri dishes and allow the medium to set. Dry the surface prior to inoculation.

Addition of Substrate

Prepare the X-Gal solution by dissolving in DMF, to give a concentration of 20mg/ml Once dissolved, spread 40µl as a surface layer over the top of the agar and allow to dry. Also spread 4µl of a solution of IPTG (200mg/ml). Alternatively, use Harlequin™ LB agar complete (HAL004), which already contains the enzyme substrate and inducer. This eliminates the potentially hazardous use of DMF and prevents variation in the colour of ß-complemented colonies due to differences in substrate concentration.

Appearance: Straw, clear gel.

pH: 7.0 ± 0.2

Minimum QC organisms - ß-gal reaction:
Escherichia coli DH5a (ATCC®53868)
Lac Z+ve (black if CHE-gal is present in the medium)
Escherichia coli DH5a, Lac Z -ve (remains cream even in the presence of CHE-gal)

Storage of Prepared Medium: Plates – up to 7 days at 2-8°C in the dark.

Inoculation: Typically surface spread over plate to detect cream colonies indicating disruption of ß-complementation. Alternatively, spread for single colonies if required.

Incubation: 37°C aerobically, for 16-18 hours. If a chromogenic substrate is used, the colour of the colonies will substantially increase with prolonged incubation (up to 24 hours).

Interpretation: Using the base medium alone, all colonies will appear cream. Alternatively, if a chromogen is included, examine for the presence of cream colonies, which indicates a successful insertion of the target DNA.

References

Miller, J.H. (1972). Experiments in Molecular Genetics. Cold Spring Harbour Laboratory. Cold Spring Harbour New ,York.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbour Laboratory. Cold Spring Harbour New, York.

LB Agar (Lennox)

LAB174

Description

This is a nutritionally rich medium containing half the sodium chloride level of LB agar (LAB168). This allows the researcher to select the optimum salt concentration for his experiment. This medium can also be used for plasmid replication experiments. Nutritionally rich media are required for molecular biology applications as the strains used are often derived from *Escherichia coli* K12, which is deficient in B vitamin production.

Formula	g/litre
Tryptone	10.0
Yeast Extract	5.0
Sodium chloride	5.0
Agar	15.0

Method for reconstitution

Weigh 35.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and add filter sterilised antibiotic as required. Pour into sterile Petri dishes and allow the medium to set. Dry the surface prior to inoculation.

Addition of Substrate

Prepare the X-Gal solution by dissolving in DMF, to give a concentration of 20mg/ml. Once dissolved, spread 40µl as a surface layer over the top of the agar and allow to dry. Also spread 4µl of a solution of IPTG (200mg/ml).

Appearance: Straw, clear gel.

pH: 7.0 ± 0.2

Minimum QC organisms - ß-gal reaction:
Escherichia coli DH5a (ATCC®53868)
Lac Z+ve (black if CHE-gal is present in the medium)
Escherichia coli DH5a, Lac Z -ve (remains cream even in the present of CHE-gal)

Storage of Prepared Medium: Plates – up to 7 days at 2-8°C in the dark.

Inoculation: Surface; either spread over entire surface for colony count or streaking for single colonies.

Incubation: 37°C aerobically for 16-18 hours.

Interpretation: Using the base medium alone, all colonies will appear cream. Alternatively, if a chromogen is included, examine for the presence of cream colonies, which indicates a successful insertion of the target DNA.

References

Lennox, E.S. (1955). Transduction of linked genetic characters of the host by bacteriophage P1. Virology 1, 190.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman. J.A., Smith, J.G. and Struhl. (1994). Current protocols in molecular biology. Vol. 1. Current protocols, New York. N.Y.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbour Laboratory. Cold Spring Harbour New, York.

LB Broth

LAB169

Description

A nutrient broth primarily used for the growth and maintenance of *Escherichia coli*. Used as the primary propagation step for donor or recipient cells, when further work is to be performed on LB Agar. This broth contains a high level of sodium chloride to aid the maintenance of plasmids. If working with temperate bacteriophages, such as lambda, the addition of magnesium sulphate ($MgSO_4.7H_2O$) at 2 grams per litre is recommended to promote phage absorption.

Formula	g/litre
Tryptone	10.0
Yeast Extract	5.0
Sodium chloride	10.0

Method for reconstitution

Weigh 25.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 15 minutes.

Appearance: Straw, clear liquid.

pH: 7.0 ± 0.2

Minimum QC organisms: *Escherichia coli* DH5 (ATCC 53868)

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20°C in the dark.

Inoculation: As per normal techniques, using a pure culture of donor/recipient cells.

Incubation: 37°C aerobically for 16-18 hours.

Interpretation: Examine all tubes for turbidity, indicating growth.

References

Miller, J.H. (1972). Experiments in Molecular Genetics. Cold Spring Harbour Laboratory. Cold Spring Harbour New ,York.

Sambrook, J., Fritsch, E.F. and Maniatis. T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbour Laboratory. Cold Spring Harbour New, York.

LB Broth (Lennox)

LAB173

Description

This is a nutrient broth containing half the sodium chloride level of LB Broth (LAB169), this allows for the addition of calcium chloride, required in some applications for efficient phage adsorption to the cell e.g. phage P1. This medium can also be used for plasmid replication experiments. Chloramphenicol can be added to achieve high plasmid copy number by inhibiting chromosomal replication.

Formula	g/litre
Tryptone	10.0
Yeast Extract	5.0
Sodium chloride	5.0

Method for reconstitution

Weigh 20.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 15 minutes.

Appearance: Straw, clear liquid.

pH: 7.5 ± 0.2

Minimum QC organisms: *Escherichia coli* DH5 (ATCC 53868)

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20°C in the dark.

Inoculation: As per normal techniques, using a pure culture of donor/recipient cells.

Incubation: 37°C aerobically for 16-18 hours.

Interpretation: Examine all tubes for turbidity, indicating growth.

References

Lennox, E.S. (1955). Transduction of linked genetic characters of the host by bacteriophage P1. Virology 1, 190.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman. J.A., Smith, J.G. and Struhl. (1994). Current protocols in molecular biology. Vol. 1. Current protocols, New York. N.Y.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbour Laboratory. Cold Spring Harbour New, York.

Luria Bertani (Hi-Salt) Broth

LAB191

Description

A nutritious medium designed for rapid bacterial growth, typically used in the detection of phage or plasmid transformed bacteria. This broth is formulated to LB Broth (LAB169), but has a higher pH for different applications.

Formula	g/litre
Tryptone	10.0
Yeast Extract	5.0
Sodium chloride	10.0

Method for reconstitution

Weigh 25.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and dispense into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Appearance: Straw, clear gel.

pH: 7.5 ± 0.2

Minimum QC organisms: *Escherichia coli* DH5 (ATCC 53868)

Storage of Prepared Medium: Plates up to 7 days at 2-8°C in the dark.

Inoculation: Dependent upon application.

Incubation: 37°C aerobically.

NZCYM Broth

LAB182

Description

This is an improved medium for increased yields of the phage Lambda. This formulation includes a higher concentration of essential elements for increased bacterial growth. To encourage multi phage insertion into the host cell, it is recommended to add 0.2% maltose (prepare a 20% solution and add 1ml per 100ml of medium), which promotes expression of *LamB* (lambda receptor). If maltose is added, do not use this medium to create phage stocks, as binding of phage particles to membrane fragments will occur because of increased *LamB* density.

Formula	g/litre
Enzymatic casein digest	10.0
Acid hydrolysed casein	1.0
Yeast extract	5.0
Magnesium Sulphate	2.0
Sodium chloride	5.0

Method for reconstitution

Weigh 23.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and dispense into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Appearance: Straw, clear liquid.

pH: 7.0 ± 0.2

Minimum QC organisms: *Escherichia coli* DH5 (ATCC 53868)

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20°C in the dark.

Inoculation: Mix a fresh overnight culture of host cells with bacteriophage and use to inoculate NZCYM Broth.

Incubation: 37°C aerobically.

References

Blattner, F., *et al.*, (1977) Science 196, 161.

NZY Broth (NZYM)

LAB181

Description

Designed for increased replication of phage Lambda. To encourage multi phage insertion into the host cell, it is recommended that 0.2% maltose be added (prepare a 20% solution and add 1ml per 100ml of medium), which promotes expression of *LamB* (lambda receptor). If maltose is added, the medium should not be used to create phage stocks, as binding of phage particles to membrane fragments will occur because of increased *LamB* density.

Formula	g/litre
Enzymatic casein digest	10.0
Yeast extract	5.0
Magnesium Sulphate	2.0
Sodium chloride	5.0

Method for reconstitution

Weigh 22.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and dispense into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Appearance: Straw, clear liquid.

pH: 7.0 ± 0.2

Minimum QC organisms: *Escherichia coli* DH5 (ATCC 53868)

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20°C in the dark.

Inoculation: Mix a fresh overnight culture of host cells with bacteriophage and use to inoculate NZY Broth.

Incubation: 37°C aerobically for 16-18 hours.

References

Blattner, F., *et al.*, (1977) Science 196, 161.

Terrific Broth

LAB183

Description

A nutritious medium that will support high bacterial cell densities, usually resulting in increased yields of DNA and recombinant proteins. The formulation requires the addition of glycerol to complete the formulation.

Formula	g/litre
Tryptone	12.0
Yeast extract	24.0
Di Potassium phosphate	9.4
Potassium di phosphate	2.2
Glycerol (added after autoclaving).	4.0/8.0 ml

Method for reconstitution

Weigh 47.6 grams of powder and disperse in 1 litre of deionised water. Add 4.0 or 8.0 ml of glycerol, swirl to mix and dispense into final containers. Allow the mixture to soak for 10 minutes, swirl to dissolve and sterilise by autoclaving at 121°C for 15 minutes.

Appearance: Straw, clear liquid.

pH: 7.0 ± 0.2

Minimum QC organisms: *Escherichia coli* DH5 (ATCC 53868)

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20°C in the dark.

Inoculation: Inoculate with a pure culture of the host strain containing the required recombinant plasmid.

Incubation: 37°C aerobically.

References

Tartoff, C.D. and Hobbs, C.A. (1987). Improved media for growing plasmid and cosmid clones. Bethesda Res. Lab. Focus, 9:205.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbour Laboratory. Cold Spring Harbour New, York.

YPD Agar

LAB176

Description
A nutritious medium used as an alternative to YPD Broth, where a solid base is required. Glucose is included to promote rapid growth.

Formula	g/litre
Tryptone	20.0
Yeast extract	10.0
Glucose	20.0
Agar	17.0

Method for reconstitution
Weigh 67.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and add filter sterilised antibiotic as required. Pour into sterile Petri dishes and allow to set. Dry the surface prior to inoculation.

Appearance: Dark straw, clear liquid.

pH: 6.5 ± 0.2

Minimum QC organisms: *Saccharomyces cerevisiae*

Inoculation: Surface; either spread over entire surface for colony count or streaking for single colonies.

Incubation: 37°C aerobically for 16-18 hours.

Interpretation: Yeasts will grow as cream colonies, size dependent upon inoculum density.

YPD Broth

LAB175

Description
A nutritious broth base recommended for the maintenance and propagation of yeasts widely used in gene insertion techniques. Glucose is included to promote rapid growth.

Formula	g/litre
Tryptone	20.0
Yeast extract	10.0
Glucose	20.0

Method for reconstitution
Weigh 50.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and dispense into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Appearance: Dark straw, clear liquid.

pH: 6.5 ± 0.2

Minimum QC organisms: *Saccharomyces cerevisiae*

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20°C in the dark.

Inoculation: As per normal techniques, using a pure culture of strain to be cultivated.

Incubation: 37°C aerobically for 16-18 hours.

Interpretation: Examine all tubes for turbidity, indicating growth.

2xYT Agar

LAB180

Description
An agar version of 2xYT broth, for the growth of host cells of filamentous single stranded bacteriophages e.g. the M13 phage.

Formula	g/litre
Tryptone	16.0
Yeast extract	10.0
Sodium chloride	5.0
Agar	15.0

Method for reconstitution
Weigh 46.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C, pour into sterile Petri dishes and allow the medium to set. Dry the surface prior to inoculation.

Appearance: Straw, clear gel.

pH: 7.0 ± 0.2

Minimum Q.C. organisms: *Escherichia coli* DH5 (ATCC®53868*)*.

Storage of Prepared Medium: Plates – up to 7 days at 2-8°C in the dark.

Inoculation: dependent upon application.

Incubation: 37˚C aerobically.

2xYT Broth

LAB179

Description
A nutritious liquid medium formulated to promote the growth of host cells, thereby encouraging increased replication and yield from filamentous single stranded bacteriophages (such as the M13 phage).

Formula	g/litre
Tryptone	16.0
Yeast extract	10.0
Sodium chloride	5.0

Method for reconstitution
Weigh 31.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 15 minutes.

Appearance: Straw, clear liquid.

pH: 7.0 ± 0.2

Minimum Q.C. organisms: *Escherichia coli* DH5 (ATCC 53868).

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20°C in the dark.

Inoculation: Mix fresh overnight culture of host cells with bacteriophage and use to inoculate 2xYTBroth.

Incubation: 37°C aerobically, typically for 4-5 hours to reduce risk of selecting deletion mutants.

Interpretation: Concentrate bacterial cells by centrifugation and transfer supernatant containing bacteriophage to a fresh tube, being careful not to disturb the pellet formed. The resulting bacteriophage stock can be stored at +4°C or -20°C.

4. Captivate™ Immunomagnetic Separation

Captivate™ is a range of antibody coated paramagnetic particles for the specific **I**mmuno**M**agnetic **S**eparation **(IMS)** of microorganisms.

This patented technology consists of microscopic paramagnetic particles. The beads have a magnetite core and a "ceramic" zirconium oxide coating. The beads are manufactured by a high speed blending process and typically cover a size diameter range of 1-4 µm, with a 2.5µm average size.

Purified antibodies to surface components of the target microorganism are covalently coupled to the bead. With careful antibody selection, a highly specific separation system for microorganisms is produced.

The pre-coated beads are designed for the IMS of target bacteria from enrichment cultures. A sample is taken from a filter stomacher bag and incubated with the ***Captivate***™ beads for 30 minutes. The bead/microorganism complexes are then removed from the sample by placing the sample in a magnetic concentrator device. This separates them from the background organisms and interfering materials. The complexes are then washed using a PBS/Tween®20 wash buffer to remove non-specifically bound material. The beads can then be plated out onto the appropriate selective agar media and incubated as described.

The IMS technique will increase the sensitivity of the methodology and, in most circumstances, results can be achieved 24 hours earlier than standard protocols.

These products can also serve as a capture system for rapid detection systems.

Special Notes on IMS Techniques.

There are important factors that affect the performance of **IMS** techniques. Thorough mixing of the particles and sample allied with efficient recovery of the beads from the sample matrix is paramount to the success of this technique. Care must be taken not to aspirate the sample vigorously as this can result in the loss of captured target organisms. Certain sample types (e.g. very fatty, particulate and viscous samples) can interfere with bead recovery. To counter-act this interference, samples can also be diluted in PBS-Tween®e.g. 1:2-1:4, reducing the effect of the matrix and allowing more efficient bead recovery. Alternatively with problem samples, after the initial magnetic separation the incomplete removal of the sample (i.e. remove 800µl) and continuation of the wash protocol as described can minimise bead losses.

Captivate™ **Product Specification**

Working concentration:	Typically 5mg/ml
Fe_3O_4content:	29-33% w/w
Antibody:	Particles coated with high avidity, affinity purified and absorbed polyclonal antibodies to cell surface antigens.
Specificity:	Reacts with target organism.
Average size:	2.5 µm (typical range 1-8µm)
Formulation:	Particles are suspended in PBS plus1% BSA pH 7.3-7.5 and 0.09% azide as preservative.
Storage:	8°C (may be shipped at ambient)
Shelf life:	2 years.

Captivate™ O157

CAP001

Description

Captivate™ O157 are magnetisable particles coated with specific antibody intended for the isolation of *E. coli* O157:H7 from food, animal feeds, beverages, pharmaceutical or environmental samples. The particles help to concentrate O157:H7 cells in mixed culture reducing the probability of missing low numbers or overgrowth of O157:H7 colonies by competing flora. In fact, immunomagnetic separation is now regarded as the gold standard method for isolation of *E. coli* O157:H7 from food and environmental samples.

E. coli O157:H7 is the primary serovar associated with food borne gastrointestinal infection, resulting in self-limiting diarrhoea, that can lead to serious disease conditions such as haemorrhagic colitis, haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopaenic purpura (TTP).

The organism itself is associated with raw meats and unpasteurised milk[1], probably due to the implication of farm animals and particularly cattle as carriers of *E. coli* O157:H7. Large outbreaks have been recorded in the United States from consumption of unpasteurised apple juice (apple cider) possibly as a result of using apples which have fallen to the ground where the potential for contamination with the organism exists[3,4].

Enrichment Protocol for *E. coli* O157:H7.

The recommended protocol for the isolation of *E. coli* O157:H7 employs a 6 hour enrichment step at 42°C in modified Tryptone Soy Broth (mTSB, LAB165) plus novobiocin (X150) followed by **IMS** (see below) and plating onto Sorbitol MacConkey Agar (LAB161 or HAL006) supplemented with or without the addition of cefixime and potassium tellurite (X161)[5-8]. It is also recommended that a further **IMS** and inoculation of SMAC plates is performed after incubation of the sample for 24 hours. Alternative enrichment protocols using different media have been described e.g. Buffered Peptone Water (LAB046) plus VCC (X546)[5-8].

Generic *Captivate*™ **IMS Procedure**

1. Add 20µl of well mixed ***Captivate***™ particles to a suitable micro tube (1.5 - 2.5ml volume).
2. To this add 1ml of the enrichment culture taking care to avoid transfer of sample debris.
3. Cap tube tightly and rotamix the suspension for 30 minutes at room temperature.
4. Insert tube into magnetic separator rack for 3 minutes to concentrate the beads to a pellet. Gently invert the rack several times to aid pelleting of the beads.
5. Carefully aspirate the supernatant from the tube and cap without removing particles, taking care to avoid splashing.
6. Remove magnet from rack or tubes from the rack and add 1ml of wash. Cap and resuspend particles by inverting several times.
7. Repeat separation and wash steps 4-6 twice more. Finally resuspend particles in 100µl of wash.
8. Remove 50µl of the complexed, resuspended particles to the plating media, streaking for single colonies. Incubate plates at 37°C for 18-24 hours and examine for typical colonies.

Phosphate Buffered Saline plus Tween®.

Formula	g/litre
Sodium chloride	8.0
Potassium chloride	0.020
Disodium hydrogen phosphate	1.15
Potassium dihydrogen phosphate	0.2
Tween® 20.	0.5

pH: 7.0 ± 0.2

Dissolve the components in deionised water and check the pH. Sterilise the solution by autoclaving at 121°C for 15 min. Allow the solution to cool and check the pH. Store in the dark and use within one month.

Interpretation: Examine the SMAC and CTSMAC plates for typical *E. coli* O157 non-sorbitol fermenting colonies that are smooth and circular, 1-3 mm in diameter that are colourless to pale orange. Confirm the colony identity with commercially available latex agglutination kits or antisera.

Product Presentation

Captivate™ O157 is available in packs of 50 test, product code CAP001-050 and 250 test, product code CAP001-250. Materials required, but not provided, include phosphate buffered saline-Tween® 20, pipettes and tips, stomacher machine and bags, magnetic separator rack and culture media. Magnetic separating racks (CAP-100-12P) and rotating mixers (CAP101-58) are also available from LAB M..

Reference

1) Padhye, N.V., and Doyle, M.P. (1992). *Escherichia coli* O157:H7: Epidemiology, Pathogenesis and Methods for Detection in Food. J.Food.Prot. **55**, 555-565.

2) Martin, M.L. *et al* (1986) Isolation of *Escherichia coli* from cattle associated with two cases of hemolytic syndrome. Lancet **ii** 1043.

3) Besser, R.E. *et al* (1993) An outbreak of diarrhoea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh pressed apple cider. JAMA **259** 2217-2220

4) McCarthy, M. (1996) *E. coli* O157:H7 outbreak in USA traced to apple juice. Lancet **348** 1299.

5)Wright, D.J., Chapman, P.A. and Siddons, C.A. (1994). Immunomagnetic separation as a sensitive method for the isolation of *Escherichia coli* O157 from food samples. Epidemiology and Infection **113**, 31-39.

6) Bolton, F.J.; Crozier, L.; Williamson, J.K. (1995) New technical approaches to *Escherichia coli* O157. PHLS Microbiol. Dig. **12** 67-71.

7) Vernozy-Rozand, C. (1997). Detection of *Escherichia coli* O157 and other VTEC in food. Journal of Applied Microbiology. **82**, 537-551.

8) Ogden, I.D.; Hepburn, N.F.; & MacRae, M. (2001). The optimisation of media used in the immunomagnetic separation methods for the detection of *Escherichia coli* O157 in foods. J. Appl.Mic. 91, 373-379.

Captivate™ O26

CAP 003

Captivate™ O111

CAP 004

Captivate™ O103

CAP 005

Captivate™ O145

CAP 006

Description

There is now growing concern that VTEC's could be playing a more significant role in human disease than currently estimated. Current microbiological methods do not permit the sensitive isolation of non-O157-VTEC. Unlike *E. coli* O157:H7, which is sorbitol negative, this group does not appear to have any common distinct biochemical properties. Therefore using a screening isolation medium such as Sorbitol MacConkey Agar does not help in the isolation of these organisms.

To address this basic problem Lab M has developed a quartet of individual immunomagnetic separation reagents; O26, O103, O111 and O145 to aid the isolation of the other common serotypes of verotoxigenic *E. coli* (VTEC). Therefore, in combination with the ***Captivate***™ **O157** reagent, the "top five" VTEC as identified by the World Health Organisation can be targeted with these reagents. The **IMS** step should greatly increase the chance of isolating these organisms.

Enrichment methods are currently being developed by researchers and consequently there are no standard protocols to recommend. Bearing this in mind, we suggest users try the same enrichment protocol that we recommend for *E. coli* O157 at 37°C and 42°C with a generic *E. coli* plating medium such as Tryptone Bile Glucuronide Agar (HAL 3) or TBA (LAB 72) plus MUG (MC406). SMAC-BCIG (HAL 6) has also been used for this application as it contains the glucuronide chromogen, which the majority of *E. coli* react with. Some workers have recommended the use of "Enterohaemolysin agar" sheep blood agar for detection of VTEC. Verocytotoxin production has been shown to be closely linked with the enterohaemolytic phenotype.

References

Bielaszewska, M. & Karch, H. (2000). Non-O157:H7 Shiga toxin (verocytotoxin)-producing Escherichia coli strains: epidemiological significance and microbiological diagnosis. World Journal of Microbiology and Biotechnology. Vol **16**, 8-9, 711-718.

Beutin, L., Montenegro, M.A., Orskov, I., Prada, J., Zimmerman, S. and Stephan, R. (1989). Close association of verocytotoxin (shiga-like toxin) production and enterohaemolysin production in strains of Esherichia coli. Journal of Clinical Microbiology **27**, 2559-2564.

Captivate™ Salmonella

CAP002

Description

This product is designed to capture and concentrate the common serotypes of Salmonella involved in human and animal disease from enriched samples. The particles are coated with affinity purified polyclonal antibody directed towards common somatic and flagellar antigens. This gives an excellent high avidity broad spectrum IMS reagent for the capture of salmonellas. Due to the large variation in Salmonella serotypes and antigen expression there is naturally strain dependent variation in the capture efficiency.

Custom Coating Service

A coating service is available for coating our IMS reagent with alternative antibodies. Prices will be calculated on an individual basis.

For more information please contact our Technical Department

Tel: +44 (0) 161 797 5729

Fax: +44 (0) 161 762 9322

Email: info@labm.com

5. Lyophilised Media Supplements

Presentation and Shelf Life

Lab M lyophilised supplements are presented in packs of 10 vials, and for the majority of the supplements each vial is sufficient for 500ml of medium. Larger and smaller volumes are indicated for relevant products.

The shelf life of freeze-dried supplements is 2-3 years provided they are stored in a refrigerator at 2-8˚C. Once rehydrated the stability of antibiotics varies greatly and will determine the shelf life of the prepared agars and broths. For this reason any unused, rehydrated, supplement should be discarded, as even deep-freezing may not prevent the rapid degradation of the antibiotics. To ensure the correct level of selective supplements the entire vial contents must be added to the stated volume of cooled, molten medium.

Rehydration

Vials should be rehydrated aseptically using a sterile needle and syringe charged with 5ml of the specified diluent for the particular supplement being added. The supplement should be rehydrated, withdrawn and added to the medium in a single process, followed by immediate disposal of the syringe into an approved container. Under no circumstances attempt to re-sheath an exposed needle.

If sterile needles and syringes are not readily available, the rubber stopper may be completely removed and, using careful aseptic technique, rehydrate the supplement using a sterile pipette.

Addition

Most antibiotics are heat labile, and so to prevent a reduction of potency the medium should be cooled to 47˚C by holding in a water bath set at this temperature.

Once the supplement has been added the medium must be gently but thoroughly mixed to ensure that the selective agents are evenly distributed. Failure to do this will result in a range of concentrations in the plates/bottles and consequent inconsistency in results. The shelf life of supplemented media is governed by the stability of the added components, and is generally shorter than unsupplemented agars and broths. For information on the shelf life of prepared media consult the individual product listings in the previous section of the manual.

Anaerobes

X090

NALIDIXIC ACID, VANCOMYCIN for the isolation of Gram negative anaerobes from clinical material.

Suitable for use with LAB090 Fastidious Anaerobe Agar. When used with other blood agar bases, e.g. LAB001 Columbia Agar, further enrichment of the medium with haemin and menadione is beneficial.

Final Concentration	mg/litre
Nalidixic acid	10
Vancomycin	2.5
Add 1 vial X090 to 500ml medium	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C together with other additives, mix gently and pour.

Reference:

Wren, M.W.D., 1980. J. Clin. Path. 33: 61-65. Multiple Selective Media for the isolation of anaerobic bacteria.

X291

NALIDIXIC ACID for the isolation of non-sporing anaerobes from clinical material.

Suitable for use with LAB090 Fastidious Anaerobe Agar. When used with other blood agar bases, e.g. LAB001 Columbia Agar, further enrichment of the medium with haemin, menadione and sodium pyruvate is beneficial. The addition of Tween®80, which, enhances the growth of anaerobic cocci, to the medium is required for N.A.T. medium. The Tween®80 may be added before sterilisation at a concentration of 0.1%.

Final Concentration	mg/litre
Nalidixic acid	10
Add 1 vial X291 to 1 litre medium	

Rehydrate contents of each vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C together with other additives, mix gently and pour.

Reference:

Wren, M.W.D., 1980. J. Clin. Path. 33: 61-65. Multiple Selective Media for the isolation of anaerobic bacteria.

X015, X215

NEOMYCIN 75 for the isolation of *Clostridium* spp. and other anaerobes.

When added to blood agar the resulting medium will allow the growth of clostridia, most *Bacteriodes fragilis* strains and some anaerobic cocci.

Final Concentration	mg/litre
Neomycin	75
Add 1 vial X015 to 500ml medium	
Add 1 vial X215 to 1 litre medium	

Reconstitute each vial by the addition of 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix gently and pour.

X016

NEOMYCIN 100 for the selective isolation of *Clostridium* spp.

When added to egg yolk medium this supplement will allow the growth of clostridia whilst inhibiting other lecithinase producing organisms.

Final Concentration	mg/litre
Neomycin	100
Add 1 vial X016 to 500ml medium	

Reconstitution as X015.

X018

KANAMYCIN 75 for the selective isolation of *Clostridium* spp. and other anaerobes.

An alternative to X015. Kanamycin is more inhibitory to anaerobic cocci.

Final Concentration	mg/litre
Kanamycin	75

Add 1 vial X018 to 500ml medium
Reconstitution as X015, X016.

References:

Lowbury, C.J.L., Lilly, H.A. (1955). A selective plate medium for *Cl. welchi*. J. Path. & Bact. 70: 105.

Collee, J.G., Watt, B. (1971). Changing approaches to the sporing anaerobes in medical microbiology. Spore Research ed. A. N. Barkeer.

Sutter, V.L., Citron, D.M., Edelstein, M.A.C., Finegold, S.M. (1985). Wadsworth Anaerobic Bacteriology Manual 4 ed. Star publishers, Belmont, California.

Wren, M.W.D. (1980). Multiple selective media for the isolation of anaerobic bacteria from clinical specimens. J. Clin. Path. 33: 61-65

X093

CYCLOSERINE, CEFOXITIN for the isolation of *Clostridium difficile* from clinical materials.

Suitable for use with LAB090 Fastidious Anaerobe Agar.

Final Concentration	mg/litre
D-Cycloserine	250
Cefoxitin	8
Add 1 vial X093 to 500ml medium	

Rehydrate contents of vial with 5ml of water. Add aseptically to sterilised medium cooled to 47˚C together with other additives, mix gently and pour.

Reference:

George, W.L., Sutter, V.L., Citron, D., Finegold, S.M. (1976). Selective and differential medium for isolation of *Clostridium difficile.*

Bacillus cereus

X074, X274

POLYMYXIN for the isolation of *B. cereus* from foods.

Suitable for the preparation of LAB073 Bacillus cereus Medium (P.R.E.P.). The addition of X073 sterile egg yolk emulsion is also required.

Final Concentration	
Polymyxin B	8mg/litre = 64,000i.u/litre
Add 1 vial X074 to 500ml medium	
Add 1 vial X274 to 2L medium	

Rehydrate contents of vial with 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C together with egg yolk emulsion, mix gently and pour.

Reference:

Micro-organisms in Food. Ed. Thatcher, F.S., Clarke, D.F. published by Univ. of Toronto Press.

X193

POLYMYXIN B for the isolation of *Bacillus cereus* from foods.

The addition of X073, Egg Yolk Emulsion, is also required. For addition to LAB193, PEMBA *Bacillus cereus* Medium.

Final Concentration	mg/litre
Polymyxin B	100,000 IU
Add 1 vial X193 to 500ml medium	

Rehydrate contents of vial by the addition of 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix gently and pour.

Burkholderia cepacia

X140

TICARCILLIN, POLYMYXIN, for the isolation of *Burkholderia (Pseudomonas) cepacia*

Suitable for use with LAB108 pseudomonas selective agar, or specific selective bases such as that described by Gilligan *et al.*

Final Concentration	mg/litre
Ticarcillin	100
Polymyxin	300,000 iu/litre
Add 1 vial to 500ml of medium	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix well and pour.

Reference:

Gilligan, P.H., Gage, P.A., Bradshaw, L.M., Schidlow, D.V., DeCicco, B.T. (1985) Isolation medium for the recovery of *Pseudomonas cepacia* from respiratory secretions of patients with cystic fibrosis. J.Clin.Microbiol. 22 (1) 5-8.

Campylobacter species

X115

Campylobacter growth supplement for the isolation of Camylobacter spp.

Suitable for use with LAB014 Nutrient Broth No. 2.

Formulation	Per vial	Conc. in medium (LAB014)
Sodium pyruvate	0.125 g	0.25 g
Sodium metabisulphite	0.125 g	0.25 g
Ferrous sulphate	0.125 g	0.25 g

Wearing latex gloves aseptically reconstitute the contents of the vial with 5 ml of sterile deionised water using a sterile pipette.

X112, X212

CEFOPERAZONE, AMPHOTERICIN for the isolation of *Campylobacter* spp. from clinical, environmental and food samples.

Suitable for use with LAB112 Campylobacter Selective Medium (blood free) or with blood agar media. Incubation at 37˚C gives better results than at 42˚C and is generally more convenient.

Final Concentration	mg/litre
Cefoperazone	32
Amphotericin	10
Add 1 vial X112 to 500ml medium	
Add 1 vial X212 to 1 litre medium	

Rehydrate contents of vial with 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix gently and pour.

Reference:

Bolton, F.J., Hutchinson, D.N., Parker, G. (1988). Reassessment of Selective Agars and Filtration Techniques for Isolation of Campylobacter Species from Faeces. Eur. J. Clin. Microbiol. Infect. Dis. 7: 155-160.

X131

Cefoperazone, Vancomycin, Trimethoprim, Cycloheximide (CVTC) Selective Supplement

Description

For the isolation of *Campylobacter* spp. from food and environmental samples by the enrichment broth technique. Developed for use with LAB135 Campylobacter Enrichment Broth. Gives higher isolation rates than Preston Broth and does not require modified atmosphere incubation.

X131 CVTC is equivalent in performance to X132 CVTN which includes natamycin in place of cycloheximide.

Formulation	Per vial	Conc. in medium (LAB135)
Cefoperazone	10mg	20mg/L
Vancomycin	10mg	20mg/L
Trimethoprim	10mg	20mg/L
Cycloheximide	25mg	50mg/L
Vials per litre of medium		**2**

Appearance

White to off-white lyophilised tablet.

Hazard classification

T + - Very toxic

Storage

2-8°C in the dark

Reconstitution

Rehydrate contents of vial with 5ml sterile 50% alcohol. Add aseptically to sterilised medium cooled to 47°C. Mix gently and dispense into sterile containers.

References

Bolton, F.J., Preston, PHLS. Personal communication (1989).

X132

Cefoperazone, Vancomycin, Trimethoprim, Natamycin (CVTN) Selective Supplement

Description

For the isolation of *Campylobacter* spp. from food and environmental samples by the enrichment broth technique. Developed for use with LAB135 Campylobacter Enrichment Broth. Gives higher isolation rates than Preston Broth and does not require modified atmosphere incubation.

X132 CVTN is equivalent in performance to X131 CVTC and includes natamycin in place of cycloheximide.

Formulation	Per vial	Conc. in medium (LAB135)
Cefoperazone	10mg	20mg/L
Vancomycin	10mg	20mg/L
Trimethoprim	10mg	20mg/L
Natamycin	12.5mg	25mg/L
Vials per litre of medium		**2**

Appearance

White to off-white lyophilised tablet.

Hazard classification

NR – Not regulated

Storage

2-8°C in the dark

Reconstitution

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47°C. Mix gently and dispense into sterile containers.

X114

Modified Preston Campylobacter Supplement for the isolation of *Campylobacter* spp.

For use with LAB015 Blood Agar Base supplemented with 5% lysed horse blood or LAB014 Nutrient Broth No.2 supplemented with 5% lysed horse blood and X115 Campylobacter Growth Supplement.

Final Concentration	Per vial	Conc. in medium
Rifampicin	5mg	10mg/L
Polymyxin B	2500 IU	5000 IU
Trimethoprim	5mg	10mg/L
Amphotericin B	5mg	10mg/L

Rehydration

Wearing latex gloves aseptically reconstitute the contents of the vial with 5ml of sterile 50% ethanol using a sterile pipette. Ensure contents of vial are well mixed before addition to culture media.

Addition

Aseptically add the reconstituted supplement to media cooled to 47°C and immediately mix gently to evenly distribute.

Storage

2-8°C. After re-hydration some antibiotics are unstable, even when frozen, and therefore unused supplement should be discarded.

Hazard classification

Not regulated.

X214

VANCOMYCIN, POLYMYXIN, TRIMETHOPRIM, to make Skirrow's medium for the isolation of *Campylobacter* spp.

Suitable for use with LAB001 Columbia Agar or other blood agar bases, supplemented with lysed horse blood.

Final Concentration	mg/litre
Vancomycin	10
Polymyxin	2500 iu/litre
Trimethoprim	5
Add 1 vial of X214 to 1 litre of medium	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, along with other additives, mix well and pour.

Reference:

Skirrow, M.B. (1977) British Medical Journal 2 11-9.

Clostridium difficile

X093

CYCLOSERINE, CEFOXITIN for the isolation of *Clostridium difficile* from clinical materials.

Suitable for use with LAB090 Fastidious Anaerobe Agar.

Final Concentration	mg/litre
D-Cycloserine	250
Cefoxitin	8
Add 1 vial X093 to 500ml medium	

Rehydrate contents of vial with 5ml of water. Add aseptically to sterilised medium cooled to 47°C together with other additives, mix gently and pour.

Reference:

George, W.L., Sutter, V.L., Citron, D., Finegold, S.M. (1976). Selective and differential medium for isolation of *Clostridium difficile.*

Clostridium perfringens

X109, X110

SULPHADIAZINE (X109). OLEANDOMYCIN PHOSPHATE, POLYMYXIN (X110).

For use with LAB109 Perfringens agar to prepare O.P.S.P. for the selective isolation of *Clostridium perfringens* from foodstuffs.

Final Concentration	mg/litre
Sulphadiazine	100
Oleandomycin	0.5
Polymyxin	10,000 i.u./litre
Add 1 vial X109 and 1 vial X110 to 500ml medium	

Rehydrate contents of vials with 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix gently and pour.

Reference:

Handford, P.M. (1974). J. Appl. Bact. 37, 559-570.

X194

D-CYCLOSERINE supplement for the isolation of *Clostridium perfringens* from foods.

For use with LAB194, Perfringens Agar Base (TSC).

Final Concentration	mg/litre
D-Cycloserine	400
Add 1 vial of X194 to 500mls medium.	

Rehydrate contents of vial by the addition of 5mls of sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix gently and pour.

Escherichia coli

X150

NOVOBIOCIN for the enrichment of *E. coli* O157:H7 from food, environmental and clinical samples.

For the addition to LAB165 O157 Broth MTSB

Final concentration	mg/litre
Novobiocin	20
Add 1 vial of X150 to 500ml of O157 Broth MTSB.	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix well and pour.

X161

CEFIXIME TELLURITE supplement for the isolation of *E. coli* O157:H7 from food, environmental and clinical samples.

For the addition to LAB161 Sorbitol MacConkey Agar (SMAC) or HAL006 (BCIG-SMAC).

Final concentration	mg/litre
Cefixime	0.05
Potassium tellurite	2.5
Add 1 vial of X161 to 500 ml of Sorbitol MacConkey Agar (SMAC) or HAL006 (BCIG-SMAC)	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix well and pour.

X546

V.C.C. Supplement for the selective enrichment of *E. coli* O157:H7 from food and other samples.

For use with Buffered Peptone Water LAB046

Final Concentration	mg/litre
Vancomycin	8.0
Cefixime	0.05
Cefsulodin	10.0
Add 1 vial of X546 to 2.25 litres of LAB046	

Rehydrate the contents of one vial with 20ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C. Mix well and dispense into 225ml aliquots.

Gardnerella vaginalis

X011

COLISTIN, NALIDIXIC ACID for the isolation of *G. vaginalis* from clinical material.

Suitable for addition to LAB001 Columbia Agar or LAB015 Blood Agar Base No. 2 to produce a selective isolation medium.

Final Concentration	mg/litre
Colistin	10
Nalidixic acid	15
Add 1 vial X011 to 500ml medium	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, together with any other additives, mix gently and pour.

Reference:

Goldberg, R.L., Washington, J.A. II (1976). “Comparison of Isolation of *Haemophilus vaginalis (Corynebacterium vaginalae)* from Peptone-Starch-Dextrose Agar and Columbia, Colistin, Nalidixic Acid Agar. J. Clin. Microbiol. 4(3): 245.

Gram Positive Cocci

X012

COLISTIN, NALIDIXIC ACID for the preparation of Columbia C.N.A. medium.

A medium selective for Gram positive cocci is obtained when this antibiotic mixture is added to LAB001 Columbia Agar.

Final Concentration	mg/litre
Colistin	10
Nalidixic acid	10
Add 1 vial X012 to 500ml medium	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, together with any other additives, mix gently and pour.

Reference:

Ellner, P.D., Stossel, C.I., Drakeford, E., Vasi, F. (1966). “A new culture medium for medical bacteriology.” Amer. J. Clin. Path. 45: 502.

X013

COLISTIN, OXOLINIC ACID for the selective isolation of streptococci from clinical material.

When added to LAB001 Columbia agar or LAB015 Blood Agar Base No. 2, X013 renders the medium selective for streptococci. Alteration in haemolysis patterns may occur when azide or crystal violet are employed as selective agents but this does not occur with X013.

Final Concentration	mg/litre
Colistin	10
Oxolinic acid	5
Add 1 vial X013 to 500ml medium	

Rehydrate contents of vial with 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C together with other additives, mix gently and pour.

Reference:

Petts, D. (1984). Colistin - Oxolinic Acid - Blood Agar: a new selective medium for streptococci. J. Clin. Microbiol. 19: 4-7.

Legionella

X195

GVPC Selective Supplement - Glycine, Vancomycin, Polymyxin B, Cycloheximide

Description

A selective supplement developed for use with LAB195 BCYE Legionella Isolation Medium for the isolation of *Legionella* spp. X195 GVPC Supplement should be used in conjunction with X196 BCYE Growth Supplement.

Formulation	Per vial	Conc. in medium (LAB195)
Glycine	1500mg	3000mg/L
Vancomycin hydrochloride	0.5mg	1mg/L
Polymyxin B sulphate	39600 IU	79200 IU/L
Cycloheximide	40mg	80mg/L
Vials per litre of medium		**2**

Appearance

Off-white lyophilised tablet.

Hazard classification

T - Toxic

Storage

2-8°C in the dark

Reconstitution

Rehydrate contents of vial with 10ml sterile deionised water. Add aseptically to sterilised medium cooled to 47°C. Mix gently and dispense into sterile containers.

References

International Standard. ISO 11731:1998(E). Water quality- Detection and enumeration of Legionella

X196

BCYE Growth Supplement

Description

L-Cysteine Hydrochloride and α-Ketoglutarate BCYE growth supplement for the isolation of *Legionella* spp. For addition to LAB195 BCYE Legionella Isolation Medium

Formulation	mg/litre
L-Cysteine Hydrochloride	400
α-Ketoglutarate	1000
Add 1 vial per 500mL of sterilised medium as appropriate.	

Hazard classification

NR – Not regulated

Storage

2-8°C in the dark

References

ISO 11731:1998(E) Water quality- Detection and enumeration of Legionella

X197

BCYE Growth Supplement (no L-cysteine)

Description

α-Ketoglutarate growth supplement for the presumptive identification of *Legionella* spp. For addition to LAB195 BCYE Legionella Isolation Medium.

Formulation	mg/litre
α-Ketoglutarate	1000
Add 1 vial per 500mL of sterilised medium as appropriate.	

Hazard classification

NR – Not regulated

Storage

2-8°C in the dark

References

ISO 11731:1998(E) Water quality- Detection and enumeration of Legionella

Listeria

X122

CCCAF Supplement
(Cefotetan, Cycloheximide, Colistin, Acriflavine, Fosfomycin)

Description

For the isolation of Listeria monocytogenes from environmental, clinical and food samples. This supplement may be added to LAB122 Listeria Isolation Medium Oxford, LAB206 Listeria Isolation Medium Oxford (ISO).

X122 CCCAF is equivalent in performance to X123 CNCAF which includes natamycin in place of cycloheximide.

Formulation	Per vial	Concentration in medium LAB122 / LAB206
Cefotetan	1 mg	2 mg/L
Cycloheximide	200 mg	400 mg/L
Colistin	10 mg	20 mg/L
Acriflavine	2.5 mg	5 mg/L
Fosfomycin	5 mg	10 mg/L
Vials per litre of medium		**2**

Appearance

White to off-white lyophilised tablet.

Hazard classification

T+ - Very toxic

Storage

2-8°C in the dark

Reconstitution

Contents of the vial should be reconstituted by the addition of sterile 50% ethanol in water. Add aseptically to sterilised medium cooled to 47°C. Mix gently then pour.

References

Curtis, *et al.* (1989) A selective differential medium for the isolation of *Listeria monocytogenes*. Lett. In Appl. Microbiol. 8:95-98.

X123

Cefotetan, Natamycin, Colistin, Acriflavine, Fosfomycin (CNCAF) Selective Supplement

Description

For the isolation of *Listeria monocytogenes* from environmental, clinical and food samples. This supplement may be added to LAB122 Listeria Isolation Medium Oxford, LAB206 Listeria Isolation Medium Oxford (ISO).

X123 CNCAF is equivalent in performance to X122 CCCAF and includes natamycin in place of cycloheximide.

Formulation	Per vial	Concentration in medium LAB122 / LAB206
Cefotetan	1 mg	2 mg/L
Natamycin	12.5 mg	25 mg/L
Colistin	10 mg	20 mg/L
Acriflavine	2.5 mg	5 mg/L
Fosfomycin	5 mg	10 mg/L
Vials per litre of medium		**2**

Appearance

White to off-white lyophilised tablet.

Hazard classification

NR – Not regulated.

Storage

2-8°C in the dark

Reconstitution

Contents of the vial should be reconstituted by the addition of sterile 50% ethanol in water. Add aseptically to sterilised medium cooled to 47°C. Mix gently then pour.

X138

N.A.C. NALIDIXIC ACID, ACRIFLAVINE, CYCLOHEXIMIDE for the selective enrichment broth culture of *Listeria monocytogenes.*

For addition to LAB138 Listeria Enrichment Broth recommended by the F.D.A. for Listeria isolation from food and environmental samples and LAB139 Buffered Listeria Enrichment Broth.

Final Concentration	mg/litre
Nalidixic acid	40
Cycloheximide	50
Acriflavine	15
Add 1 vial of X138 to 500ml medium	

Reconstitute contents of vial by the addition of sterile 50% ethanol in water. Add aseptically to sterilised medium cooled to 47˚C, mix gently then pour.

Reference:

Lovett et al. (1987). *Listeria monocytogenes* in raw milk: detection incidence and pathogenicity. J. Food Protect. 50: 188-192.

X139

N.A.N. NALIDIXIC ACID, ACRIFLAVINE, NATAMYCIN.

An alternative natamycin based supplement for the selective enrichment broth culture of *Listeria* spp.

For addition to LAB 138, *Listeria* Enrichment Broth and LAB139, Buffered *Listeria* Enrichment Broth.

Final Concentration	mg/litre
Nalidixic acid	40
Acriflavine	15
Natamycin	25
Add 1 vial of X139 to 500ml medium.	

Rehydrate contents of vial by the addition of 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix gently and dispense.

X144

P.A.C. supplement for the enrichment and isolation of *Listeria* spp from food and environmental samples.

For the addition to LAB144 Palcam Broth and Lab 148 Palcam Agar

Final concentration	mg/litre
Polymyxin	10
Acriflavine	5
Ceftazidime	20
Add 1 vial of X144 to 500ml of Palcam Broth or Agar	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, along with other additives, mix well and pour.

X164, X564

HALF FRASER supplement for the primary enrichment of *Listeria* spp from food and environmental samples.

For addition to LAB164 Fraser Broth Base

Final Concentration	mg/litre
Ferric ammonium citrate	500
Acriflavine	12.5
Nalidixic acid	10
Add 1 vial of X164 to 450ml of Fraser Broth Base	
Add 1 vial of X564 to 2.25 litres of Fraser Broth Base	

Rehydrate contents of vial with 2ml 50% methanol (5ml for X564). Add aseptically to sterilised medium cooled to 47˚C, mix well and pour.

X072

POLYMYXIN B, CEFTAZIDIME supplement for the isolation of *Listeria monocytogenes*.

For addition to LAB172, LMBA

Final Concentration	mg/litre
Polymyxin B	10
Ceftazidime	20
Add 1 vial X072 and 1 vial of X072N to 500ml medium.	

Rehydrate contents of vial by the addition of 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix gently and pour.

X072N

NALIDIXIC ACID supplement for the isolation of *Listeria monocytogenes*.

For addition to LAB172, LMBA

Final Concentration	mg/litre
Nalidixic acid	40
Add 1 vial X072N and 1 vial of X072 to 500ml medium.	

Rehydrate contents of vial by the addition of 5 ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix gently and pour.

X165

FRASER supplement for the secondary enrichment of *Listeria* spp from food and environmental samples.

For addition to LAB164 Fraser Broth Base

Final Concentration	mg/litre
Ferric ammonium citrate	500
Acriflavine	25
Nalidixic acid	20
Add 1 vial of X165 to 500ml of Fraser Broth Base	

Rehydrate contents of vial with 2ml 50% methanol. Add aseptically to sterilised medium cooled to 47˚C, mix well and pour.

X155

UVMI. Supplement for the primary enrichment of *Listeria* spp from food and environmental samples.

For addition to LAB 155 UVM Broth Base

Final Concentration	mg/litre
Nalidixic acid	20
Acriflavine	12
Add 1 vial of X155 to 500ml of UVM Broth Base	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix well and pour.

Neisseria gonorrhoeae

X070

L.C.A.T. LINCOMYCIN, COLISTIN, AMPHOTERICIN, TRIMETHOPRIM for the isolation of *Neisseria* spp. from clinical material.

L.C.A.T. is often preferred to X068 V.C.N.T. for the isolation of *N. gonorrhoeae* because of the emergence of vancomycin sensitive strains. The antifungal agent amphotericin is more readily soluble and therefore a more active antifungal than nystatin. L.C.A.T. is quoted as the selective agent for New York City G.C. agar but can readily be substituted for V.C.N. or V.C.N.T. in Thayer Martin G.C. agar.

Final Concentration	mg/litre
Lincomycin	1
Colistin	6
Amphotericin	1
Trimethoprim	6.5
Add 1 vial X070 to 500ml medium	

Rehydrate contents of vial with 5ml sterile 25% alcohol in water. Add aseptically to sterilised medium cooled to 47˚C together with other additives, mix gently and pour.

Reference:

Young, H. (1978). Cultural Diagnosis of Gonorrhoea with modified N.Y.C. Medium. Brit. Journ. Ven. Dis. 54: 36-40.

X069

L.C.T. LINCOMYCIN, COLISTIN, TRIMETHOPRIM. A variant of L.C.A.T. with the amphotericin omitted to permit the growth of yeasts.

Concentrations and rehydration as L.C.A.T.
Add 1 vial X069 to 500ml medium

X068

V.C.N.T. VANCOMYCIN, COLISTIN, NYSTATIN, TRIMETHOPRIM for Thayer Martin Medium.

The addition of trimethoprim in V.C.N.T. inhibits the swarming of *Proteus* spp. which occasionally make interpretation difficult.

Final Concentration	mg/litre
Vancomycin	3
Colistin	7.5
Nystatin	12.5
Trimethoprim	5
Add 1 vial X068 to 500ml medium	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47°C together with other additives, mix gently and pour.

Reference:

Thayer, J.D. and Martin, J.E. (1966). Improved medium selective for the cultivation of *N. gonorrhoeae* and *N. meningitidis*. Public Health rep. 81: 559-562.

X271

GROWTH SUPPLEMENT, to improve the isolation of *Neisseria* spp. from selective media.

For addition to GC agar base LAB067.

Final Concentration	mg/litre
L-cystine	11
L-cysteine	259
Thiamine HCl	0.03
Ferric nitrate	0.2
Co-Carboxylase	1
NAD	1.0
Guanine HCl	0.3
Adenine	10
L-glutamine	100
PABA	0.13
Vitamin B12	0.1
Add 1 vial to 1 litre of medium	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, along with other additives, mix well and pour.

Pseudomonas species

X108

MODIFIED C.F.C. – CEPHALOTHIN, FUCIDIN, CETRIMIDE for the selective isolation of *Pseudomonas* spp.

When added to LAB108 Pseudomonas Agar, to prepare C.F.C. medium this supplement can be used to select pseudomonads from food and environmental samples.

Final Concentration	mg/litre
Cephalothin	50
Fucidin	10
Cetrimide	10
Add 1 vial X108 to 500ml medium	

Rehydrate contents of vial with 5ml of sterile 50% alcohol. Add aseptically to sterilised medium cooled to 47˚C, mix gently and pour.

Reference:

Mead, G.C. and Adams, B.W. (1977). Br. Poult. Sci. 18: 661-667

X107

C.N. CETRIMIDE, NALIDIXIC ACID for the isolation of *Pseudomonas aeruginosa.*

Suitable for use with LAB108 Pseudomonas Agar to make the medium selective for *Ps. aeruginosa.*

Final Concentration	mg/litre
Cetrimide	200
Nalidixic acid	15
Add 1 vial X107 to 500ml medium	

Rehydrate contents of vial with 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix gently and pour.

Reference:

Goto, S., Enomoto, S. 1970. Jap. J. Microbiol. 14: 65-72.

Pre-Incubation Test (P-INC)

X019, X219

PENICILLIN, NISIN, CRYSTAL VIOLET, for accelerated shelf life determination of dairy products.

The Pre-incubation test uses a selective mixture to inhibit Gram positive organisms whilst allowing the growth of Gram negative bacteria, the main cause of post-pasteurisation contamination and a major factor in determining the shelf life of the product. The technique is also useful for monitoring plant hygiene.

Final Concentration	mg/litre
Penicillin	20,000iu/litre
Nisin	40,000iu/litre m
Crystal violet	2.0
Add 1 vial of X019 to 200ml of Milk Agar LAB019	
Add 1 vial of X219 to 1 litre of Milk Agar LAB019	

Rehydrate contents of 1 vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix thoroughly and pour plates.

Method A

Pre-incubate test material at 21˚C for 24hr. Prepare suitable dilution series, and inoculate Milk Agar plates containing P-INC supplement. Incubate at 21˚C for 24hr, and count all colonies (some may be small, use of a hand lens is recommended). Calculate the CFU/ml and using the tables of Griffith's *et al* the shelf life can be determined.

Method B

Rehydrate X219 with 1ml of deionised water only, add 0.1ml to the test material and incubate at 20˚C for 24hr. Prepare suitable dilution series, and inoculate Milk Agar plates. Proceed as for Method A above.

References:

Griffiths, M.W., and Phillips, J.D. (1985) J.Appl.Bact. 57, 107.

Griffiths, M.W., and Phillips, J.D., and Muir, D.D. (1980) J. Soc. Dairy Technol. 33, 8.

Griffiths, M.W., and Phillips, J.D., and Muir, D.D. (1981) J. Soc. Dairy Technol. 34, 142.

Griffiths, M.W., and Phillips, J.D., and Muir, D.D. (1984) J. Soc. Dairy Technol. 37, 22.

Griffiths, M.W., and Phillips, J.D., and Muir, D.D. (1984) Rapid detection of post-pasteurised contamination. Hannah Research Inst. Bulletin No.10.

Griffiths, M.W., and Phillips ,J.D., and Muir, D.D. (1984) Dairy Ind. Int. 50 (3) 25

Griffiths, M.W., and Phillips, J.D., and Muir, D.D. (1984) Post-pasteurisation contamination - the major cause of failure of fresh dairy products. Hannah Research Inst.

Griffiths, M.W., and Phillips, J.D., and Muir, D.D. (1986) Aust. J. Dairy Technol. 41, 77-79.

Salmonella

X150

NOVOBIOCIN, for the isolation of *Salmonella* using semi-solid technology.

For addition to LAB150 MSRV and LAB 537 Diassalm

Final Concentration	mg/litre
Novobiocin	20 (MSRV)
Novobiocin	10 (Diassalm)
Add 1 vial to 500ml (MSRV)	
Add 1 vial to 1 litre (Diassalm)	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix well and pour.

References:

De Smedt, J.M., and Bolderdijk, R.F., (1986) Dynamics of salmonella isolation with modified semi-solid Rappaport Vassiliadis medium. J.Food Protection 50 658-661

Van Netten, P., Van Der Zee H., and Van Der Moosdijk A., (1991) The use of diagnostic selective semi-solid medium for the isolation of Salsmonella enteritidis from poultry. Proceedings of the 10th symposium on the quality of poultry meat. Spelderholt Beckbergen 56-67.

Staphylococci

X086

RPF Supplement
Rabbit Plasma Fibrinogen Supplement

Description

Bovine fibrinogen, rabbit plasma, trypsin inhibitor and potassium tellurite for the isolation of *Staphylococcus aureus*.

For addition to LAB285 Baird-Parker Medium Base (ISO) and LAB085 Baird-Parker Medium Base.

Final Concentration	amount / vial	amount / litre
Bovine Fibrinogen	0.375mg	3.75mg
Rabbit Plasma	2.5ml	25ml
Trypsin Inhibitor	2.5mg	25mg
Potassium Tellurite	2.5mg	25mg

Storage

Store at 2-8 ºC.

Prepared media: Use on day of preparation.

Method for reconstitution

Add 1 vial of X086 to 90mL of medium.

Rehydrate contents of vial by the addition of 10mL sterile deionised water. Add aseptically to sterilised media (cooled to 47ºC), mix gently to evenly distribute and pour.

X192

OXACILLIN, POLYMYXIN B supplement for the isolation of Methicillin Resistant *Staphylococcus aureus* (MRSA).

For addition to LAB192, ORSIM (Oxcacillin Resistant *Staphylococcus* Isolation Medium).

Final Concentration	mg/litre
Oxacillin	2
Polymyxin B	25,000 I.U
Add 1 vial of X192 to 500ml medium.	

Rehydrate contents of vial by the addition of 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix gently and dispense.

Streptococci

X013

COLISTIN, OXOLINIC ACID for the selective isolation of streptococci from clinical material.

When added to LAB001 Columbia agar or LAB015 Blood Agar Base No. 2, X013 renders the medium selective for streptococci. Alteration in haemolysis patterns may occur when azide or crystal violet are employed as selective agents but this does not occur with X013.

Final Concentration	mg/litre
Colistin	10
Oxolinic acid	5
Add 1 vial X013 to 500ml medium	

Rehydrate contents of vial with 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C together with other additives, mix gently and pour.

Reference:

Petts, D. (1984). Colistin - Oxolinic Acid - Blood Agar: a new selective medium for streptococci. J. Clin. Microbiol. 19: 4-7.

Yeasts and Moulds

X009, X209

CHLORAMPHENICOL for the selective isolation of yeasts and moulds from food, environmental and clinical specimens.

Chloramphenicol's broad antibiotic spectrum suppresses most contaminating bacteria allowing the yeasts and moulds to grow. It can be added to such media as LAB009 Sabouraud Dextrose Agar, LAB036 Rose Bengal Chloramphenicol Agar, LAB037 Malt Extract Agar and LAB117 Dermatophyte Test Medium to increase their selectivity whilst not lowering the pH. Reduction of pH will increase the selectivity of a yeast and mould medium but will also inhibit some yeasts as well as having a deleterious effect on the agar gel.

Final Concentration	mg/litre
Chloramphenicol	100
Add 1 vial X009 to 500ml medium	
Add 1 vial X209 to 1 litre medium	

Rehydrate contents of vial with 5ml of Ethyl or Methyl alcohol. Add aseptically to sterilised medium cooled to 47˚C, mix gently and pour.

References:

Jervis, B. (1973). Rose Bengal Chlortetracycline agar with other media for the selective isolation and enumeration of moulds and yeasts in foods. J. Appl. Bact. 36 Pages 723-727.

X089

OXYTETRACYCLINE for O.G.Y.E. medium.

For use with LAB089 Oxytetracycline Glucose Yeast Extract Agar for the enumeration of yeasts and moulds from foodstuffs. Highly proteinaceous foods and incubation above 30˚C will inactivate oxytetracycline.

Final Concentration	mg/litre
Oxytetracycline	100
Add 1 vial X089 to 500ml medium	

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix gently and pour.

References:

Mossel, D.A.A., *et al*. (1970). O.G.Y.E. for the selective enumeration of moulds and yeasts in food and clinical material. J. Appl. Bact. 35: 454-457.

Yersinia

X120

C.I.N. - CEFSULODIN, IRGASAN, NOVOBIOCIN for the isolation of *Yersinia* spp. from clinical and environmental material.

For addition to LAB120 Yersinia C.I.N. Agar Base used in the selective isolation of *Y. enterocolitica.*

Final Concentration	mg/litre
Cefsulodin	15
Irgasan	4
Novobiocin	2.5
Add 1 vial X120 to 500ml medium	

Rehydrate contents of vial with 5ml of 30% sterile alcohol. Add aseptically to sterilised medium cooled to 47˚C, mix gently and pour.

References:

Schiemann, D.A. (1979). Synthesis of a selective medium of Yersinia enterocolitica. Can. J. Microbiol. 25 (2): 1298.

Schiemann, D.A. (1980). Isolation of toxigenic Yersinia enterocolitica from retail pork products. J. Food Prot. 43: 360.

Schiemann, D.A. (1982). Development of a two-step enrichment procedure for recovery of Yersinia enterocolitica from food. Appl. Microbiol. 43 (1): 14.

6. Agars, Peptones, Extracts & Other Media Constituents

Sourcing

The Lab M range of media constituents are selected on the basis of quality and performance from the world's leading suppliers. It is a deliberate policy not to invest in our own peptone manufacturing facility in order to allow our microbiologist freedom to choose the best ingredients available on the international market.

Agars

A range of agars are offered to suit all microbiological applications. Koch originally used gelatin to solidify culture media, but the superior properties of agar resulted in its universal adoption as the gelling agent of choice. Careful selection of agars is vital as they can interact with nutrient components in a beneficial or deleterious manner.

Peptones and Extracts

Like agars, peptones and extracts are biologically variable products requiring careful selection. They provide the amino acids and peptides required by micro-organisms for growth as well as other vital growth factors such as minerals, vitamins and nucleic acid fractions.

To ensure we use only the best available peptones and extracts these materials are exhaustively tested. Growth parameters are obtained by classical microbiological techniques and by automated growth rate analysis. Chemical and physical properties are also closely monitored. Lab M can select specific peptones for special purposes such as vaccine production and fermentation processes. If more information is required on special services please contact Lab M or your local agent.

Acid Hydrolysed Casein

MC007

A soluble protein hyrolysate obtained by digesting casein with hot acid. It is almost free from growth factors, vitamins and antagonists, and these qualities make it suitable for use as a protein source in media for antibiotic and vitamin assays.

Typical Analysis	
Appearance	cream/white powder
Solubility in water at 5%	total
Clarity	clear and colourless
pH of 2% solution	6.0 ± 0.5
Total Nitrogen	8.3% ± 0.5
Total Amino Nitrogen	6.1% ± 0.5

Agar No. 1 Bacteriological

MC002

A high clarity agar with good gelling properties and a low concentration of metal ions. This agar is suitable for all bacteriological purposes including sensitivity testing and pour plate techniques. A firm gel is obtained at working concentrations of 1.0 to 1.5%. No significant precipitation is observed on reheating or prolonged holding at 65˚C.

Typical Analysis	
Gel strength (Nikan)	650-1000g/m2
Colourimetry (1.5% soln at 65˚C)	> 0.28 at 340nm
	> 0.02 at 525nm
Melting point	> 85˚C
Setting point	32-35˚C
pH	6.5-7.4
Moisture	< 10%
Total ash	< 3%
Calcium	< 0.02%
Magnesium	< 0.02%
Sodium chloride	< 1.0%
Iron	< 0.01%
Insoluble ash	< 0.1%
Sulphate	1.5%
Salmonella	Absent
TVC	$< 10^3$/g
Spores	< 2/g

Agar No. 2 Bacteriological

MC006

A bacteriological agar which gives a firm gel at working concentrations of 1.0 to 1.5% which is reasonably clear. This agar is recommended for all culture media except sensitivity testing media and those where absolute clarity is advantageous.

Typical Analysis	
Gel strength (Nikan)	650-1000g/m^2
Colourimetry (1.5% soln at 65˚C)	> 0.3 at 340nm
	>0.04 at 525nm
Appearance	cream/white powder
Melting point	>85˚C
Setting point	32-35˚C
pH	6.5-7.4
Moisture	<12%
Total ash	<3.5%
Calcium	< 0.5%
Magnesium	< 0.1%
Sodium chloride	<1.0%
Iron	< 0.01%
Insoluble ash	< 0.1%
Sulphate	< 3.0%
Salmonella	Absent
TVC	$< 10^3$/g
Spores	< 2/g

Agar No.4

Plant Tissue Culture Grade

MC029

Lab M Agar No.4 has been selected specifically for use as a gelling agent in plant tissue culture techniques. The product is selected primarily on gel strength, a parameter of particular importance for this application, and then tested to ensure it meets the parameters set by a major plant producer. The agar contains no nutrients for plant growth and is designed to be incorporated into classical formulations such as Murashige and Skoog, as well as customer's own formulations.

Typical analysis	
Ash	2.30%
Acid Insoluble Ash	0.16%
Calcium	0.31%
Magnesium	0.12%
Iron	0.018%
Total Nitrogen	0.15%
Recommended Concentration	0.75 - 1.5%
Melting Point	88-91˚C
Setting Point	32-33˚C
Mesh	80
pH (1.5% at 20˚C)	7.0 ± 0.2
Gel Strength (1.5% W/V)	>700g/cm^2

Bacteriological Peptone

MC024

An economical source of nutrients provided by a balanced mixture of meat peptones and tryptone. The growth requirements of most non fastidious organisms will be fulfilled by the range of amino acids, peptides and proteoses in this mixture.

Typical Analysis	
Appearance	cream powder
Solubility in water at 5%	total
Clarity	clear, pale straw colour
pH of 2% solution	7.2 ± 0.2
Total Nitrogen	12% ± 0.5
Amino Nitrogen	5% ± 0.5

Balanced Peptone No. 1

MC004

A rich mixture of tryptone and meat peptones which fulfills the nutritional demands of a wide variety of micro-organisms. This peptone is used in many Lab M culture media formulations.

Typical Analysis	
Appearance	beige powder
Solubility in water at 5%	total
Clarity	clear, pale straw colour
pH of 2% solution	7.2 ± 0.2
Total Nitrogen	12.8% ± 0.5
Amino Nitrogen	5.1% ± 0.5

Beef Extract

MC019

This complements the nutritive properties of peptones in culture media and is often used as an added enrichment. Beef extract can be used as a direct replacement for meat peptones and, as it contains no carbohydrates, can be used as a component of media for fermentation studies.

Typical Analysis	
Appearance	light brown powder
Solubility in water at 5%	total
Clarity	clear, light brown colour
pH of 2% solution	7.0 ± 0.2
Total Nitrogen	12.0% ± 0.5
Amino Nitrogen	1.6% ± 0.5

Bile Salts No. 3

MC025

A refined bile salt, comprising mainly sodium cholate and sodium desoxycholate. It is used as a selective agent in culture media such as Violet Red Bile Agar for the enumeration of coliforms, MacConkey Agar No. 3 for the isolation and differentiation of enteric organisms, and SS Agar for the isolation of enteric pathogens. This fraction of bile is highly active, allowing maximum selection of organisms of enteric origin at relatively low concentrations (0.15%).

Typical Analysis	
Appearance	white powder
Solubility in water at 2%	total
Clarity	clear
pH of a 2% solution	8.0 ± 0.5

FMV (Foot and Mouth Vaccine) Peptones

MC033

Description

A special blend of peptones developed for use in the production of Foot and Mouth vaccine.

Typical analysis:	
Appearance	cream powder
Solubility in water at 2%	total
Clarity	clear, straw colour
pH of 2% solution	7.0 ± 0.2 Total
Nitrogen	10.0 - 14.5%
Amino Nitrogen	2.5 - 5.5%

Gelatin Powder

MC015

Description
A collagenous protein used for the solidification of culture media and for the detection and differentiation of certain proteolytic bacteria.

Typical Analysis:	
Appearance	buff crystalline powder
pH	5.7

Glucose (Dextrose)

MC013

Description
Glucose for use in microbiological culture media.

IPTG (Isopropyl-β-D-Thiogalactopyranoside)

MC402

Description
Isopropyl-β-D-Thiogalactopyranoside (IPTG) is used as an inducer of the lac Z operon. Incorporation of this compound into media containing X-β-Galactoside (MC405), or equivalent chromogenic compound, enhances the colour development of organisms capable of fermenting lactose. This combination of compounds is especially useful in transformation experiments involving *Escherichia coli* where disruption of the lac Z operon is used as a marker for DNA insertion.

Lactalbumin Hydrolysate

MC040

Description
Lactalbumin is a protein removed from the whey, left after removal of casein from milk. Lactalbumin hydrolysate is a pancreatic digest of these proteins, containing high levels of essential amino acids. It can be used for tissue culture media and for production of vaccines of viral origin. Other uses include growth of lactobacilli, clostridial spores and certain fermentation procedures.

Typical analysis:	
Appearance	cream powder
Solubility in water at 5%	total
Clarity	clear, pale straw colour
pH of 2% solution	7.0 ± 0.2
Total Nitrogen	12.0% ± 0.5
Amino Nitrogen	5.5 ± 0.5

Lactose

MC020

Description
Lactose for use in microbiological culture media.

Lecithin (Soya Bean Lecithin)

MC041

Description
A deoiled, powdered soybean lecithin for use in microbiological culture media.

Composition
A mixture of polar (phospho- and glyco-) lipids and a small amount of carbohydrates.

Acetone insolubles	min.	97%
Oil	max.	1.5%
Phosphatidylcholine		20-24%
Phosphatidylethanolamine		18-22%
Phosphatidylinositol		12-15%
Phosphatidic acid		4-7%
Fatty acids (total content) including saturated fatty acids, monounsaturated fatty acids & polyunsaturated fatty acids	approx.	50%

Typical analysis:	
pH (1% aqueous solution)	6-7
Total plate count	<1000/g
Solubility in:	
water	dispersible
fats / oils	soluble

Appearance
Fine, beige powder

Hazard classification
NR – Not regulated

Storage
Store at 10-25˚C. Do not store above 25˚C.

This component is suitable for use in the preparation of microbiological culture media. Other uses and applications are subject to validation by the end user.

Liver Digest

MC034

Liver digest is prepared by the controlled hydrolysis of liver. It is rich in vitamins and essential amino acids and has excellent nutritional properties and especially favours the growth of strict anaerobes and other fastidious microorganisms. Due to its capacity to stimulate sugar metabolism in saccharolytic organisms it is perfectly suited for the growth of a broad range of organisms such as *Clostridium*, *Leuconostoc*, *Bacillus*, homo and hetero-fermentative Lactic acid bacteria, as well as yeasts and filamentous moulds.

Typical Analysis	
Appearance	light brown powder
Solubility in water at 5%	total
Clarity	clear, brown colour
pH of 2% solution	6.0 - 7.0
Total Nitrogen	9.5 - 11.5%
Amino Nitrogen	4.0 - 6.0%

Storage
Dehydrated culture media: 10-25°C away from direct sunlight.

Malt Extract

MC023

A water soluble extract of malted barley suitable for use in the cultivation of yeasts and moulds. Malt extract has a very high carbohydrate content and consequently is very sensitive to over heating which will cause a darkening of the medium.

Typical Analysis	
Appearance	yellow/brown powder
Solubility in water at 5%	total
Clarity	clear, light brown colour
pH of 2% solution	5.2 ± 0.2
Maltose	55%
Other Carbohydrates	40%
Protein	5%

Maltose Monohydrate

MC022

Description
Maltose for use in microbiological culture media.

Mannitol

MC014

Description
D-Mannitol for use in microbiological culture media.

Meat Peptone

MC018

Description
A highly nutritious enzymatic digest of meat for use in microbiological culture media

Typical Analysis:	
Appearance	cream powder
Solubility in water at 5%	total
Clarity	clear, pale straw colour
pH of 2% solution	6.7 - 7.3
Total Nitrogen	11.0 - 13.0%
Amino Nitrogen	2.5 - 5.0%

MUG (4-Methylumbelliferyl-ß-D-Glucuronide)

MC406

Description
MUG is a fluorogenic compound used for the specific detection of *E. coli* in bacteriological culture media. MUG reagent is cleaved by the enzyme glucuronidase to release fluorescent 4-methylumbelliferone that can be detected under long wave UV light (366nm) as a blue/green fluorescence. MUG can be incorporated into a range of culture media to enhance detection of *E. coli*.

Mycological Peptone

MC009

A mixture of peptones with a high carbohydrate content suitable for the rapid growth and colonial development of yeasts and moulds. Bacterial growth is inhibited by the low pH of this peptone.

Typical Analysis	
Appearance	beige powder
Solubility in water at 5%	total
Clarity	clear, pale straw colour
pH of 2% solution	5.4 ± 0.1
Total Nitrogen	13% ± 0.5
Amino Nitrogen	1.4% ± 0.5

Ox Bile

MC010

Description
Ox bile is a dehydrated, purified fresh bile used as a selective agent in bile media, such as Brilliant Green Bile 2% Broth (LAB051).

Proteose Peptone A

MC011

An enzymatic digest of meat adapted to encourage the production of toxins by *Corynebacterium diphtheriae,* staphylococci, salmonellae, and clostridia. This peptone is highly nutritious and suitable for use in culture media for fastidious organisms such as *Neisseria, Haemophilus* and *Pasteurella* species.

Typical Analysis	
Appearance	cream powder
Solubility in water at 5%	total
Clarity	clear, light straw colour
pH of 2% solution	7.0 ± 0.2
Total Nitrogen	12.0% ± 0.5
Amino Nitrogen	5.8% ± 0.5

Skim Milk Powder

MC027

A bacteriological grade of thermophile free spray dried skim milk. Used in Milk Plate Count Agar LAB115 and in media for diagnostic tests involving the digestion or coagulation of casein and the fermentation of lactose. Recommended working concentration 10%.

Typical Analysis	
Appearance	white powder
Clarity	opaque white suspension
Total Nitrogen	5.3% ± 0.5
Lactose	48.0% ± 0.5

Sodium Chloride (Bacteriological)

MC017

Description
Sodium chloride for use in microbiological culture media.

Sodium Desoxycholate

MC026

Sodium desoxycholate is a specific bile acid, derived from deconjugated bile salts. Leifson showed that desoxycholic acid had the most inhibitory effect on bacterial growth, and that this could be enhanced by the removal of magnesium ions by chelating with sodium citrate. These components comprise the selective agents in DCA, DCA (Hynes) and DCLS.

Typical Analysis	
Appearance	white powder
pH of 2% solution	8.3 ± 0.5
Solubility in water at 2%	total
Moisture	<5%
Heavy metals	<20 ppm
Sodium cholate	<2%

Sodium Thioglycollate

MC016

Description
Sodium thioglycollate for use in bacteriological culture media. It is used to lower the oxidation-reduction potential of the medium and for the neutralisation of mercurial compound preservatives.

Soy Peptone

MC003

Prepared using the enzyme papain to digest soyabean meal, this peptone is a rich source of nutrients with a high carbohydrate content. Most organisms will grow rapidly in this peptone but some bacteria will produce high levels of acid leading to auto-sterilisation unless an adequate buffering system is incorporated.

Typical Analysis	
Appearance	cream powder
Solubility in water at 5%	total
Clarity	clear, straw colour
pH of 2% solution	7.1 ± 0.2
Total Nitrogen	9.0% ± 0.5
Amino Nitrogen	1.6% ± 0.5

Special Tryptone

MC035

Description
Special tryptone is a high quality source of peptides produced by enzymatic digest of casein. It is a uniform, high quality peptone providing superior growth characteristics. Special tryptone is a refined hydrolysate, with very high solubility and clarity in solution. It is recommended for laboratory media and fermentation, especially where high clarity of solution is required.

Typical Analysis:	
Appearance	cream powder
Solubility in water at 5%	total
Clarity	clear, pale straw colour
pH of 2% solution	6.7 - 7.0
Total Nitrogen	12.5 - 13.5%
Amino Nitrogen	5.5 - 7.0%

Tryptone

MC005

An enzymatic hydrolysate of casein, rich in peptones and amino acids (including tryptophane). This peptone can be utilised by most bacteria as a growth substrate. This peptone conforms to the U.S.P. requirements for a pancreatic digest of casein.

Typical Analysis	
Appearance	cream powder
Solubility in water at 5%	total
Clarity	clear, pale straw colour
pH of 2% solution	7.2 ± 0.5
Total Nitrogen	13.0% ± 0.5
Amino Nitrogen	4.9% ± 0.5

Tryptose

MC008

A blend of peptones suitable for the cultivation of most fastidious organisms including *Neisseria gonorrhoeae, Streptococcus milleri* and *Brucella* spp. especially where rapid or profuse growth is required such as in blood culture media and blood agars.

Typical Analysis	
Appearance	beige powder
Solubility in water at 5%	total
Clarity	clear, light straw colour
pH of 2% solution	7.2 ± 0.2
Total Nitrogen	12.5% ± 0.5
Amino Nitrogen	4.9% ± 0.5

X-β-Galactoside (5-Bromo-4-Chloro-3-Indolyl-β-D-Galactopyranoside)

MC405

Description

X-β-galactoside (5-Bromo-4-chloro-3-indolyl-β-D-galactoside) is used as a chromogenic substrate for the detection of organisms capable of fermenting lactose (lac Z-positive organisms). In combination with IPTG (MC402), X-β-Galactoside can be used in transformation experiments involving *Escherichia coli* where disruption of the lac Z operon is used as a marker for DNA insertion.

Xylose

MC032

Description

Xylose for use in microbiological culture media.

Yeast Extract Powder

MC001

Prepared by the autolysis of *Saccharomyces cerevisae* under thermostatically controlled conditions to protect the B vitamins and other heat labile constituents. This extract provides a mixture of amino acids, peptides, vitamins and carbohydrates making it suitable for many applications.

Typical Analysis	
Appearance	yellow powder
Solubility in water at 5%	total
Clarity	clear, pale yellow
pH of 2% solution	7.0 ± 0.2
Total Nitrogen	10.5% ± 0.5
Amino Nitrogen	5.3% ± 0.5

TYPICAL ANALYSIS									
	MC007 Acid Hydrolysed Casein	**MC024 Bacteriological Peptone**	**MC004 Balanced Peptone No. 1**	**MC009 Mycological Peptone**	**MC011 Proteose Peptone A**	**MC003 Soy Peptone**	**MC005 Tryptone**	**MC008 Tryptose**	**MC001 Yeast Extract**
Total Nitrogen	8.3%	12.0%	12.5%	12.6%	12.0%	9.0-9.4%	12.8%	12.7%	10.5%
Total AminoNitrogen	6.1%	5.0%	5.1%	1.4%	5.8%	1.6-1.8%	4.8%	4.9%	5.3%
Amino N/Total N.	73.4%	41.7%	40.8%	11.1%	48.0%	17.7%	37.5%	38.7%	50.4%
Total Amino Acid Assay (mg/g)									
Lysine	53.5	62.0	75.3	38.9	48.2	43.1	79.7	77.5	49.0
Histidine	18.7	21.0	26.8	12.7	15.6	16.2	31.2	29.0	14.0
Arginine	22.1	32.0	41.0	55.0	50.6	39.2	37.5	39.2	27.0
Aspartic Acid	44.1	69.0	58.4	69.9	63.9	74.4	62.8	60.6	52.0
Threonine	23.5	40.0	31.1	17.7	27.5	21.7	36.9	34.0	33.0
Serine	30.0	40.0	40.3	26.2	36.2	27.0	50.3	45.3	34.0
Glutamic Acid	130.0	160.0	138.9	103.5	100.2	110.0	184.0	161.5	73.0
Proline	52.2	46.0	61.0	74.5	55.6	28.0	82.1	71.6	26.0
Glycine	11.1	29.0	44.5	105.9	83.38	22.6	15.6	30.0	25.0
Alanine	19.0	39.0	38.1	49.9	52.3	23.1	26.9	32.5	51.0
Cystine	1.1	1.0	1.1	2.7	8.4	5.3	2.2	1.6	6.0
Valine	35.2	45.0	45.3	22.9	35.2	23.7	59.2	52.3	37.0
Methionine	10.4	8.0	19.1	7.0	12.3	6.2	25.0	22.1	9.0
Isoleucine	27.9	33.0	43.8	18.7	23.3	26.8	58.5	51.1	73.0
Leucine	30.9	65.0	65.3	32.0	55.5	38.6	83.5	74.4	73.0
Tyrosine	12.7	12.0	9.8	12.8	13.3	16.8	14.7	12.2	12.0
Phenylalanine	17.0	34.0	31.8	20.2	27.2	22.7	42.4	37.1	25.0
Tryptophane	-	3.0	4.9	1.9	4.6	3.7	6.6	5.7	9.0

7. Sterile Additives and Ready Prepared Media

Availability

Sterile additive products are offered in ready-to-use format. Each product has been prepared with an appropriate sterilisation procedure, e.g. aseptic preparation, filtration or irradiation, which will not affect the performance of the product. All sterile reagents should be stored at 2-8ºC away from light.

The ready prepared media offer a cost effective alternative to preparation of large quantities of small volume dispensed media.

Egg Yolk Emulsion

X073

Description

A sterile emulsion of egg yolks for use in bacteriological culture media. It may be added directly to nutrient media for the identification of *Clostridium, Bacillus* and *Staphylococcus* species by their lipase and/or lecithinase activity.

Presented in 100ml bottles, add 100ml to 900ml of Bacillus cereus medium, or 50ml to Blood Agar Base LAB028 containing Fildes extract and serum.

Technique

For detection of lecithinase activity (especially in the investigation of `bitty cream' conditions) add 0.5 or 1.0ml of the emulsion to 10ml of sterile Blood Agar Base (LAB028) or Nutrient Broth No.2 (LAB014). In order to clear the medium, raise the final salt concentration by the addition of 1% of sodium chloride. After incubation for up to 5 days at 35ºC, lecithinase-producers render the broth opalescent, whilst, on the solid medium, the colonies are surrounded by opaque zones.

Egg Yolk Tellurite Emulsion

X085

Description

A sterile egg yolk emulsion containing potassium tellurite for use in Baird-Parker Medium Base (ISO) LAB285 or Baird-Parker Medium Base LAB085.

Formulation	mg/litre
Potassium tellurite	0.20% w/v
Egg yolk emulsion	20%
Final concentration of tellurite in medium is 0.01% w/v.	

Appearance

Yellow, opaque solution with resuspendable deposit.

Storage

Store at room temperature before first opening. Once opened store at 2-8°C. A deposit may form during storage. This is normal and will not affect the product. Mix well before use.

Method for reconstitution

Add 50mL (5%) X085 to 1 litre of sterilised media, tempered to 48ºC.

Lactic Acid 10%

X037

Description

A sterile solution of 10% lactic acid added to culture media to reduce the pH, in order to suppress bacterial growth.

Directions

Add 1 vial (5ml) to 1 litre of Malt Extract Agar LAB037.

Add 2 vials (10ml) to 1 litre of Potato Dextrose Agar LAB098.

Addition of X037 should be carried out after sterilisation and cooling the medium to 47ºC.

Listeria Selective Diagnostic Supplement

X010 / X210

Description

A selective diagnostic supplement containing cycloheximide, nalidixic acid and phosphatidylinostiol for the isolation and presumptive identification of *Listeria monocytogenes*. For use with HAL010 Harelquin™ Listeria Chromogenic Agar.

Final Concentration	Concentration Per vial		Conc. in medium
	X010	X210	(mg/litre)
Cycloheximide	25 mg	125 mg	50
Nalidixic acid	10 mg	50 mg	20
Phosphatidylinositol	~300 mg	~1500 mg	~600

Hazard classification

NR – Not Regulated

Instructions for use

Pre-heat X010/X210 to 48-50°C and aseptically add to sterilised medium cooled to 48-50°C.

- Add 1 vial X010 and 1 vial of X072 to 475mL medium **OR**
- Add 1 vial of X210 and 5 vials of X072 to 2375mL medium.

Storage

2-8°C in the dark

References

ISO 11290-1:1997 Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Listeria monocytogenes* - Part 1: Detection method. Incorporating Amendment 1.

Potassium Tellurite Solution 1%

X043

Description

Potassium Tellurite Solution for use with Modified Giolitti and Cantoni Broth (ISO) LAB219 for the detection and enumeration of coagulase-positive staphylococci.

Formulation	Per vial	Conc. in medium
Potassium tellurite	10 g/L	0.1 g/L

Addition

Aseptically add to media tempered to 44-47ºC sufficient volume of X043 1% potassium tellurite to give a final concentration of 0.1g/L. For example, add 0.1ml X043 to 9ml of single strength base or add 0.2ml X043 to 10ml of double strength base.

Appearance

Clear, colourless liquid

Hazard classification

NR – Not regulated

Storage

2-8°C in the dark

References

ISO 6888-3:2003 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 3: Detection & MPN technique for low numbers.

Potassium Tellurite Solution 3.5%

X027

Description

A sterile solution of 3.5% potassium tellurite. A selective agent for addition to Hoyles's Medium (LAB027), for the selective isolation and differentiation of *Corynebacterium diphtheriae*.

Directions

Add 1 vial (2ml) to 200ml of Hoyle's Medium (LAB027).

Urea Solution 40%

X130, X135

Description

A sterile solution of 40% urea, for addition to Urea Broth Base (LAB131) and Urea Agar Base (LAB130) for the detection of urease production by *Proteus* spp.

Directions

Add 1 vial X130 (5ml) to 95ml of Urea Broth Base (LAB131) and Urea Agar Base (LAB130). X135 contains 100ml of solution for production of larger volumes of Urea Broth or Agar.

NOTES

NOTES

8. INDEXES

By product code (V1.0)

Dehydrated Culture Media

Harlequin™ Dehydrated Culture Media

Media Constituents

MC009	Mycological Peptone	Page 120
MC010	Ox Bile	Page 120
MC011	Proteose Peptone A	Page 120
MC013	Glucose (Dextrose)	Page 119
MC014	Mannitol (D-Mannitol)	Page 120
MC015	Gelatin Powder	Page 119
MC016	Sodium Thioglycollate	Page 121
MC017	Sodium Chloride (Bacteriological)	Page 121
MC018	Meat Peptone	Page 120
MC019	Beef Extract	Page 118
MC020	Lactose	Page 119
MC022	Maltose Monohydrate	Page 120
MC023	Malt Extract	Page 120
MC024	Bacteriological Peptone	Page 118
MC025	Bile Salts No.3	Page 118
MC026	Sodium Desoxycholate	Page 121
MC027	Skim Milk Powder	Page 121
MC029	Agar No.4 - Plant Tissue Culture Grade	Page 118
MC033	FMV Peptones (Foot and Mouth Vaccine)	Page 118
MC034	Liver Digest	Page 119
MC035	Special Tryptone	Page 121
MC040	Lactalbumin Hydrolysate	Page 119
MC041	Lecithin	Page 119
MC402	IPTG (Isopropyl thiogalacatopyranoside) dioxane free	Page 119
MC405	X-gal (X-B-Galactoside)	Page 122
MC406	MUG (4-methylumbelliferyl-B-D-glucuronide)	Page 120

Captivate™

CAP001	*Captivate™ O157*	Page 105
CAP002	*Captivate™ Salmonella*	Page 106
CAP003	*Captivate™ O26*	Page 106
CAP004	*Captivate™ O111*	Page 106
CAP005	*Captivate™ O103*	Page 106
CAP006	*Captivate™ O145*	Page 106

Supplements & Additives

X009	Chloramphenicol supplement	Page 115
X010	HAL010 Listeria selective diagnostic supplement	Page 124
X011	Colistin / Nalidixic acid selective supplement	Page 110
X012	Colistin & Nalidixic acid selective supplement	Page 110
X013	Colistin & Oxolinic acid selective supplement	Page 111,115
X015	Neomycin 75mg	Page 107
X016	Neomycin 100mg	Page 107
X018	Kanamycin 75mg	Page 107
X019	P-INC supplement (PNCV)	Page 114
X027	Potassium Tellurite 3.5%	Page 125
X037	Lactic acid 10% 5ml per 500ml	Page 124
X043	Potassium Tellurite solution 1%	Page 124
X068	V.C.N.T. selective supplement	Page 114
X069	L.C.T. selective supplement	Page 113
X070	L.C.A.T. selective supplement	Page 113
X072	Polymyxin, Ceftazidime selective supplement	Page 123
X072N	Nalidixic acid selective supplement	Page 113
X073	Egg Yolk Emulsion	Page 124
X074	Polymixin B	Page 108
X085	Egg Yolk Tellurite	Page 124
X086	RPF Supplement	Page 115
X089	Oxytetracycline supplement	Page 116
X090	Nalidixic Acid & Vancomycin	Page 107
X093	Cycloserine/Cefoxitin selective supplement	Page 108,109
X107	CN supplement	Page 114
X108	CFC supplement	Page 114
X109	Sulphadiazine supplement	Page 110
X110	Oleandomycin / Polymixin supplement	Page 110
X112	Cefoperazone /Amphotericin selective supplement	Page 108
X114	Modified Preston Campylobacter supplement	Page 109
X115	Campylobacter growth supplement	Page 108
X120	CIN selective supplement	Page 116
X122	C.C.C.A.F. selective supplement	Page 112
X123	C.N.C.A.F. selective supplement	Page 112
X130	Urea 40% 5ml per 95ml	Page 125
X131	CVTC supplement	Page 109
X132	CVTN supplement	Page 109
X135	Urea 40% 100 ml	Page 125
X138	N.A.C. selective supplement	Page 112
X139	N.A.N. selective supplement	Page 112
X140	Cepacia selective supplement	Page 108
X144	P.A.C. selective supplement	Page 112
X150	Novobiocin selective supplement	Page 110,115
X155	UVM I supplement	Page 113
X161	Cefixime Tellurite supplement	Page 110
X164	Half Fraser Supplement	Page 113
X165	Fraser Supplement	Page 113
X192	O.R.S.I.M. selective supplement	Page 115
X193	Polymixin B	Page 108
X194	D-Cycloserine supplement	Page 110
X195	GVPC selective supplement	Page 111
X196	BCYE growth supplement	Page 111
X197	BCYE growth supplement (no L-cysteine)	Page 111
X209	Chloramphenicol supplement	Page 115
X210	HAL010 Listeria selective diagnostic supplement	Page 124
X212	Cefoperazone/Amphotericin selective supplement	Page 108
X214	VPT selective supplement	Page 109
X215	Neomycin 75mg	Page 107
X219	P-INC supplement (PNCV)	Page 114
X271	GC growth supplement	Page 114
X274	Polymixin B	Page 113
X291	Nalidixic Acid (1 vial per litre)	Page 107
X546	VCC supplement	Page 110
X564	Half Fraser Supplement	Page 113

By product name

Dehydrated Culture Media

Harlequin™ Dehydrated Culture Media

Media Constituents

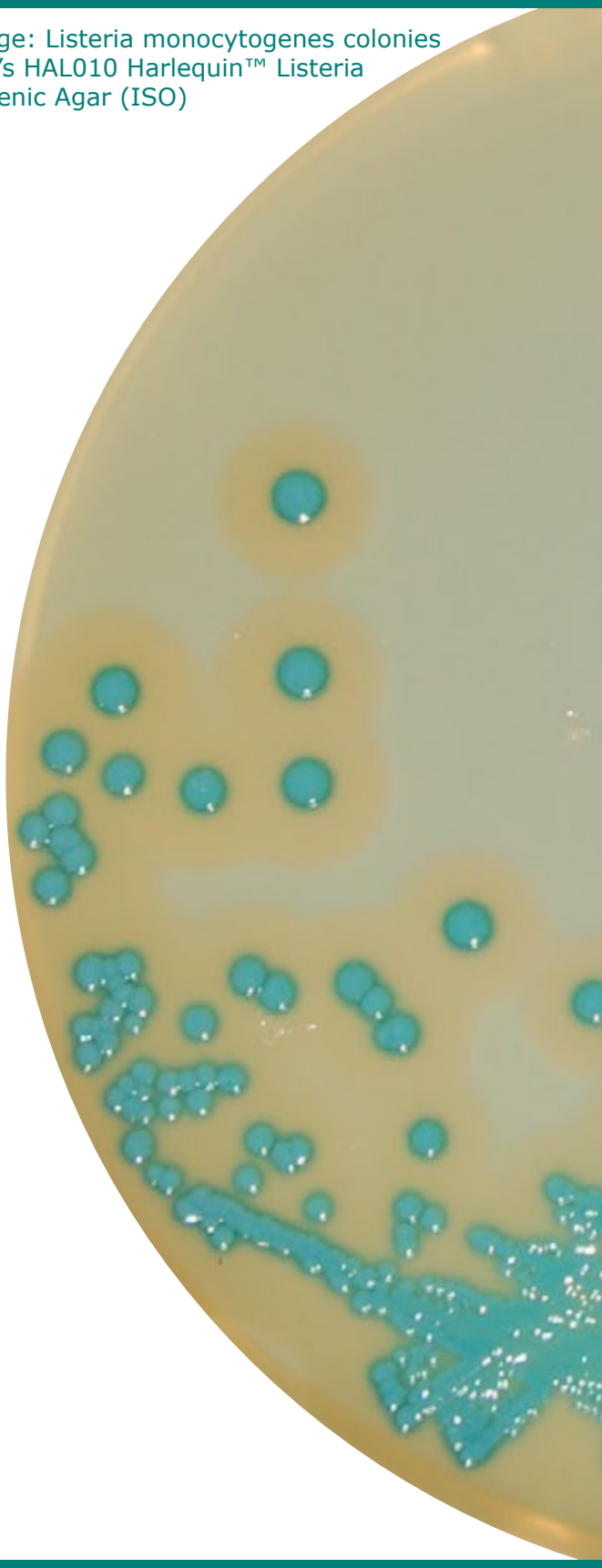
Captivate™

Supplements & Additives

NOTES

Lab M Limited Topley House • 52 Wash Lane • Bury • Lancashire • BL9 6AS • UK
Telephone: **+44 (0) 161 797 5729** • Email: **info@labm.com**
Facsimile: **+44 (0) 161 762 9322** • Web: **www.labm.com**

Main image: Listeria monocytogenes colonies on Lab M's HAL010 Harlequin™ Listeria Chromogenic Agar (ISO)



Your Local Distributor is