Lactobacillus MRS Agar(MRS Agar) M641I

Intended use

Recommended for the isolation and enumeration of lactic acid bacteria from meat and meat products.The composition and performance criteria of this medium are as per the specifications laid down in ISO 1995, Draft ISO/DIS 13721.

Composition**

Ingredients	Gms / Litre
HM extract B#	8.000
Peptone	10.000
Yeast extract	5.000
Ammonium citrate	2.000
Sodium acetate	5.000
Magnesium sulphate, heptahydrate	0.200
Manganese sulphate, tetrahydrate	0.050
Dipotassium phosphate	2.000
Glucose, anhydrous	20.000
Polysorbate 80 (Tween 80)	1.000
Agar	12.000
Final pH (at 25°C)	5.7±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 65.13 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Lactobacilli MRS medium is based on the formulation of deMan, Rogosa and Sharpe (1) with slight modification. It supports luxuriant growth of all Lactobacilli from oral cavity (1), dairy products (6), foods (8), faeces (7) and other sources (5). Lactobacillus MRS Agar is recommended by ISO Committee (2).

Peptone and HM extract B supplies nitrogenous and carbonaceous compounds, long chain amino acids and other essential growth nutrients. Yeast extract provides vitamin B complex and glucose is the fermentable carbohydrate and energy source. Polysorbate 80 supplies fatty acids required for the metabolism of Lactobacilli. Sodium acetate and ammonium citrate inhibit Streptococci, moulds and many other microorganisms. Phosphates provide good buffering action in the media.

Lactobacilli are microaerophillic and generally require layer plates for aerobic cultivation on solid media. When the medium is set, another layer of un-inoculated MRS Agar is poured over the surface to produce a layer plate (5). Lactobacilli isolated on MRS Agar should be further confirmed biochemically

Type of specimen

Clinical samples: faeces; Food and dairy samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,8,9).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard

precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Biochemical identification required for confirmation of *lactobacillus* species.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to light yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium

Medium to dark amber coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 6.51% w/v aqueous solution at 25°C. pH : 5.7±0.2

pH

5.50-5.90

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours (longer if neccesary) (with 5% CO2)

Organism	Inoculum (CFU)	Growth	Recovery
Lactobacillus acidophilus ATCC 4356 (00098)*	50-100	luxuriant	>=50%
Lactobacillus casei ATCC 9595	50-100	luxuriant	>=50%
Lactobacillus fermentum ATCC 9338	50-100	luxuriant	>=50%
Lactobacillus plantarum ATCC 8014	50-100	luxuriant	>=50%
Lactococcus lactis subsp. lactis ATCC 19435 (00016*)	50-100	luxuriant	>=50%
Lactococcus sakei ATCC 15521 (00015*)	50-100	luxuriant	>=50%
Pediococcus damnosus ATCC 29358	50-100	luxuriant	>=50%
Pediococcus pentosaceus ATCC 33316 (00158*)	50-100	luxuriant	<=10%
Bifidobacterium bifidum# ATCC 11863	50-100	luxuriant	<=10%
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibition	0%
Bacillus cereus ATCC 11778 (00001*)	>=10^4	inhibition	0%

Key : # Growth under anaerobic conditions for 72 hours, *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1.deMan J., Rogosa M. and Sharpe M., 1960, J. Appl. Bacteriol., 23:130.

2.International Organization for Standardization (ISO), 1995, Draft ISO/DIS, 13721

3.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

5.MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol.1, Williams and Wilkins, Baltimore.

6.Marshall R.T. (Ed.), 1992, Standard Methods for the Examination of Dairy Products, 16th ed., APHA, Washington,D.C.

7.Sabine and Vaselekos, 1965, Nature, 206:960.

8.Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

9.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 04 / 2019

IVD — In vitro diagnostic medical device

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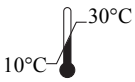

Storage temperature

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Hugh Leifson Medium

M826

Intended use:

Recommended for detecting aerobic and anaerobic breakdown of glucose.

Composition**

Ingredients	Gms / Litre
Peptone	2.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	0.300
Dextrose (Glucose)	10.000
Bromothymol blue	0.050
Agar	2.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 19.35 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Dispense into test tubes in duplicate for aerobic and anaerobic fermentation. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the tubed medium in an upright position

Principle And Interpretation

Hugh Leifson Medium was formulated by Hugh and Leifson (2). They described the taxonomic significance of fermentative and oxidative metabolism of carbohydrates in gram-negative intestinal bacteria.

There are two ways of utilizing carbohydrates by microorganisms, namely fermentation and oxidation. This property may be frequently used for the differentiation of some bacteria.

The medium contains a high concentration of carbohydrate and low concentration of peptone to avoid the possibility of an aerobic organism utilizing peptone and producing an alkaline condition which would neutralize slight acidity produced by an oxidative organism (1,5). Dipotassium phosphate promotes fermentation and acts as pH controlling buffer. Agar concentration enables the determination of motility and aids in distribution of acid throughout the tube produced at the surface of medium. Bromthymol blue is the pH indicator.

Type of specimen

Clinical samples- Swabs of mouth, mucosae, oropharynx and upper respiratory tract; Food and dairy samples

Specimen Collection and Handling

The tubes for aerobic and anaerobic fermentation are inoculated and the agar surface of one tube of duplicate is covered with layer of sterile paraffin oil, about 25 mm thickness and incubated at 37°C. Oxidative organisms produce acid in unsealed tube with little or no growth and no acid formation in sealed tube while fermentative organisms produce acid in both sealed and unsealed tubes. If acid is produced, it is indicated by change in colour from greenish blue to yellow throughout the medium. Liquid paraffin tube used should be dry sterilized at 160-170°C for 2 hours. Wet sterilization with high pressure is not sufficient for the purpose.

Warning and Precautions :

In Vitro diagnostic Use. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidleines should be followed while handling clincal specimens. Saftey guidelines may be referred in individual safety data sheets.

Limitations :

1. Other biochemicla tests must be performed in conjunction for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Light yellow to bluish green homogeneous free flowing powder

Gelling
Semisolid,comparable with 0.2% Agar gel.

Colour and Clarity of prepared medium
Greenish blue coloured, clear to slightly opalescent gel forms in tubes as butts

Reaction
Reaction of 1.94% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Motility	Aerobic fermentation	Anaerobic fermentatoin
#Klebsiella aerogenes ATCC 13048 (00175*)	50-100	positive,growth away from stabline causing turbidity	acid (yellow) and gas production	acid (yellow) and gas production
Escherichia coli ATCC 25922 (00013*)	50-100	positive, growth away from stabline causing turbidity	acid (yellow) and gas production,	acid (yellow) and gas production
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	positive,growth away from stabline causing turbidity	acid (yellow) production	unchanged (green) or alkaline (blue)
Salmonella Typhi ATCC 6539	50-100	positive, growth away from stabline causing turbidity	acid (yellow) and gas production	acid (yellow) and gas production
Shigella sonnei ATCC 25931	50-100	negative, growth along the stabline, surrounding medium	acid (yellow) production	acid (yellow) and gas production

Key : *Corresponding WDCM numbers. (#) Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Finegold S. M., Martin W. J., and Scott E. G., 1978, Bailey and Scotts Diagnostic Microbiology, 5th Ed., The C.V. Mosby Co., St. Louis.
2. Hugh and Leifson, 1953, J. Bacteriol., 66:24.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

5. MacFaddin J.F., 1985, Media for Isolation-Cultivation-Identification- Maintenance of Medical Bacteria,Vol. I, Williams and Wilkins, Baltimore.

Revision : 02/2021

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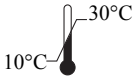

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PNY Medium

M835

Intended Use:

Recommended for cultivation and isolation of *Lactobacillus* species.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
Yeast extract	5.000
Dextrose (Glucose)	5.000
Potassium dihydrogen phosphate	0.500
Dipotassium hydrogen phosphate	0.500
Magnesium sulphate	0.250
Manganese sulphate	0.010
Ferrous sulphate	0.010
Sodium chloride	0.010
Zinc sulphate	0.001
Copper sulphate	0.001
Cobalt sulphate	0.001
Agar	15.000
Final pH (at 25°C)	6.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 31.28 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Lactobacilli grow in a variety of habitats, wherever high levels of soluble carbohydrate, protein background products, vitamins and a low oxygen tension occur (1). These sites include the oral cavity, the intestinal tract (8,2), the vagina (5), food products (6) and dairy products (7). PNY Medium is formulated for isolation and cultivation of *Lactobacillus* species. Peptone and yeast extract provide amino acids, other nitrogenous nutrients, vitamin B complex etc. Dextrose is the fermentable carbohydrate. The phosphates form buffering system while sodium chloride maintains osmotic equilibrium. Other salts supply essential nutrients for the growth of the organisms.

Type of specimen

Clinical samples - Swabs from oral cavity, Faeces, etc.; Food and dairy samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Further biochemical and serological tests must be carried out for complete identification.
2. Individual organisms differ in growth due to nutritional variations.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow coloured homogeneous free flowing powder

Gelling

Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light yellow coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 3.1% w/v aqueous solution at 25°C. pH : 6.0±0.2

pH

5.80-6.20

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours in presence of 3-5% CO2.

Organism	Inoculum (CFU)	Growth
Lactobacillus casei ATCC 9595	50-100	luxuriant
Lactobacillus leichmannii ATCC 4797	50-100	luxuriant
Lactobacillus plantarum ATCC 8014	50-100	luxuriant

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Balows A., Truper H. G., Dworkin M., Harder W., Schleifer K. H., (Eds.), The Prokaryotes, 2nd Edi, 1992, Springer-Verlag
2. Wiseman R. F, Sarles W. B, Benton D. A, Harper A. E and Elvehjem C.A., 1956, J. Bacteriol., 72:723.
3. Ellis R. F. and Sarles W. B., 1958, J. Bacteriol., 75:272.
4. Rogosa M. and Sharpe M. E., 1960, J. Gen. Microbiol., 23:197
5. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
6. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
8. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 03/2022

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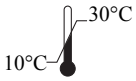	Storage temperature
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Clostridium Difficile Agar Base

M836

Intended Use:

Recommended for selective isolation of *Clostridium difficile* from food and certain pathological specimens.

Composition**

Ingredients	Gms / Litre
Proteose peptone	40.000
Disodium hydrogen phosphate	5.000
Potassium dihydrogen phosphate	1.000
Magnesium sulphate	0.100
Sodium chloride	2.000
Fructose	6.000
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 34.55 grams in 500 ml purified / distilled water. Heat gently to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add rehydrated contents of 1 vial of Clostridium Difficile Supplement (FD010) together with 7% (v/v) defibrinated Horse blood or Sheep blood. Mix well and pour into sterile Petri plates.

Principle And Interpretation

The spectrum of disease caused by *Clostridium difficile* (a pathogenic *Clostridium* affecting the bowel) ranges from pseudomembranous colitis (PMC) through antibiotic associated colitis (AAC). It also includes chronic inflammatory bowel diseases, post-operative diarrhoea and non-antibiotic associated diarrhoea (1). Smith and King (6) first reported the presence of *C.difficile* in human infections. George et al (2) recommended the use of a fructose-containing medium with egg yolk for the isolation of *C.difficile* from faecal specimens. The medium was made inhibitory to the accompanying flora by the addition of the selective agents namely, D-cycloserine and cefoxitin.

This medium does not contain neutral red indicator, as in the original formulation, as it is recommended for use with sheep or horse blood (2). Clostridium Difficile Agar Base is used for the primary isolation of *C.difficile*from faecal specimens.The medium composition is designed so as to obtain luxuriant growth of *C.difficile*. The selective agents D-cycloserine and cefoxitin used in the medium inhibit the growth of majority of *Enterobacteriaceae* and also *Enterococcus faecalis,* Staphylococci, gram-negative anaerobic bacilli and *Clostridium* species other than C. difficile, which may be found abundantly in faecal samples. Addition of 7% v/v horse blood to the base increases the recovery of C. difficile and also increases its colony size.

Spread a part of the faecal sample on the medium to obtain isolated colonies. Incubate the plates anaerobically at 37°C for 18 - 48 hours. C. difficile forms grayish white, irregular, raised and opaque colonies, 4-6 mm in diameter, after 48 hours.

Typical gram stain morphology of C. difficile may not be seen in colonies taken from this medium due to the presence of antibiotics. Subculture on Blood Agar (M073) to obtain characteristic morphology. *C.difficile* colonies will not exhibit the typical fluorescence and colour of colony on this medium whereas other Clostridia can give fluorescence. Therefore, for complete identification and confirmation, other tests like gram staining, morphology, biochemicals, specific cytotoxin and clinical observation should be carried out.

Type of specimen

Clinical samples - Stool sample; Food samples.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Further biochemical and serological tests must be carried out for further identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Basal medium: Light amber coloured clear to slightly opalescent gel. After addition 7% v/v defibrinated horse blood: Cherry red coloured, opaque gel forms in Petri plates.

Reaction
Reaction of 6.91% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
Cultural characteristics observed under anaerobic condition with added Clostridium Difficile Supplement(FD010) and 7% v/v defibrinated horse blood, after an incubation at 35-37°C for 48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Clostridium difficile ATCC 11204	50-100	good-luxuriant	>=50%	greyish-white
Shigella flexneri ATCC 12022	>=10^4	inhibited	0%	
Escherichia coli ATCC 25922	>=10^4	inhibited	0%	
Staphylococcus aureus ATCC 25923	>=10^4	inhibited	0%	

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 14th Ed., Churchill Livingstone.
2. George W. L., Sutter V. L., Citron D., and Finegold S. M., 1979, J.Clin. Microbiol., 9:214
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
6. Smith L. D. S. and King E. O., 1962, J. Bacteriol., 84:65.

Revision :02 / 2019

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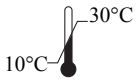

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Perfringens Agar Base (T. S. C. /S. F. P. Agar Base) M837

Intended Use:

Perfringens Agar Base with the addition of selective supplement and enrichment, it is used for the presumptive identification and enumeration of *Clostridium perfringens.*

Composition**

Ingredients	Gms / Litre
Tryptose	15.000
HM peptone B #	5.000
Soya peptone	5.000
Yeast extract	5.000
Sodium metabisulphite	1.000
Ferric ammonium citrate	1.000
Agar	15.000
Final pH (at 25°C)	7.6±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Beef extract

Directions

Suspend 23.5 grams in 475 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°) for 15 minutes. Cool to 45-50°C. Add 25 ml of Egg Yolk Emulsion (FD045) and rehydrated contents of 1 vial of S.F.P. Supplement (FD013) / T.S.C. Supplement (FD014). Alternatively if fluorogenic detection is desired add rehydrated contents of Clostridium perfringens supplements (FD243) instead of FD013/FD014. Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Tryptose Sulphite Cycloserine Agar (TSC) was originally formulated by Harmon et al (1) for the enumeration of *C. perfringens* from food. TSC Agar has been documented as one of the most useful media for the quantitative recovery of *C. perfringens* while suppressing growth of other facultative anaerobes (2). TSC Agar Base (with FD014) or SFP Agar Base (with FD013) is comparable in performance for isolation of C. perfringens (3,4). Perfringens Agar Base is also recommended by APHA (5). Perfringens Agar Base can be made selective either by addition of D-cycloserine (FD014) (1, 2) or Kanamycin and Polymyxin B (FD013) (6).

Tryptose, Soya peptone, yeast extract, HM peptone B provide nitrogenous compounds, carbon, sulphur, vitamin B complex and trace elements essential for clostridial growth. Sodium metabisulphite and ferric ammonium citrate act as an indicator of sulphite reduction, indicated by black coloured colonies. D-Cycloserine (FD014), Kanamycin and Polymyxin B (FD013) help in the selective isolation of *C. perfringens* by inhibiting accompanying flora. Egg yolk emulsion serves as a source of lecithin utilized by *C. perfringens* (M837).

Type of specimen

Clinical- stool, abscess; Food samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8). For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Further biochemical and serological tests must be carried out for further identification.
2. Some organism may show poor growth due to nutritional variation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Light yellow to brownish yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% Agar gel
Colour and Clarity of prepared medium
Basal medium :Amber coloured clear to slightly opalescent gel. After Addition of Egg Yolk Emlusion (FD045) : Yellow coloured opaque gel forms in Petri plates
Reaction
Reaction of 4.7% w/v aqueous solution at 25°C. pH : 7.6±0.2
pH
7.40-7.80
Cultural Response
Cultural characteristics observed under anaerobic condition with added TSC Supplement (FD014)/S.F.P Supplement (FD013)/Clostridium Perfringens Supplement (FD243) and Egg Yolk Emulsion (FD045), after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Sulphite Reduction	Lecithinase/ Haloes	Fluorescence
Clostridium perfringens ATCC 12924	50-100	luxuriant	>=50%	positive, blackening of medium	Positive reaction, opaque zone around the colony	Positive Reaction
Clostridium sordellii ATCC 9714	>=10^4	inhibited	0%			

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

1. Harmon S. M., Kauttar D.A. and Peiler J. T., 1971, Appl. Microbiol., 22:688.
2. Harmon S. M. and Kautter D.A., 1987, J. Asso. Off. Anal. Chem., 70: 994.
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Revision : 04/2022

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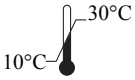

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Yersinia Selective Agar Base

M843

Intended use

Recommended for the selective isolation and enumeration of *Yersinia enterocolitica* from clinical specimens and food samples.

Composition**

Ingredients	Gms / Litre
Peptone, special	20.000
Yeast extract	2.000
Mannitol	20.000
Sodium pyruvate	2.000
Sodium chloride	1.000
Magnesium sulphate	0.010
Sodium deoxycholate	0.500
Neutral red	0.030
Crystal violet	0.001
Agar	12.500
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 29.02 grams in 500 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add reconstituted contents of 1 vial of Yersinia Selective Supplement (FD034). Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Yersinia enterocolitica is widely distributed in lakes and reservoirs. Epizootic outbreaks of diarrhea, lymphadenopathy, pneumonia and spontaneous abortions occur in various animals. It is the most common species of *Yersinia* recovered from clinical specimens. *Y. enterocolitica* is biochemically more active at room temperature than at 37°C. Yersinia Selective Agar Base with added Yersinia Selective Supplement is used to isolate *Y. enterocolitica* from clinical and non-clinical specimens. The formulation is based on CIN Agar of Schiemann (1,2) and is recommended by ISO Committee (3). Schiemann (1) modified his previous formula of CIN medium by replacing bile salts with sodium deoxycholate.

The medium differentiates between mannitol fermenting and non-fermenting bacteria. Microorganisms that ferment the sugar mannitol acidify the medium and cause a localized drop in pH around the colonies. In presence of neutral red, the colonies take red colour. Mannitol negative organisms form colourless and translucent colonies. The medium is selective due to the of presencesodium deoxycholate and crystal violet, which inhibit gram-positive and a number of gram-negative bacteria. Addition of antibiotic supplement makes it highly selective for *Yersinia.* Typical colonies of *Y. enterocolitica* will form dark red colonies resembling bull's eye, which are normally surrounded by a transparent border. Colony size, smoothness and ratio of the border to center diameter may vary among different serotypes.

For the isolation of *Y. enterocolitica* by direct plating and pour plating, inoculate the specimen directly onto the medium. Incubate at 22-32°C for 24-48 hours or suspend the sample (food, faeces, etc.) in sterile Phosphate Buffer Saline and incubate for upto 21 days (4) at 4°C. Periodically subculture samples onto Yersinia Agar Plate and incubate as above.

Type of specimen

Clinical samples - faeces ; Food and dairy samples.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (4,7,8).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be

referred in individual safety data sheets.

Limitations :

1. *Serratia liquefaciens, Citrobacter freundi* and *Enterobacter agglomerans* may resemble *Y.enterocolitica* that can be further identified by biochemical tests.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.25% Agar gel.

Colour and Clarity of prepared medium

Orange red coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 5.8% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural characteristics observed with added Yesinia Selective Supplement (FD034) after an incubation at 22-32°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%	
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	>=10^4	inhibited	0%	
Proteus mirabilis ATCC 25933	>=10^4	inhibited	0%	
Pseudomonas aeruginosa ATCC 27853 (00025*)	>=10^4	inhibited	0%	
Yersinia enterocolitica ATCC 27729	50-100	good-luxuriant	>=50%	transluscent with dark pink centre & bile precipitate.
Yersinia enterocolitica ATCC 23715 (00160*)	50-100	good-luxuriant	>=50%	transluscent with dark pink centre & bile precipitate.
Yersinia enterocolitica ATCC 9610 (00038*)	50-100	good-luxuriant	>=50%	transluscent with dark pink centre & bile precipitate.

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1.Schiemann D. A., 1979, Can. J. Microbiol., 25: 1298.
2.Schiemann D. A., 1980, Can. J. Microbiol., 26: 1232.
3.International Organization for Standardization (ISO), 1994 Draft ISO/DIS 10273.
4.Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.
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Revision : 05/2022

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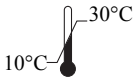

Storage temperature


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Diphtheria Virulence Agar Base

M882

Intended Use:

Recommended for determining toxigenicity of *Corynebacterium diphtheriae.*

Composition**

Ingredients	Gms / Litre
Proteose peptone	20.000
Sodium chloride	2.500
Agar	15.000
Final pH (at 25°C)	7.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37.5 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 55-60°C. Aseptically add 2 ml sterile KL Virulence Enrichment (FD072) and 0.5 ml sterile 1% Potassium Tellurite (FD052) to a 100 mm Petri plate and quickly add 10 ml of sterile Diphtheria Virulence Agar Base. Before the medium solidifies, place a filter paper strip saturated with potent Diphtheria antitoxin across the diameter of the plate. Allow the strip to sink to the bottom of the plate. Inoculate the plate with heavy inoculum across the strip.

Principle And Interpretation

Corynebacterium diptheriae is a principle human pathogen and owes its pathogenicity to the production of a potent exotoxin active on a variety of tissue including heart muscles and peripheral nerves (2). Toxin diffusing from a streak culture of suspected *C. diphtheriae* is demonstrated by the formation of a white line of precipitate where it meets with diphtheria antitoxin diffusing from a strip of filter paper embedded in the agar. In vitro toxigenicity (virulence) of *C. diphtheriae* was first described by Elek (3). Eleks technique was further improved by King, Frobisher and Parsons (7) by the use of a standardized medium. This medium gave results comparable with animal inoculation test. Also it was found that proteose peptone supported toxin production in addition to maintaining the consistency of results. Hermann et al (4) developed a non-serum based enrichment to overcome the irregularities encountered during the usage of horse, sheep or rabbit serum based enrichments. These non-serum based enrichments consist of Acicase™, tween 80 and glycerol (8).

Upon incubation of the inoculated plate, a line of precipitin is observed for toxigenic strains.

Proteose peptone provides the carbon and nitrogen sources required for good growth of a wide variety of organisms and also for toxin production. Sodium chloride maintains the osmotic balance of the medium. Agar is incorporated as the solidifying agent. Potassium tellurite inhibits most gram-negative bacteria except *Corynebacterium* species, *Streptococcus mitis, Streptococcus salivarius* and Enterococci. *Staphylococcus epidermidis* may exhibit growth. False positive results may also be encountered. Therefore, a positive control has to always be run in parallel (9). *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis* may also produce line of precipitation (1).

Type of specimen

Clinical samples - Throat swab

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. False positive results may also be encountered. Hence, a positive control has to always be run in parallel (9)

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Medium amber coloured, slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.75% w/v aqueous solution at 25°C. pH : 7.8±0.2

pH

7.60-8.00

Cultural Response

Cultural characteristics observed with added KL Virulence Enrichment (FD072) and 0.5 ml of 1% Potassium tellurite solution (FD052) after an incubation at 35-37°C for 24-72 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Line of precipitin
Bacillus subtilis subsp. *spizizenii ATCC 6633* (00003*)	>=10^4	inhibited	0%	
Corynebacterium diphtheriae type gravis	50-100	luxuriant	>=50%	positive
Corynebacterium diphtheriae type intermedius	50-100	luxuriant	>=50%	positive
Corynebactrium diphtheriae type mitis	50-100	luxuriant	>=50%	positive
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	none-poor	<=10%	

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1. Branson, 1972, Methods in Clinical Bacteriology, Charles C. Thomas,Springfield, III
2. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology,1996, 14th Edition, Churchill Livingstone
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Revision :02 / 2019

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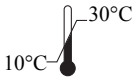

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Andrade Peptone Water

M885

Intended Use:

A basal medium which; with carbohydrate addition is used to study fermentation reactions.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Sodium chloride	5.000
Andrade indicator	0.100
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 15.1 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely and dispense in test tubes containing inverted Durhams tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to room temperature and aseptically add sterile stock solution of carbohydrate to a final concentration of 0.5% to 1.0% (w/v).

Principle And Interpretation

Bacteria differ widely in their ability to metabolize carbohydrates and related compounds. Carbohydrate fermentation reactions aids in the differentiation and identification of various bacteria. Andrade Peptone Water is the most commonly used media for carbohydrate fermentation (5). Desired carbohydrate is added to the medium, which is inoculated with the test organism. If the test organism metabolizes the added carbohydrate, acids are produced, thereby lowering the pH of the medium. This causes a subsequent colour change of the indicator, from colourless to pink to red. If the added carbohydrate is not metabolized, the medium remains pale tan to straw coloured. Gas produced during fermentation is collected in the Durhams tube.

The peptone used in the medium is free from fermentable carbohydrates (1,5) and the medium is also free from nitrates which may interfere with gas production. Andrade indicator is a solution of acid fuchsin which when titrated with sodium hydroxide; changes colour from pink to yellow. The Andrade indicator changes colour from yellow to pink as the pH decreases (5). The medium is pink when hot but becomes straw coloured on cooling. Test carbohydrate solutions should be sterilized separately and aseptically added to sterile Andrade Peptone Water. Use fresh cultures of organisms only which have been presumptively identified by Gram staining and colony morphology. The biochemical identification of organisms capable of growing in this medium is made by various sugar fermentation results (1,2,6).

Type of specimen

Food samples, Pure isolate

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (7).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets

Limitations

1. Fresh cultures should be used to avoid errorneous results.
2. For final identification further biochemical tests are required.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow coloured with pink tinge, homogeneous free flowing powder

Colour and Clarity of prepared medium

Light pink to straw coloured clear solution without any precipitate

Reaction

Reaction of 1.51% w/v aqueous solution at 25°C. pH : 7.4±0.2

Cultural response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Acid in absence of dextrose	Gas in absence of dextrose	Acid with added dextrose	Gas with added dextrose
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	negative reaction	negative reaction	positive reaction, colour changes to pink red	positive reaction
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	negative reaction	negative reaction	positive reaction, colour changes to pink red	positive reaction
Proteus vulgaris ATCC 13315	50-100	luxuriant	negative reaction	negative reaction	positive reaction, colour changes to pink red	positive reaction
Salmonella Typhi *ATCC 6539*	50-100	luxuriant	negative reaction	negative reaction	positive reaction, colour changes to pink red	negative reaction
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	luxuriant	negative reaction	negative reaction	positive reaction, colour changes to pink red	positive reaction
Shigella flexneri ATCC 12022 (00126*)	50-100	luxuriant	negative reaction	negative reaction	positive reaction, colour changes to pink red	negative reaction
Shigella sonnei ATCC 25931	50-100	luxuriant	negative reaction	negative reaction	positive reaction, colour changes to pink red	negative reaction

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C0in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

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2. Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Co., St. Louis.
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6. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R.H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
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Revision : 02/2020

WL Differential Agar

M1060

WL Differential Agar is recommended for selective isolation and enumeration of bacteria encountered in breweries and industrial fermentations.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	5.000
Yeast extract	4.000
Dextrose	50.000
Monopotassium phosphate	0.550
Potassium chloride	0.425
Calcium chloride	0.125
Magnesium sulphate	0.125
Ferric chloride	0.0025
Manganese sulphate	0.0025
Bromo cresol green	0.022
Cycloheximide	0.004
Agar	20.000
Final pH (at 25°C)	5.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 80.26 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. If desired, to obtain a pH of 6.5, add 1% solution of sodium bicarbonate before sterilization.

Warning : Cycloheximide is very toxic. Avoid skin contact or aerosol formation and inhalation.

Principle And Interpretation

WL (Wallerstein Laboratory) media are formulated as described by Green and Gray for the examination of materials encountered in brewing and for industrial fermentations containing mixed flora of yeast and bacteria (1, 2). Bakers yeast counts can be carried out in this medium at a pH 5.5. By adjusting the pH to 6.5, the medium can be used for obtaining counts of Baker and distillers yeast (3).

WL Nutrient and WL Differential Media are used in combination. One plate of WL Nutrient Agar and two plates of WL Differential Agar are prepared (3). The WL Nutrient Agar plate is incubated aerobically to give a total yeast count while one WL Differential Agar plate gives the count of acetic acid bacteria, *Flavobacterium* , *Proteus* and thermophilic bacterial count when incubated aerobically. The other WL Differential Agar Plate is incubated anaerobically for the growth of lactic acid bacteria and *Pediococcus* . While determining microbial counts using these media, temperature and time of incubation will vary depending on the nature of material under test. Temperatures of 25°C are employed for brewing materials while 30°C are employed for bakers yeast and alcohol fermentation mash analyses.

WL Differential medium contain yeast extract, which serves as a source of trace elements, vitamins and amino acids. Casein enzymic hydrolysate is used as a source of nitrogen, amino acids and carbon. Dextrose is the source of carbohydrate. Buffering of the medium is done by monopotassium phosphate. Potassium chloride, calcium chloride and ferric chloride are essential ions that help to maintain the osmotic balance. Magnesium sulphate and manganese sulphate are sources of divalent cations. Bromocresol green is a pH indicator. Yeasts and moulds are inhibited by cycloheximide (actidione).

Quality Control

Appearance

Light yellow to light green homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% Agar gel.

Colour and Clarity of prepared medium
Bluish green coloured clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 8.03% w/v aqueous solution at 25°C. pH : 5.5±0.2

pH
5.30-5.70

Cultural Response
M1060: Cultural characteristics observed after an incubation for 40-48 hours at 35-37°C for bacteria and at 30 ± 2°C for yeasts.

Organism	Inoculum (CFU)	Growth	Recovery
Cultural Response			
Escherichia coli ATCC 25922	50-100	luxuriant	>=50%
Lactobacillus fermentum ATCC 9338	50-100	good	40-50%
Proteus mirabilis ATCC 25933	50-100	good	40-50%
Saccharomyces cerevisiae ATCC 9763	>=10^3	inhibited	0%
Saccharomyces uvarum ATCC 28098	>=10^3	inhibited	0%

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
1. Green S. R. and Gray P. P., 1950, Wallerstein Lab. Commun., 12:43
2. Green S. R. and Gray P. P., 1950, Wallerstein Lab. Commun., 13:357
3. MacFaddin J. F., 1985, Media for Isolation- Cultivation- Identification- Maintenance of Medical Bacteria, Vol.1,Williams & Wilkins, Baltimore, Md.

Revision : 02 / 2015

Listeria Identification Agar Base (PALCAM)

M1064

Intended use

Recommended for selective isolation and identification of *Listeria* species from clinical and non-clinical samples.

Composition**

Ingredients	Gms / Litre
Peptone	23.000
Starch	1.000
Sodium chloride	5.000
Mannitol	10.000
Ammonium ferric citrate	0.500
Esculin	0.800
Dextrose (Glucose)	0.500
Lithium chloride	15.000
Phenol red	0.080
Agar	13.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 34.44 grams in 500 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add rehydrated contents of 1 vial of Listeria Selective Supplement (PALCAM) (FD061). Mix well and pour into sterile Petri plates.

Principle And Interpretation

The genus *Listeria* constitutes *Listeria monocytogenes, Listeria ivanovii, Listeria seeligeri, Listeria welshimerii, Listeria innocua, Listeria grayi, Listeria murrayi* and *Listeria denitrificans.* Among these, *L. monocytogenes* and *L. ivanovii* are associated with diseases in humans. The pathogenicity of *L.ivanovii* is uncertain. *L. monocytogenes* is found in a wide variety of habitats, including the normal microflora of healthy ruminants, gastrointestinal tract of asymptomatic humans and environmental sources including river water, sewage, soil, silage, fertilizers and decaying vegetation (8).

Listeria Identification Agar also known as Polymyxin-Acriflavin-Lithium chloride-Ceftazidime-Aesculin-Mannitol (PALCAM) Agar was formulated by Van Netten et al (5) and is recommended for the isolation of *L. monocytogenes* from foods. PALCAM medium is highly selective due to the presence of lithium chloride, ceftazidime, polymyxin B and acriflavin hydrochloride. PALCAM medium is a differential diagnostic medium utilizing two indicator systems, as esculin and ferric citrate and mannitol and phenol red.

Peptone serves as carbon, nitrogen substances, long chain amino acids, vitamins and essential growth nutrients for the organisms. Dextrose (Glucose), starch and mannitol are the carbohydrate and energy sources. Sodium chloride maintains the osmotic equilibrium of the medium. Phenol red is the pH indicator dye that exhibits changes in the pH of the medium. *L. monocytogenes* hydrolyzes esculin to form esculetin and dextrose. Esculetin reacts with ammonium ferric citrate and forms a brown-black complex seen as a black halo around colonies. *L.monocytogenes* does not ferment mannitol but contaminants such as Enterococci and Staphylococci ferment mannitol and is indicated by colour change from red to yellow. Under microaerophilic conditions, strict aerobes such as *Bacillus* species and *Pseudomonas* species are inhibited. The addition of egg yolk (2.5% v/v) to PALCAM Agar has been reported to aid repair of damaged cells (7). Medium containing blood when overlaid on PALCAM Agar enables to differentiate and enumerate haemolytic *Listeria* species (6). Depending upon the type of sample used, selective enrichment broth should be used prior to inoculation onto PALCAM Agar. Generally Listeria Selective Enrichment Medium is used for dairy products and Listeria Selective Enrichment Medium UVM (M890A), Fraser Secondary Enrichment Broth (M1083) are used for meats and poultry. On PALCAM Agar, colonies of *Listeria* appear as grey-green with a black precipitate, following inoculation and incubation at 35°C for 24-48 hours under

aerobic or microaerophilic conditions.

Type of specimen

Clinical samples - blood , body fluids, Food samples; Water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,3).
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (4).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (1).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. The medium is not differential, so further biochemical testing is required for identification between *Listeria* species.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.3% Agar gel.

Colour and Clarity of prepared medium

Red coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 6.9% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed under microaerophilic condition, with added Listeria Selective Supplement (FD061), after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colony characteristics
Enterococcus faecalis ATCC 29212 (00087)*	50-100	none-poor	<=10%	grey colonies with a brown-green halo
Listeria monocytogenes ATCC 19111 (00020)*	50-100	luxuriant	>=50%	grey-green with black center and a black halo
Listeria monocytogenes ATCC 19112	50-100	luxuriant	>=50%	grey-green with black center and a black halo
Listeria monocytogenes ATCC 19117	50-100	luxuriant	>=50%	grey-green with black center and a black halo
Listeria monocytogenes ATCC 19118	50-100	luxuriant	>=50%	grey-green with black center and a black halo

Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	50-100	none-poor	<=10%	yellow colonies with yellow halo

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
3. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.
5. Van Netten P., Peralse I, Van de Mosdik A., Curtis G.D.W., Mossel D. A.A., 1989, Int. J. Food Microbiol., 8(4):299.
6. Van Netten P., van Gaal B. and Mossel D. A. A., 1991, Lett. Appl.Microbiol, 12:20.
7. Veld P.H. and de Boer E., 1991, Int. J. Food Microbiol., 13:295.
8. Watkin J., Sleath K. P., J. Appl. Bacteriol., 50: 1-9, 1981.

Revision : 02 / 2019

IVD	In vitro diagnostic medical device
CE	CE Marking
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Sabouraud Chloramphenicol Agar

M1067

Intended use

Recommended for the selective cultivation of yeasts and moulds from clinical and non-clinical samples.

Composition**

Ingredients	Gms / Litre
Tryptone	5.000
Peptone	5.000
Dextrose (Glucose)	40.000
Chloramphenicol	0.050
Agar	15.000
Final pH (at 25°C)	5.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 65.05 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Caution: Some pathogenic fungi may produce infective spores which are easily dispersed in air, so examination should be carried out in safety cabinet.

Principle And Interpretation

Sabouraud Chloramphenicol Agar is cited as Medium C and recommended for cultivation of yeasts and moulds. This medium was described originally by Sabouraud (7) for the cultivation of fungi, particularly useful for the fungi associated with skin infections. The medium is often used with antibiotics such as Chloramphenicol (1) for the isolation of pathogenic fungi from materials containing large numbers of fungi or bacteria.

Tryptone and peptone provide nitrogenous and carbonaceous compounds, long chain amino acids, and other essential growth nutrients. Dextrose provides an energy source. Chloramphenicol inhibits a wide range of Gram-positive and Gram-negative bacteria which makes the medium selective for fungi (5). The low pH favors fungal growth and inhibits contaminating bacteria from clinical specimens (6).

Type of specimen

Clinical samples - Blood; Food and dairy samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2,8,9).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Certain pathogenic fungi may show poor growth on this medium.
2. Presence of chloramphenicol may inhibit certain pathogenic fungi.
3. Overheating of the medium may result in low productivity and softening of gel.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light amber coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 6.5% w/v aqueous solution at 25°C. pH : 5.6±0.2

pH

5.40-5.80

Cultural Response

Cultural characteristics observed after an incubation at 20-25°C for 48-72 hours (Incubate for 7 days for Trichophyton species).

Organism	Inoculum (CFU)	Growth	Recovery
Aspergillus brasiliensis ATCC 16404 (00053*)	50-100	good-luxuriant	
Candida albicans ATCC 10231 (00054*)	50-100	good-luxuriant	>=50%
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%
Lactobacillus casei ATCC 334	>=10^4	inhibited	0%
Saccharomyces cerevisiae ATCC 9763 (00058*)	50-100	good-luxuriant	>=50%
Trichophyton rubrum ATCC 28191	50-100	good-luxuriant	
Escherichia coli NCTC 9002	>=10^4	inhibited	0%
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	0%

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store the dehydrated powder and prepared medium on receipt between 15-25°C in a tightly closed container. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use.
Product performance is best if used within stated expiry period.

Disposal

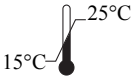
User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Ajello L., 1957, J. Chron. Dis., 5:545.
2. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
4. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

5. Lorian (Ed.),1980, Antibiotics In Laboratory Medicine, Williams and Wilkins, Baltimore.
6. Murray, P. R 2005, In Manual of Clinical Microbiology, 7th ed., ASM, Washington, D.C.
7. Sabouraud K., 1892, Ann. Dermatol. Syphilol, 3:1061.
8. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.
9. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 04/ 2019

IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
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C.L.E.D. Agar Base w/o Indicator

M1146

Intended Use:

C.L.E.D. Agar w/o Indicator (with added Bromo Thymol Blue) is recommended for isolation, enumeration and presumptive identification of bacterial flora in the urinary tract.

Composition**

Ingredients	Gms / Litre
Peptone	4.000
Tryptone	4.000
HM Peptone B #	3.000
Lactose	10.000
L-Cystine	0.128
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 36.1 grams in 998 ml purified/ distilled water. Add rehydrated contents of 1 vial of Bromo Thymol Blue Supplement (FD091). Heat, to boiling, to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121° C) for 15 minutes. Mix well and pour into sterile Petri plates.

Principle And Interpretation

On a solid medium, Sandy's reported that swarming of *Proteus* species could be controlled by restricting the electrolytes (1).

Formerly swarming of *Proteus* was controlled by adding alcohol, surface-active agent, sodium azide, boric acid etc. to the medium (1). Later on Sandys medium was modified by Mackey and Sandy's (2), by replacing mannitol by lactose and sucrose and elevating concentration of agar and bromothymol blue. This formulation was further modified by the same authors and called C.L.E.D. (Cystine-Lactose-Electrolyte-Deficient) by deleting the sucrose and by including L-cystine for promoting the growth of cystine dependent dwarf coliform colony (3). This medium is recommended for use in urine bacteriology, promoting the growth of all urinary pathogens. C.L.E.D. Medium is also recommended for dipstick procedures and as dip inoculum transport medium for urine specimens (2,3,4).

Peptone, HM Peptone B and tryptone provides nitrogen and carbon source, long chain amino acids, vitamins and other essential growth nutrients. Lactose is the fermentable sugar. L-cystine supports the growth of dwarf coliform colony. Bromo thymol blue is the pH indicator which turns yellow at acidic pH.

Bacteriuria may be quantitated by inoculating the surface of an agar medium by proper dilution. Inoculate the medium immediately after urine collection. It can also be inoculated by calibrated loop or duplicate dilution pour plate methods (5,6). *Shigella* species may not grow on this medium. Initiation of antibiotic therapy, before collection sample, low urine pH (less than 5) etc. may result in low bacterial count from infected patients.

Type of specimen

Clinical: Urine

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8).

Warning and Precautions

In Vitro diagnostic use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. This medium is recommended for urine infection. Low urine count may be a result of antibiotic therapy, low pH of urine.
2. Recovery depends on the urine count.
3. Inoculate the medium immediately after urine collection.
4. *Shigella* species may not grow on this medium.
5. For better results, the medium should not be incubated for more than 24 hours because if lactose fermenters predominate the entire medium may turn yellow masking the presence of non-lactose fermenters.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder.

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

With addition of Bromo Thymol Blue Supplement (FD091): Green coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 3.61% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed with added Bromothymol Blue Supplement(FD091),after an incubation at 35-37ºC for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	>=70%	yellow, opaque, center slightly deeper yellow
Enterococcus faecalis ATCC 29212 (00087*)	50-100	good-luxuriant	>=70%	slight yellowish or greenish
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	good-luxuriant	>=70%	yellow to whitish blue
Proteus vulgaris ATCC 13315	50-100	good-luxuriant	>=70%	blue
Staphylococcus aureus subsp. *aureus ATCC 25923* (00034*)	50-100	good-luxuriant	>=70%	deep yellow
Salmonella Typhi *ATCC 6539*	50-100	good-luxuriant	>=70%	bluish

Storage and Shelf Life

Store between 10- 30°C in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label. On opening, product should be properly stored dry,after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

1. Sandys, 1960, J. Med. Lab. Technol., 17:224.
2. Mackey and Sandys, 1965, Br. Med. J., 2:1286.
3. Mackey and Sandys, 1966, Br. Med. J., 1:1173.
4. Dixson J. M. S. and Clark M. A., 1968, Conc. Med. Assoc. J., 99 (15)
5. Benner E. J., 1970, Appl. Microbiol., 19(3), 409
6. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
8. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 04 / 2022

IVD — In vitro diagnostic medical device

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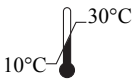

Storage temperature

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Tryptone Soya Yeast Extract Agar

M1214

Tryptone Soya Yeast Extract Agar is recommended for confirmation of *Listeria* in Henry's light.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	17.000
Papaic digest of soyabean meal	3.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	2.500
Dextrose	2.500
Yeast extract	6.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 51 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Tryptone Soya Yeast Extract Agar is formulated as per APHA (1) for the isolation and cultivation of *L. monocytogenes* from foods. ISO Committee (2) has recommended this medium for confirmation of *Listeria* species and can also be used for the cultivation and maintenance of a wide variety of heterotrophic microorganisms (3).

Casein enzymic hydrolysate and papaic digest of soyabean meal provide amino acids and other complex nitrogenous substances. Dextrose is the energy source. Dipotassium hydrogen phosphate buffers the medium. Yeast extract is the rich source of vitamin B complex.

According to FDAs enrichment procedure (4) for isolation of *L. monocytogenes* from dairy products, the sample to be tested is inoculated in enrichment broth and incubated at 30°C for 24-48 hours. This culture is streaked on Modified McBride Listeria Agar (M891) with cycloheximide or Lithium-Phenylethanol-Moxalactam (LPM) Agar (M1228) and incubated at 35°C for 48 hours. Presumptive *Listeria* colonies are selected under 45° transillumination and colonies are further purified on Tryptone Soya Yeast Extract Agar under the light illumination. *Listeria* colonies are dense white to iridescent white appearing as crushed glass. Other colonies tend to be yellowish or orange.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Yellow coloured clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 5.1% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

Cultural Response
M1214: Cultural characteristics observed after an incubation at 30-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery

Cultural Response

Listeria monocytogenes ATCC 19111	50-100	good-luxuriant	>=70%
Listeria monocytogenes ATCC 19118	50-100	good-luxuriant	>=70%

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Vanderzant C. and Splittstoesser D. F., (Eds.), 1992, Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed., APHA, Washington, D.C.
2. International Organization for Standardization (ISO), 1993, Draft, ISO/DIS 10560.
3. Atlas R. M. 2004, 3rd Ed., Handbook of Microbiological Media, Parks, L.C. (Ed.), CRC Press, Boca Raton.
4. FDA, Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, DC.

Revision : 02 / 2015

HiCrome™ Candida Differential Agar Base M1297AR

Intended use

HiCrome™ Candida Differential Agar Base is selective and differential medium for rapid isolation and identification of *Candida* species from mixed cultures from clinical and non-clinical samples

Composition**

Ingredients	Gms / Litre
Peptone	4.000
Chromogenic mixture	13.600
Agar	13.600
Final pH (at 25°C)	6.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 15.6 grams in 500 ml purified / distilled water. Add the rehydrated contents of one vial of HiCrome Candida Differential Selective Supplement (FD283R). Heat to boiling with frequent agitation to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Perry and Miller (4) reported that *Candida albicans* produces an enzyme b -N-acetyl- galactosaminidase and according to Rousselle et al (5) incorporation of chromogenic or fluorogenic hexosaminidase substrates into the growth medium helps in identification of C. albicans isolates directly on primary isolation. HiCrome™ Candida Differential Agar Base incorporates two chromogens X-NAG which detects the activity of hexosaminidase and BCIP which detects phosphatase activity. HiCrome™ Candida Differential Agar Base is a selective and differential medium, which facilitates rapid isolation of yeasts from mixed cultures and allows differentiation of *Candida* species namely *C.albicans, C.krusei, C.tropicalis* and *C.glabrata* on the basis of colouration and colony morphology. On this medium results are obtained within 48 hours and it is useful for the rapid and presumptive identification of common yeasts in Mycology and Clinical Microbiology Laboratory. Peptone provides nitrogenous, carbonaceous compounds and other essential growth nutrients. Chloramphenicol from the supplement suppresses the accompanying bacterial flora. *C.albicans* appear as light green coloured smooth colonies, *C.tropicalis* appear as blue to metallic blue coloured raised colonies. *C.glabrata, C.kefyr, C.parapsilosis* colonies appear as cream to white, beige/yellow due to natural pigmentation and some alkaline phosphatase activity, while *C.krusei* appear as pink-purple, fuzzy, dry colonies.

Type of specimen

Clinical samples - Blood; Food and dairy samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,3).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,6).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use . Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Slight variation in colour for isolates may be observed as the reaction is based on the enzyme present in organism.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to beige homogeneous free flowing powder

Gelling
Firm, comparable with 1.36% Agar gel

Colour and Clarity of prepared medium
Light amber coloured, opaque gel forms in Petri plates

Reaction
Reaction of 3.12% w/v aqueous solution at 25°C. pH : 6.0±0.2

pH
5.80-6.20

Cultural Response
Cultural characteristics observed with added HiCrome Candida Differential Selective Supplement (FD283R) after an incubation at 30-35°C for 40-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Candida albicans ATCC 10231 (00054*)	50-100	good-luxuriant	>=50%	light green
Candida glabrata ATCC 15126	50-100	good-luxuriant	>=50%	cream to white
#Teunomyces krusei ATCC 24408	50-100	good-luxuriant	>=50%	purple, fuzzy
Candida tropicalis ATCC 750	50-100	good-luxuriant	>=50%	blue to purple
Candida kefyr ATCC 66058	50-100	good-luxuriant	>=50%	cream to white with slight purple centre
Candida utilis ATCC 9950	50-100	good-luxuriant	>=50%	pale pink to pinkish purple
Candida parapsilosis ATCC 22019	50-100	good-luxuriant	>=50%	white to cream
Candida membranifaciens ATCC 20137	50-100	good-luxuriant	>=50%	white to cream
Candida dubliensis NCPF 3949	50-100	good-luxuriant	>=50%	pale green
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Staphylococcus aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%	

Key : *Corresponding WDCM numbers. # - Formerly known as *Candida krusei*

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.

2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

4. Perry J. L. and Miller G. R., 1987, J. Clin. Microbiol., 25: 2424 -2425.

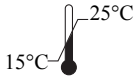
5. Rousselle P., Freydiere A., Couillerot P., de Montclos H. and GilleY., 1994, J. Clin. Microbiol. 32:3034-3036.

6. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

Revision : 04/2020

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

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HiCrome™ UTI Agar

M1353R

Intended Use:

Recommended for presumptive identification of microorganisms mainly causing urinary tract infections

Composition**

Ingredients	Gms / Litre
Peptone	15.000
Chromogenic mixture	26.800
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 56.8 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Urinary tract infections are bacterial infections affecting parts of urinary tract. The common symptoms of urinary tract infection are urgency and frequency of micturition, with associated discomfort or pain. The common condition is cystitis, due to infection of the bladder with a uropathogenic bacterium, which most frequently is *Escherichia coli*, but sometimes *Staphylococcus saprophyticus* or especially in hospital-acquired infections, *Klebsiella* species, *Proteus mirabilis*, other coliforms, *Pseudomonas aeruginosa* or *Enterococcus faecalis* (1). HiCrome™ UTI Agar is formulated on basis of work carried out by Pezzlo (8) Wilkie et al (11), Friedman et al (3), Murray et al (7), Soriano and Ponte (10) and Merlino et al (6). HiCrome™ UTI Agar (M1353R) is similar to M1353 with a slight difference in chromogenic mixture to improve the colour characteristic of media. These media are recommended for the detection of urinary tract pathogens where HiCrome™ UTI Agar has broader application as a general nutrient agar for isolation of various microorganisms. It facilitates and expedites the identification of some gram-negative bacteria and some gram-positive bacteria on the basis of different contrasted colony colours produced by reactions of genus or species specific enzymes with two chromogenic substrates. The chromogenic substrates are specifically cleaved by enzymes produced by *Enterococcus* species, *E.coli* and coliforms. Presence of amino acids like phenylalanine and tryptophan from peptones helps for detection of tryptophan deaminase activity, indicating the presence of *Proteus* species, *Morganella* species and *Providencia* species.

One of the chromogenic substrate is cleaved by ß-glucosidase possessed by Enterococci resulting in formation of blue colonies. *E.coli* produce pink colonies due to the enzyme ß-D-galactosidase that cleaves the other chromogenic substrate.

Further confirmation of *E.coli* can be done by performing the indole test. Coliforms produce purple coloured colonies due to cleavage of both the chromogenic substrate. Colonies of *Proteus, Morganella* and *Providencia* species appear brown because of tryptophan deaminase activity. Peptone provides nitrogenous, carbonaceous compounds and other essential growth nutrients. This medium can be made selective by supplementation with antibiotics for detecting microorganisms associated with hospital borne infections.

Type of specimen

Clinical samples : urine, faeces, Food samples, Water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (9).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(1)
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Since it is an enzyme-substrate based reaction, the intensity of colour may vary with isolates.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
White to cream homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
White coloured, opaque gel with precipitate forms in Petri plates

Reaction

Reaction of 5.68% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=70%	blue, small
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=70%	pink-purple
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	>=70%	blue to purple, mucoid
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	luxuriant	>=70%	colourless (greenish pigment may be observed)
Proteus mirabilis ATCC 12453	50-100	luxuriant	>=70%	light brown
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	luxuriant	>=70%	golden yellow

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

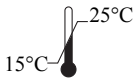
1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
2. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology,1996, 14th Edition, Churchill Livingstone.
3. Friedman M. P. et al, 1991, J. Clin. Microbiol., 29:2385-2389.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual

of Clinical Microbiology, 11th Edition. Vol. 1.
6. Merlino et al, 1995, Abstr. Austr. Microbiol. 16(4):17-3.
7. Murray P., Traynor P. Hopson D., 1992, J. Clin. Microbiol., 30:1600-1601.
8. Pezzlo M., 1998, Clin. Microbiol. Rev., 1:268-280.
9. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
10. Soriano F., Ponte C., 1992, J. Clin. Microbiol., 30:3033-3034.
11. Wilkie M. E., Almond M. K., Marsh F. P., 1992, British Medical Journal 305:1137-1141.

Revision : 02 / 2021

In vitro diagnostic medical device

CE Marking



Storage temperature

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HiCrome™ Enterococci Broth

M1376

Intended use

Recommended for the identification and differentiation of Enterococci from water samples and clinical samples.

Composition**

Ingredients	Gms / Litre
Peptone, special	10.000
Sodium chloride	5.000
Sodium azide	0.300
Chromogenic substrate	0.040
Polysorbate 80 (Tween 80)	2.000
Disodium hydrogen phosphate	1.250
Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37.18 grams (double strength) or 18.59 grams (single strength) in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121° C) for 15 minutes.

Principle And Interpretation

HiCrome™ Enterococci Broth is formulated on the basis of the work carried out by Althous et al (1), Amoras (2), Litsky et al (6), and Manafi and Sommer (7) and Snyder and Lichstein (8). These media is recommended for the rapid detection of Enterococci from water samples. The presence of *Enterococcus* group, which is a subgroup of the faecal Streptococci, serves as a valuable bacterial indicator for determining the extent of faecal contamination (1, 9) and it is more specific than the detection of coliforms, which may originate from non-faecal sources. The enzyme ß-glucosidase produced by Enterococci cleaves the chromogenic substrate, resulting in a bluish green colour.

The medium contains peptone special, which provides carbonaceous, nitrogenous compounds, long chain amino acids, vitamins and other essential nutrients. Sodium chloride maintains the osmotic balance of the medium. Sodium azide inhibits the accompanying microflora, especially gram-negative organisms. Polysorbate 80 acts as a source of fatty acids.

Type of specimen

Clinical samples - faeces; water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(3).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Some species may show poor growth due to nutritional variations.
2. Slight colour variations may be observed depending upon the utilization of the substrate by the organism.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light amber coloured, clear solution in tubes

Reaction
Reaction of 1.86% w/v aqueous solution at 25°C. pH : 7.5±0.2

pH
7.30-7.70

Cultural Response
Cultural characteristics observed after an incubation at 35- 37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Colour of Medium
Escherichia coli ATCC 25922 (00013*)	50-100	none-poor	light yellow
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	Light blue-green
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	none-poor	light yellow
Staphylococcus aureus subsp. *aureus ATCC 25923* (00034*)	50-100	none-poor	light yellow

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1. Althous, H., Dott, W., Havemeister, G, Muller, H.E, a. Sacre,C., 1982, Zbl. Bakt. Hyg. I. Abt. Orig. A. 252:154-165.
2. Amoras I, 1995, Poster presentation congress of Spanish Society of Microbiology, Madrid.
3. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke,G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. Litsky, W., Mallmann, W.L., a Fifield, C.W. 1953, Amer. J. Pbl. Hlth. 43:873-879.
7. Manafi M., and Sommer R, 1993, Wat. Sci. Tech. 27:271-274.
8. Snyder M.L., and Lichstein, H.C. 1940, J. Infect. Dis. 67. 113-115
9. Standard Methods for the Examination of Water and Wastewater, 20th Edition, Edited by L.S. Clesceri, A.E. Greenberg and A.D. Eaton, Published by APHA, AWWA and WEF (1998).

Revision : 03/ 2020

IVD — In vitro diagnostic medical device

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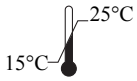

Storage temperature

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Neutralizing fluid

M1420

This medium is recommended by European Pharmacopoeia and British Pharmacopoeia, to be used to neutralize the activity of antimicrobial agents.

Composition**

Ingredients	Gms / Litre
Peptone (meat or casein)	1.000
Egg lecithin	3.000
Histidine hydrochloride	1.000
Sodium chloride	4.300
Potassium dihydrogen phosphate	3.600
Disodium hydrogen phosphate dihydrate	7.200

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 20.1 grams in 1000 ml distilled water containing 30 gm of polysorbate 80. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Neutralising fluid is used to neutralize the activity of antimicrobial agents generally present in pharmaceutical materials. This is required to neutralize the effect of antimicrobials while testing the sterility of such materials. This medium may be added to Buffered Sodium Chloride Peptone Solution, pH 7.0 before sterilization. If utilized their efficacy and non-toxicity towards microorganisms are demonstrated (1, 2).

The neutralising agents present in the medium neutralises the activity of antimicrobial agents present in various pharmaceutical products which may interfere with microbial limit tests or sterility testing analysis. Egg lecithin and polysorbate 80 act as neutralis-ing agents. Sodium chloride maintains osmotic equilibrium and phosphates serve as buffering agents.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured opalescent solution in tubes

Cultural Response

M1420: Cultural characteristics observed when subcultured on Tryptone Soya Agar (M290), after an incubation at 35-37°C for 40-48 hours

Organism	Inoculum (CFU)	Growth
Bacillus subtilis ATCC 6633	50-100	good
Escherichia coli ATCC 25922	50-100	good
Pseudomonas aeruginosa ATCC 27853	50-100	good
Staphylococcus aureus ATCC 25923	50-100	good
Salmonella Typhimurium ATCC 14028	50-100	good

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. European Pharmacopoeia, 2005, European Department, Directorate for the Quality of Medicines of the Council of Europe, Vol. 5, pp -161.
2. British Pharmacopoeia, 2004, The Stationery office British Pharmacopoeia

Revision : 1 / 2011

CE