Technical Data

Beta Lactamase Mixture Supplement (Sterile) **FD268G**

An innovative enzyme based product that can efficiently inactivate wide range of antibiotics like Penicillins, Cephalosporins of first, second, third and fourth generation and Penems.

Composition :
(per vial contains)

Ingredients

Cephalosporinase	> 50 IU activity
Penicillinase	>500 IU activity

1 IU is defined as the amount of enzyme needed to hydrolyze 1µmole of Penicillin G (Penicillinase) or 1µmole of Cephalosporin C (Cephalosporinase) per minute at 25ºC and pH 7.0.
1 IU of Penicillinase corresponds to 600 Levy Units or 75 Pollock Units.

Directions :

Rehydrate the contents of 1 vial with 5 ml of sterile distilled water. Add appropriate amount of solution depending on the application. Remaining solution can be stored at 2-8º C for 4 weeks.

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Date: 2022.03.28 09:49:36 EEST
Reason: MoldSign Signature
Location: Moldova

Glycerol, Hi-LR™ GRM081

Product Identifier

CAS No.	:	56-81-5
EC No.	:	200-289-5
Molecular Formula	:	$C_3H_8O_3$
Molecular Weight	:	92.09
Synonym	:	1,2,3-Propanetriol; Glycerin; Propane-1,2,3-triol
HS Code	:	2905 45 00
Storage	:	Below 30°C
Shelf life	:	4 years

Technical Specification

Appearance	:	Colourless to faint yellow syrupy, very hygroscopic clear viscous liquid
Solubility	:	1 mL miscible in 1 mL of water
FTIR	:	Matches with the standard pattern
Refractive index (n 20/D)	:	1.4700 - 1.4750
Density (at 25°C)	:	1.245 - 1.255 g/mL
Sulphated ash	:	<= 0.01%
Sugars (Glucose)	:	<= 0.004%
Assay (NaOH Titration/GC)	:	98.00 - 102.00%

Safety Information

UN No.	:	Not dangerous goods
Class	:	-
Packing Group	:	-
RTECS	:	MA8050000
WGK	:	1

Grams Stain-Kit

K001

Intended Use

Grams Stain Kit is used for differentiation of bacteria on the basis of their gram nature.

Composition**

Ingredients

Gram's Crystal Violet (S012)(Solution A)	-
Crystal Violet	2.000 gm
Ethyl alcohol,95%	20.000 ml
Gram's Crystal Violet (S012)(Solution B)	-
Ammonium oxalate	0.800 gm
Distilled Water	80.000 ml

Solution A and B are mixed and stored for 24 hours before use.The resulting stain is stable.

Gram's Decolourizer(S032)	-
Ethyl alcohol, 95%	50.0 ml
Acetone	50.0 ml
Gram's Iodine(S013)	-
Iodine	1.000 gm
Potassium iodide	2.000 gm
Distilled water	300.000 ml
Safranin,0.5% w/v(S027)	-
Safranin O	0.500 gm
Ethyl alcohol, 95%	100.000 ml

**Formula adjusted, standardized to suit performance parameters

Directions

1)Prepare a thin smear on clear, dry glass slide.

2)Allow it to air dry and fix by gentle heat.

3)Flood with Gram's Crystal Violet (S012) for 1 minute. (If over staining results in improper decolourization of known gram-negative organisms,use less crystal violet).

4)Drain the stain.

5)Flood the smear with Gram’s Iodine (S013). Allow it to remain for 1 minute.

6)Decolourize with Gram's Decolourizer (S032) until the blue dye no longer flows from the smear.

7)Wash with tap water.

8)Counter stain with 0.5% w/v Safranin (S027). Allow it to remain for 1 minute.

9)Wash with water.

10)Allow the slide to air dry or blot dry between sheets of clean bibulous paper and examine under oil immersion objective.

Principle And Interpretation

The Gram stain is a differential staining technique most widely applied in all microbiology disciplines laboratories. It is one of the most important criteria in any identification scheme for all types of bacterial isolates. Different mechanisms have been proposed to explain the gram reaction. There are many physiological differences between gram-positive and gram-negative cell walls (1). Ever since Christian Gram has discovered Gram staining, this process has been extensively investigated and redefined. In practice,a thin smear of bacterial cells is stained with crystal violet, then treated with an iodine containing mordant to increase the binding of primary stain (2). A decolourizing solution of alcohol or acetone is used to remove the crystal violet from cells which bind it weakly and then the counterstain (like safranin) is used to provide a colour contrast in those cells that are decolourized.

Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50–90% of cell envelope), and as a result are stained purple by crystal violet, whereas gram-negative bacteria have a thinner layer (10% of cell envelope), so do not retain the purple stain and are counter-stained pink by safranin.In a properly stained smear by gram staining procedure, the gram-positive bacteria appear blue to purple and gram negative cells appear pink to red.

Type of specimen

Clinical samples - Blood, urine, CSF, pus, wounds, lesions, body tissues, sputum etc. ; food & dairy samples ; Water 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 (5, 6).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(7).
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 guidleines should be followed while handling clincal specimens. Saftey guidelines may be referred in individual safety data sheets

Limitations :

1. Use results of Gram stains in conjunction with other clinical and laboratory findings. Use additional procedures (e.g., special stains, inclusion of selective media, etc.)to confirm findings suggested by gram-stained smears (8).
2. False Gram stain results may be related to inadequately collected specimens or delay in transit.
3. Careful adherence to procedure and interpretive criteria is required for accurate results. Accuracy is highly dependent on the training and skill of microscopists (9).
4. The sensitivity of Gram stain is 10^5 cells/ml or 10^4 if the specimen has been prepared with the cytocentrifuge (10). This is particularly applicable to the smear of a drop of urine, where an average of the one bacterial cell per field from an examination of 20 fields correspond to a count of >= 10^5 cfu/ml.

Performance and Evaluation

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

Quality Control

Microscopic examination

Gram staining is carried out and observed under oil immersion lens.

Results

Gram-positive organisms : Violet coloured
Gram-negative organisms : Pinkish red coloured

Storage and Shelf Life

Store between 10- 30°C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

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. Lamanna and Mallette, 1965, Basic Bacteriology, 3rd ed., Williams and Wilkins Co.,Baltimore.
2. Salton, 1964, The Bacterial Cell Wall, Elsevier, Amsterdam.
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. Downes F. P. and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, 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. Rice E.W., Baird, R.B., Eaton A. D., Clesceri L. S. (Eds.), 2012, Standard Methods for the Examination of Water and Wastewater, 22nd ed., APHA, Washington, D.C.
8. Brown,M.S.,and T.C. Wu. 1986. The Gram stain morphology of fungi, mycobacteria, and Pneumocytis carinii. J.Med .Technol3:495-499.
9. Washington, J.A.1986.Rapid diagnosis by microscopy. Clin.Microbiol. Newsl.8:135-137.
10. Shanhooltzer,C.J.,P. Schaper ,and L.R. Peterson.1982.Concentrated Gram stain smear prepared with a cytospin centrifuge. J.clin. Microbiol.16:1052-1056

Revision : 02 / 2018

IVD	In vitro diagnostic medical device
CE	CE Marking
30°C 10°C	Storage temperature
	Do not use if package is damaged
	HiMedia Laboratories Pvt. Limited, 23 Vadhani Industrial Estate, LBS Marg,Mumbai-86,MS,India
EC REP	CE Partner 4U ,Esdoornlaan 13, 3951 DB Maarn The Netherlands, www.cepartner 4u.eu

Technical Data

LA006

Spatula - 200

Product Number		Packing
LA006	:	50 NO
LA006	:	100 NO

Product Information

Product Code	:	LA006
Product Name	:	Spatula - 200

Technical Specification

Description	:	Spatula made up of stainless steel material, individually packed, having flat surface on one side and small scoop on the other side.
Size	:	Length =200 mm
Use	:	Useful for weighing of dry powders.

Transport Information

ADR/RID	:	Not Dangerous Goods
IMDG	:	Not Dangerous Goods
IATA	:	Not Dangerous Goods

HiMedia Laboratories Pvt. Ltd. A-516,Swastik Disha Business Park,Via Vadhani Ind. Est., LBS Marg, Mumbai-400086, India.
Customer care No.: 022-6147 1919 Email: techhelp@himedialabs.com Website: www.himedialabs.com

Technical Data

Sterile Fluid Thioglycollate Medium

LQ026

Recommended for Sterility testing of biologics and for cultivation of aerobes ,anaerobes and microphiles.

Composition**

Ingredients	Gms / Litre
Pancreatic digest of casein	15.000
Yeast extract	5.000
Dextrose	5.500
Sodium chloride	2.500
L-Cystine	0.500
Sodium thioglycollate	0.500
Resazurin sodium	0.001
Agar	0.750

**Formula adjusted, standardized to suit performance parameters

Directions

Label the ready to use LQ026 bottle. Remove the top of he cap. Disinfect the part of the rubber stopper which is now exposed. Draw sample with the sterile or disposable needle and syringe. Transfer the sample immediately into the LQ026 bottle by puncturing the rubber stopper with the injecting needle. Keep the bottle in an upright position preferably in a biological safety cabinet, place an alcohol swab over the rubber stopper and insert the venting needle with filter through it (Use sterile venting needle (LA038). Insertion and withdrawal of the needle should be done in a straight line. discard the needle and mix the contents by gently inverting the bottle 2-3 times. Do Not vent the bottle for anaerobic cultures. Incubate at .

Principle And Interpretation

Brewer (1) formulated Fluid Thioglycollate Medium for rapid cultivation of aerobes as well as anaerobes including microaerophiles by adding a reducing agent and small amount of agar. The USP (2), BP (3), EP (4) and AOAC (5) have recommended the media for sterility testing of antibiotics, biologicals and foods and for determining the phenol coefficient and sporicidal effect of disinfectants. However, it is intended for the examination of clear liquid or water-soluble materials. Fluid Thioglycollate Medium is also routinely used to check the sterility of stored blood in blood banks (10). Dextrose, pancreatic digest of casein, yeast extract, L-cystine provide the growth factors necessary for bacterial multiplication. L-cystine and sodium thioglycollate allows *Clostridium* to grow in this medium even under aerobic conditions(11). Also the small amount of agar used in the medium favors the growth of aerobes as well as anaerobes in the medium, even if sodium thioglycollate is deleted from the medium(1). Sodium thioglycollate act as a reducing agent and neutralizes the toxic effects of mercurial preservatives and peroxides formed in the medium, thereby promoting anaerobiosis, and making the medium suitable to test materials containing heavy metals. (9,10). Any increase in the oxygen content is indicated by a colour change of redox indicator, resazurin to red (6,7,8). The small amount of agar helps in maintaining low redox potential for stabilizing the medium (9).

Quality Control

Appearance
Sterile clear Fluid Thioglycollate Medium in glass bottle.

Colour
Light straw coloured solution with upper 10% or less medium pink on standing.

Quantity of Medium
100 ml of medium in glass bottle.

pH
6.90- 7.30

Sterility test
Passes release criteria

Growth Promotion Test

In accordance with the harmonized method of USP/EP/BP.

Cultural response
Cultural characteristics observed after an incubation at 30-35°C for not more than 3 days.

Organism	Inoculum (CFU)	Growth
Growth at 30-35°C for <= 3 days incubated anaerobically		
Bacteroides vulgatus ATCC 8482	50 -100	luxuriant
Clostridium sporogenes ATCC 19404	50 -100	luxuriant
Clostridium sporogenes ATCC 11437	50 -100	luxuriant
Clostridium sporogenes NBRC 14293	50 -100	luxuriant
Clostridium perfringens ATCC 13124	50 -100	luxuriant
Bacteroides fragilis ATCC 23745	50 -100	luxuriant
Growth at 30-35°C for <= 3 days		
Staphylococcus aureus ATCC 25923	50 -100	luxuriant
Staphylococcus aureus ATCC 6538	50 -100	luxuriant
Escherichia coli ATCC 8739	50 -100	luxuriant
Escherichia coli NCTC 9002	50 -100	luxuriant
Salmonella Typhimurium ATCC 14028	50 -100	luxuriant
Salmonella Abony NCTC 6017	50 -100	luxuriant
Bacillus subtilis ATCC 6633	50 -100	luxuriant
Pseudomonas aeruginosa ATCC 27853	50 -100	luxuriant
Pseudomonas aeruginosa ATCC 9027	50 -100	luxuriant
Micrococcus luteus	50 -100	luxuriant
Streptococcus pneumoniae ATCC 6305	50 -100	luxuriant
Escherichia coli ATCC 25922	50 -100	luxuriant

Storage and Shelf Life

Store between 15-25°C. Use before expiry date on the label.

Reference

1. Brewer, 1940, J. Am. Med. Assoc., 115:598. 2. The United States Pharmacopoeia, 2009, The United States Pharmacopoeial Convention, Rockville, MD. 3. British Pharmacopoeia, 2009, The Stationery office British Pharmacopoeia 4. European Pharmacopoeia, 2009, European Dept. for the quality of Medicines. 5. Williams H., (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC, Washington, D.C 6. Marshall, Gunnison and Luxen, 1940, Proc. Soc. Exp. Biol. Med., 43:672. 7. Nungester, Hood and Warren, 1943, Proc. Soc. Exp. Biol. Med., 52:287. 8. Portwood, 1944, J. Bact., 48:255. 9. MacFaddin J.F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore. 10. Federal Register, 1992, Fed. Regist., 21:640.2.17. 11. Quastel and Stephenson, 1926, J.Biochem., 20:1125.

Revision : 1 / 2011

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Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory,diagnostic,research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

Technical Data

Sterile Soyabean Casein Digest Medium

LQ027

Sterility test media prepared in accordance with harmonized methods of USP, EP, BP, JP & IP.

Composition**

Ingredients	Gms / Litre
Pancreatic digest of casein	17.000
Papaic digest of soybean (soyabean)	3.000
Sodium chloride	5.000
Dibasic potassium phosphate	2.500
Glucose monohydrate	2.500
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Label the ready to use LQ027 bottle. Do not unscrew cap. remove the top of he screw cap. Disinfect the part of the rubber stopper which is now exposed. Draw sample with the sterile or disposable needle and syringe. Transfer the sample immediately into the LQ027 bottle by puncturing the rubber stopper with the injecting needle. Keep the bottle in an upright position preferably in a biological safety cabinet, place an alcohol swab over the rubber stopper and insert the venting needle with filter through it (Use sterile venting needle (LA038). Insertion and withdrawal of the needle should be done in a straight line. discard the needle and mix the contents by gently inverting the bottle 2-3 times. Do Not vent the bottle for anaerobic cultures. Incubate at specified temperature for 18-24 hours and further for seven days.

Principle And Interpretation

Soybean Casein Digest Medium is recommended as a sterility testing medium in accordance with the harmonized method of USP/EP/BP/JP/IP (1,2,3,4,5). It is used for the sensitivity testing of antimicrobial agents by the tube dilution method (5). It is also employed in diagnostic research in microbiology. This medium is used as a diluent and suspending medium for preparation of samples or test strains. It is also employed in sample preparation for testing of products, wherein incubation is carried out, only to serve sufficient resuscitation of the cell, while avoiding multiplication of the organism. The combination of pancreatic digest of casein and papaic digest of soybean meal makes this medium nutritious by providing amino acids and long chain peptides for the growth of microorganisms. Natural sugars in soybean promote growth of fastidious organism. Glucose monohydrate is the fermentable source of carbon and dibasic potassium phosphate serves as the buffer in the medium. Sodium chloride maintains the osmotic balance of the medium. This medium is recommended for sterility checking and for studying total aerobic microbial count in verification of microbiological testing procedures employed for sterility checking.

Quality Control

Appearance
Sterile Soyabean Casein Digest Medium in a glass bottle.

Colour
Light yellow coloured clear solution

Quantity of Medium
100 ml of medium in glass bottle.

pH
7.10-7.50

Growth Promotion Test
Growth Promotion is carried out in accordance with the harmonized method of USP/EP/BP/JP.

Stability test
Light yellow coloured clear solution without any precipitation or sedimentation at room temperature for 7 days

Growth promoting properties
Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu(at 30-35°C for 18-24 hours for bacteria and 5 days for fungal).

Sterility Testing + Validation
The medium is tested with suitable strains of microrganisms inoculating <=100cfu and incubating at 20-25°C for not more than 3 days in case of bacteria and not more than 5 days in case of fungi.

Sterility test
Passes release criteria

Cultural Response

Cultural characteristics observed after an incubation as specified.

Organism	Growth	Incubation period	Inoculum (CFU)	Incubation temperature
Growth promoting				
Micrococcus luteus ATCC 9341	luxuriant	18 -24 hrs	50 -100	30 -35 °C
Streptococcus pneumoniae ATCC 6305	luxuriant	18 -24 hrs	50 -100	30 -35 °C
Candida albicans ATCC 10231	luxuriant	<=5 d	50 -100	20 -25 °C
Escherichia coli ATCC 8739	luxuriant	18 -24 hrs	50 -100	30 -35 °C
Pseudomonas aeruginosa ATCC 9027	luxuriant	18 -24 hrs	50 -100	30 -35 °C
Pseudomonas aeruginosa ATCC 27853	luxuriant	18 -24 hrs	50 -100	30 -35 °C
Bacillus subtilis ATCC 6633	luxuriant	18 -24 hrs	50 -100	30 -35 °C
Salmonella Typhimurium ATCC 14028	luxuriant	18 -24 hrs	50 -100	30 -35 °C
Salmonella Abony NCTC 6017	luxuriant	18 -24 hrs	50 -100	30 -35 °C
Candida albicans ATCC 2091	luxuriant	<=5 d	50 -100	20 -25 °C
**Aspergillus brasiliensis ATCC 16404*	luxuriant	<=5 d	50 -100	20 -25 °C
Escherichia coli ATCC 25922	luxuriant	18 -24 hrs	50 -100	30 -35 °C
Escherichia coli NCTC 9002	luxuriant	18 -24 hrs	50 -100	30 -35 °C
Staphylococcus aureus ATCC 6538	luxuriant	18 -24 hrs	50 -100	30 -35 °C
Staphylococcus aureus ATCC 25923	luxuriant	18 -24 hrs	50 -100	30 -35 °C
Sterility Testing- Growth promotion+Validation				
Staphylococcus aureus ATCC 6538	luxuriant	<=3 d	50 -100	20 -25 °C
Staphylococcus aureus ATCC 25923	luxuriant	<=3 d	50 -100	20 -25 °C
Escherichia coli ATCC 8739	luxuriant	<=3 d	50 -100	20 -25 °C
Escherichia coli ATCC 25922	luxuriant	<=3 d	50 -100	20 -25 °C
Escherichia coli NCTC 9002	luxuriant	<=3 d	50 -100	20 -25 °C
Pseudomonas aeruginosa ATCC 9027	luxuriant	<=3 d	50 -100	20 -25 °C
Pseudomonas aeruginosa ATCC 27853	luxuriant	<=3 d	50 -100	20 -25 °C
Bacillus subtilis ATCC 6633	luxuriant	<=3 d	50 -100	20 -25 °C
Micrococcus luteus ATCC 9341	luxuriant	<=3 d	50 -100	20 -25 °C

Salmonella Typhimurium ATCC 14028	luxuriant	<=3 d	50 -100	20 -25 °C
Salmonella Abony NCTC 6017	luxuriant	<=3 d	50 -100	20 -25 °C
Streptococcus pneumoniae ATCC 6305	luxuriant	<=3 d	50 -100	20 -25 °C

Storage and Shelf Life

Store between 15-25°C. Use before expiry date on the label.

Reference

1.The United States Pharmacopoeia, 2011, The United States Pharmacopoeial Convention. Rockville, MD.
2.British Pharmacopoeia, 2011, The Stationery office British Pharmacopoeia.
3.European Pharmacopoeia, 2011, European Dept. for the quality of Medicines.
4.Japanese Pharmacopoeia, 2008.
5.Indian Pharmacopoeia, 2007, Govt. of India, the controller of Publication, Delhi, India.
6.Wright and Welch, 1959-60, Antibiotics Ann., 61.

Revision : 1 / 2011

Technical Data

Diluting Fluid K

LQ122L

Diluent in testing of pharmaceuticals in accordance with USP.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Beef extract	3.000
Polysorbate 80	10.000

**Formula adjusted, standardized to suit performance parameters

Principle And Interpretation

Diluting Fluid K is recommended as rinsing fluid for membrane filter method used in validation tests for bacteriostasis and fungistasis activity of pharmaceutical articles before carrying out sterility test procedures as per USP (1). After filtering the specified quantity of the test specimen the membrane is rinsed with measured portions of rinsing or diluting fluid.
This rinse is inoculated with known number of test bacteria and fungi as specified in pharmacopoeia. The resultant growth is compared with positive control to determine presence of fungistasis or bacteriostasis activity in test specimen.

Quality Control

Appearance
Sterile clear Diluting Fluid K in bottle.

Colour
Light yellow coloured medium

Quantity of medium

300 ml of medium in bottle

pH
6.70- 7.10

Sterility test
Passes release criteria.

Growth Promotion Test
In accordance with the harmonized method of USP.

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

Organism	Inoculum (CFU)	Growth
Escherichia coli ATCC 25922	50-100	good
Staphylococcus aureus ATCC 25923	50-100	good
Staphylococcus aureus ATCC 6538	50-100	good
Candida albicans ATCC 10231	50-100	good

Storage and Shelf Life

Store between 2-8°C. Use before expiry date on the label.

Reference

1. The United States Pharmacopoeia / National Formulary, USP31 / NF26, 2008, Asian Edition, US Pharmacopeial convention Inc., Rockville, MD.

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Technical Data

Diluting Fluid D

LQ510L

Diluent in testing of pharmaceuticals in accordance with USP.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	1.000
Polysorbate 80	1.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Diluting fluid is used as the diluting or rinsing solution for membrane filter techniques in pharmaceutical products. Measured portions of Diluting fluid D should be used to rinse the membrane after filtration. Inoculate this rinse with 50-100 cfu of test organisms. Simultaneously run a positive control of the same medium. Incubate both the set of medium at the specified time and temperature. Compare the growth obtained for the rinse with that obtained in the positive control after incubation.

Principle And Interpretation

Diluting Fluid D is recommended as rinsing fluid for membrane filter method used in validation tests for bacteriostasis and fungistasis activity of pharmaceutical articles before carrying out sterility test procedures as per USP (1). After filtering the specified quantity of the test specimen, the membrane is rinsed with measured portions of rinsing or diluting fluid.
This rinse is inoculated with known number of test bacteria and fungi as specified in pharmacopoeia. The resultant growth is compared with positive control to determine presence of fungistasis or bacteriostasis activity in test specimen. This medium is recommended for articles containing lecithin or oil or for devices labeled as ‘sterile pathway”(1)

Quality Control

Appearance
Sterile clear Diluting Fluid D in bottle.

Colour
Light amber coloured medium

Quantity of medium
300ml of medium in bottle

pH
6.90- 7.30

Growth Promotion Test
In accordance with the harmonized method of USP.

Sterility test
Passes release criteria.

Cultural Response
LQ510L: Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth
Cultural response		
Candida albicans ATCC 10231	50-100	good
Escherichia coli ATCC 25922	50-100	good
Escherichia coli ATCC 8739	50-100	good
Staphylococcus aureus ATCC 25923	50-100	good
Staphylococcus aureus ATCC 6538	50-100	good

Storage and Shelf Life

Store between 2-8°C. Use before expiry date on the label.

Reference

1. The United States Pharmacopoeia / National Formulary, USP34 / NF29, 2011, The US Pharmacopeial convention Inc., Rockville, MD.

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Nutrient Agar

M001

Intended use

Nutrient Agar is used as a general purpose medium for the cultivation of less fastidious microorganisms, can be enriched with blood or other biological fluids.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
Sodium chloride	5.000
HM peptone B#	1.500
Yeast extract	1.500
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 28.0 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. If desired ,the medium can be enriched with 5-10% blood or other biological fluids. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Nutrient media are basic culture media used for maintaining microorganisms, cultivating fastidious organisms by enriching with serum or blood and are also used for purity checking prior to biochemical or serological testing (5,6). Nutrient Agar is ideal for demonstration and teaching purposes where a more prolonged survival of cultures at ambient temperature is often required without risk of overgrowth that can occur with more nutritious substrate. This relatively simple formula has been retained and is still widely used in the microbiological examination of variety of materials and is also recommended by standard methods. It is one of the several non-selective media useful in routine cultivation of microorganisms (1,7). It can be used for the cultivation and enumeration of bacteria which are not particularly fastidious. Addition of different biological fluids such as horse or sheep blood, serum, egg yolk etc. makes it suitable for the cultivation of related fastidious organisms. Peptone, HM peptone B and yeast extract provide the necessary nitrogen compounds, carbon, vitamins and also some trace ingredients necessary for the growth of bacteria. Sodium chloride maintains the osmotic equilibrium of the medium.

Type of specimen

Clinical samples - Blood; Food and dairy samples; Water 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 (1,7,8).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(2)
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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

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 yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 2.8% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

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

Organism	Inoculum (CFU)	Growth	Recovery
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	>=70%
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	good-luxuriant	>=70%
Salmonella Typhi *ATCC 6539*	50-100	good-luxuriant	>=70%
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	good-luxuriant	>=70%
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant	>=70%
Salmonella Enteritidis *ATCC 13076* (00030*)	50-100	good-luxuriant	>=70%
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	good-luxuriant	>=70%
Yersinia enterocolitica ATCC 9610 (00038*)	50-100	good-luxuriant	>=70%
Yersinia enterocolitica ATCC 23715 (00160*)	50-100	good-luxuriant	>=70%

Key : *Corresponding WDCM numbers.

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. 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 (3,4).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. 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.
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. Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
6. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins,Baltimore.
7. 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.
8. 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 : 05 / 2021

IVD — In vitro diagnostic medical device

CE Marking

30°C / 10°C — Storage temperature

Do not use if package is damaged

HiMedia Laboratories Pvt. Limited,
23 Vadhani Industrial Estate,
LBS Marg,Mumbai-86,MS,India

EC REP

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DB Maarn The Netherlands,
www.cepartner 4u.eu

Nutrient Broth

M002

Intended use

Nutrient Broth is used for the general cultivation of less fastidious microorganisms, can be enriched with blood or other biological fluids.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
Sodium chloride	5.000
HM peptone B#	1.500
Yeast extract	1.500
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 13.0 grams 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

Nutrient media are basic culture media used for maintaining microorganisms, cultivating fastidious organisms by enriching with serum or blood and are also used for purity checking prior to biochemical or serological testing (5,6). Nutrient Broth has the formula originally designed for use in the Standard Method for Examination of Water and Waste water. It is one of the several non-selective media useful in routine cultivation of microorganisms (1,7). It can be used for the cultivation and enumeration of bacteria which are not particularly fastidious. Addition of different biological fluids such as horse or sheep blood, serum, egg yolk etc. makes it suitable for the cultivation of related fastidious organisms.

Peptone, HM peptone B and yeast extract provide the necessary nitrogen compounds, carbon, vitamins and also some trace ingredients necessary for the growth of bacteria. Sodium chloride maintains the osmotic equilibrium of the medium.

Type of specimen

Clinical samples - Blood; Food and dairy samples; Water 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 (1,7,8).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(2)
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. This medium is general purpose medium and may not support the growth of fastidious organisms.

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 yellow coloured clear to slightly opalescent solution

Reaction

Reaction of 1.3% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

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

Organism	Inoculum (CFU)	Growth
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	good-luxuriant
Salmonella Typhi *ATCC 6539*	50-100	good-luxuriant
Staphylococcus aureus aubsp.aureus ATCC 25923 (00034*)	50-100	good-luxuriant
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant

Key : *Corresponding WDCM numbers.

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 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

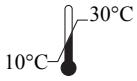
1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
3. Isenberg, H.D. Clinical 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. Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
6. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore.
7. 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.

8. 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 : 09 / 2021

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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Antibiotic Assay Medium No.1 (Seed Agar)

M003

Intended use

Antibiotic Assay Medium No.1 (Seed Agar) is used in the microbiological assay of beta-lactam and other antibiotics.

Composition**

Ingredients	Gms / Litre
Peptone	6.000
Tryptone	4.000
Yeast extract	3.000
HM peptone B #	1.500
Dextrose (Glucose)	1.000
Agar	15.000
Final pH (at 25°C)	6.6±0.2

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

Directions

Suspend 30.5 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 45-50°C. Mix well and pour into sterile Petri plates. *Advice: Recommended as a inoculum medium for Amikacin, Bacitracin, Capreomycin, Cephalothin, Cephaperin, Chloramphenicol, Chlortetracycline, Cloxacillin, Cycloserine, Colistimethate sodium, Colistin, Demeclocycline, Dihydrostreptomycin, Erythromycin ,Framycetin, Gentamicin, Kanamycin, Kanamycin B, Kanamycin sulphate, Lymecycline, Methacycline, Nafcillin, Neomycin, Netilmicin, Novobiocin, Oxytetracycline, Paromomycin, Penicillin-G, Rifamycin sodium, Rolitetracycline, Sisomycin Spiramycin, Streptomycin Tetracycline, Tobramycin, Troleandomycin, Tylosin*

Principle And Interpretation

The potency of an antibiotic can be determined by chemical, physical and biological means. An assay is made to determine the ability of an antibiotic to kill or inhibit the growth of living microorganisms. Biological tests offer the most convenient means of performing an assay (7), since a reduction in the antimicrobial activity of a specific antibiotic reveals changes not usually displayed by chemical methods (9). Antibacterial susceptibility testing may be performed by either dilution (turbidimetric) or diffusion methods. The choice of methodology is often based on many factors, including relative ease of performance, flexibility and use of automated or semi-automated devices for both identification and susceptibility testing (6). Grove and Randall have elucidated those antibiotic assays and media in their comprehensive treatise on antibiotic assays (3). Antibiotic Assay Medium No.1 is used in the microbiological assay of ß-lactam and other antibiotics. These media are prepared according to the specifications detailed in various pharmacopoeias (1,2,9) and by the FDA (8).

Freshly prepared plates should be used for antibiotic assays. Test organisms are inoculated in sterile seed agar cooled to 40-45°C and spread evenly over the surface of solidified base agar. After incubation the concentration of the antibiotic being assayed is determined by measuring the zone of inhibition obtained, with that of reference standard antibiotic. All conditions in the microbiological assay must be carefully controlled. The use of standard culture media in the test is one of the important steps for good results.

Nutrients and growth factors are supplied by the ingredients like peptone, Tryptone, yeast extract and HM Peptone B. Dextrose is supplemented as a carbon and energy source.

Type of specimen

Pharmaceutical preparations

Specimen Collection and Handling

For pharmaceutical samples follow appropriate techniques for handling specimens as per established guidelines (1,2,9).
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. Freshly prepared plates must be used or it may result in erroneous results.

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

Yellow coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 3.05% w/v aqueous solution at 25°C. pH : 6.6±0.2

pH
6.40-6.80

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

Organism	Inoculum (CFU)	Growth	Recovery	Inoculum medium	Assay medium	Assay medium Inoculum &
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)	50-100	luxuriant	>=70%	Framycetin, Josamycin,Josamycin propionate, Kanamycin B,Spiramycin, Streptomycin,Vancomycin	Streptomycin, Vancomycin	
Bordetella bronchiseptica ATCC 4617	50-100	luxuriant	>=50%	Colistimethate sodium,Colistin,Polymyxin B		
Escherichia coli ATCC 10536	50-100	luxuriant	>=70%	Chloramphenicol		
Bacillus cereus var mycoides ATCC 11778 (00001)*	50-100	luxuriant	>=70%	Oxytetracycline, Tetracycline		
Bacillus pumilis ATCC 14884	50-100	luxuriant	>=70%	Chlortetracycline,Framycetin, Kanamycin sulphate		
Klebsiella pneumoniae ATCC 10031	50-100	luxuriant	>=70%	Capreomycin,Dihydrostreptomycin, Neomycin,Streptomycin, Troleandomycin		

Micrococcus luteus ATCC 9341	50-100	luxuriant	>=70%	Erythromycin, Rifamycin sodium	Erythromycin Bacitracin
Micrococcus luteus ATCC 10240	50-100	luxuriant	>=70%		
Pseudomonas aeruginosa ATCC 25619	50-100	luxuriant	>=70%	Carbenicillin	
Staphylococcus aureus ATCC 29737	50-100	luxuriant	>=70%	Amikacin, Cephothin, Cephapirin, Chlotetracycline, Cloxacillin, Cycloserine,Demeclocycline, Doxycycline, Kanamycin, Methacycline, Nafcillin, Oxytetracycline, Penicillin-G, Rolitetracycline, Tetracycline, Tobramycin,Tylosin Gentamicin,Neomycin,Netilmicin,	Cephalothin,Cephapirin,Cloxacillin, Nafcillin,Penicillin-G
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	luxuriant	>=70%	Novobiocin,Sisomycin,Paromomycin	

*- Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and use freshly prepared medium. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1. British Pharmacopoeia, 2020,The Stationery office British Pharmacopoeia
2. European Pharmacopoeia, 2020, European Department, for the Quality of Medicines
3. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc.New York.
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. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
7. Pelczar M. J. Jr., Reid R. D., Chan E. C. S., 1977, Microbiology, 4th Edi, Tata McGraw-Hill Publishing Company Ltd, New Delhi.
8. Tests and Methods of Assay of Antibiotics and Antibiotic containing Drugs, FDA, CFR, 1983 Title 21, Part 436, Subpart D, Washington, D.C.: U.S. Government Printing Office, paragraphs 436, 100-436, 106, p. 242- 259 (April 1).
9. The United States Pharmacopoeia, 2019. The United States Pharmacopoeial Convention,Rockville, MD.

Revision : 05/2021

Kligler Iron Agar

M078

Intended Use:

Recommended for the differential identification of gram-negative enteric bacilli from clinical and non clinical samples on the basis of the fermentation of dextrose, lactose and H_2S production.

Composition**

Ingredients	Gms / Litre
Peptone	15.000
HM Peptone B #	3.000
Yeast extract	3.000
Proteose peptone	5.000
Lactose	10.000
Dextrose	1.000
Ferrous sulphate	0.200
Sodium chloride	5.000
Sodium thiosulphate	0.300
Phenol red	0.024
Agar	15.000
Final pH (at 25°C)	7.4±0.2

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

Directions

Suspend 57.52 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Mix well and distribute into into tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubes to cool in slanted position to form slopes with about 1inch butts.

Best reactions are obtained on freshly prepared medium. Do not use screw capped tubes or bottles.

Principle And Interpretation

Kligler Iron Agar is a combination of the lead acetate medium described by Kligler (9) and Russels Double Sugar Agar (7) and is used as a differentiation medium for typhoid, dysentery and allied bacilli (3). Bailey and Lacey substituted phenol red for andrade indicator previously used as pH indicator (3). Kligler Iron Agar differentiates lactose fermenters from the non-fermenters. It differentiates *Salmonella* Typhi from other Salmonellae and also *Salmonella* Paratyphi A from *Salmonella* Scottmuelleri and *Salmonella* Enteritidis (4). Fermentation of dextrose results in production of acid, which turns the indicator from red to yellow. Since there is little sugar i.e. dextrose, acid production is very limited and therefore a reoxidation of the indicator is produced on the surface of the medium, and the indicator remains red. However, when lactose is fermented, the large amount of acid produced, avoids reoxidation and therefore the entire medium turns yellow.

Kligler Iron Agar, in addition to peptone, HM peptone B and yeast extract, contains lactose and glucose (dextrose), which enables the differentiation of species of enteric bacilli. Phenol red is the pH indicator, which exhibits a colour change in response to acid produced during the fermentation of sugars. The combination of ferrous sulphate and sodium thiosulphate enables the detection of hydrogen sulfide production, which is evidenced by a black color either throughout the butt, or in a ring formation near the top of the butt. Lactose non-fermenters (e.g., *Salmonella* and *Shigella*) initially produce a yellow slant due to acid produced by the fermentation of the small amount of glucose (dextrose). When glucose (dextrose) supply is exhausted in the aerobic environment of the slant, the reaction reverts to alkaline (red slant) due to oxidation of the acids produced. The reversion does not occur in the anaerobic environment of the butt, which therefore remains acidic (yellow butt). Lactose fermenters produce yellow slants and butts because of lactose fermentation. The high amount of acids thus produced helps to maintain an acidic pH under aerobic conditions. Tubes showing original colour of the medium indicates the fermentation of neither glucose (dextrose) nor lactose. Gas production (aerogenic reaction) is detected as individual bubbles or by splitting or displacement of the agar by the formation of cracks in the butt of the medium.

Pure cultures of suspected organisms from plating media such as MacConkey Agar (M081), Bismuth Sulphite Agar (M027) or Deoxycholate Citrate Agar (M065), SS Agar (M108) etc. are inoculated on Kligler Iron Agar for identification.

Type of specimen

Isolated microorganism from clinical, food, dairy and water 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 (1,10,11).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(2)
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. Results should be noted after 18-24 hours. Else it might result in erroneous results.
2. Straight wire loop should be used for inoculation.
3. Pure isolates should be used to avoid erroneous results.

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 1.5% Agar gel

Colour and Clarity of prepared medium
Red coloured, clear to slightly opalescent gel forms in tubes as slants

Reaction
Reaction of 5.75% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

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

Organism	Inoculum (CFU)	Growth	Gas	H2S	Slant	Butt
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	positive reaction	negative reaction, no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
#Klebsiella aerogenes ATCC 13048 (00175*)	50-100	luxuriant	positive reaction	negative reaction, no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium

Citrobacter freundii ATCC 8090	50-100	luxuriant	positive reaction	positive reaction, blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
Proteus vulgaris ATCC 6380	50-100	luxuriant	negative reaction	positive reaction, blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Klebsiella pneumoniae ATCC 13883 (00087*)	50-100	luxuriant	positive reaction	negative reaction,no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
Salmonella Paratyphi A *ATCC 9150*	50-100	luxuriant	positive reaction	negative reaction,no blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Salmonella Schottmuelleri *ATCC 10719*	50-100	luxuriant	positive reaction	positive reaction, blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Salmonella Typhi *ATCC 6539*	50-100	luxuriant	negative reaction	positive reaction, blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Salmonella Enteritidis *ATCC 13076* (00030*)	50-100	luxuriant	positive reaction	positive reaction, blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Shigella flexneri ATCC 12022 (00126*)	50-100	luxuriant	negative reaction	negative reaction,no blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	luxuriant	negative reaction	negative reaction, blackening of medium	alkaline reaction, red colour of the medium	alkaline reaction,red colour of the medium
Yersinia enterocolitica ATCC 27729	50-100	luxuriant	variable reaction	negative reaction,no blackening of medium	alkaline reaction,red colour of the medium	acidic reaction, yellowing of the medium
*Enterobacter cloacae ATCC 13047 (*00083*)	50-100	luxuriant	positive reaction	negative reaction,no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium

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. 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 (5,6).

Reference

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

2.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.

3.Bailey S. F. and Lacey G. R., 1927, J. Bacteriol., 13:183.

4.Ewing, 1986, Edwards and Ewings Identification of the Enterobacteriaceae, 4th Ed., Elsevier Science Publishing Co., Inc., N.Y.

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

6. 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.

7.Kligler I. J., 1917, Am. J. Publ. Health, 7:1041.

8.Kligler I. J., 1918, J. Exp. Med., 28:319.

9.Russell F. F., 1911, J. Med. Res., 25:217.

10. 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.

11. 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 : 02 / 2018

IVD	In vitro diagnostic medical device
CE	CE Marking
30°C 10°C	Storage temperature
	Do not use if package is damaged
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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.

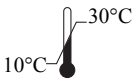
8.Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

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

CE Marking



Storage temperature

Do not use if package is damaged

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Iron Sulphite Agar

M868

Intended Use:

Recommended for detection of thermophilic anaerobic organisms causing sulphide spoilage in food.

Composition**

Ingredients	Gms / Litre
Tryptone	10.000
Sodium sulphite	0.500
Iron (III) citrate	0.500
Agar	15.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 26 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

Iron Sulphite Agar is a modification of Cameron Sulphite Agar developed by the National Canners Association of America (7). It was shown by Beerens (2) that 0.1% sulphite concentration in the original formula was inhibitory to some strains of *Clostridium sporogenes*. This observation was later confirmed by Mossel et al (5), who consequently showed that 0.05% sulphite concentration was not inhibitory to the organisms. Most clostridia have sulfite reductase in their cytoplasm but they are unable to expel them to the exterior. So when H_2S is produced from sulfite, the colony becomes dark due to the formation of precipitates of iron sulfide from citrate.

Tryptone provides nitrogen and other nutrients necessary to support bacterial growth. Sulphite-reducing bacteria usually produce black colonies as a result of the reduction of sulphite to sulphide, which reacts with the iron (III) salt.

For the detection of organisms causing sulphide spoilage, two methods can be followed:

a) Deep-Shake Culture Method: Dispense the medium in 10 ml amounts in tubes. Inoculate the sample when the medium is at about 50°C. Allow to set and incubate at 55°C for 24-48 hours.
Typical thermophilic species -*Desulfotomaculum nigrificans*, produces distinct black spherical colonies in the depth of the medium.

b) Attenborough and Scarr (1) Method: In this method, diluted samples of sugar or any other food are filtered through membrane filters.

These filters are then rolled up and placed in tubes containing just sufficient Iron Sulphite Agar (at 50°C) to cover them. The medium is allowed to set and then incubated at 55-56°C for 24-48 hours. After incubation, the number of black colonies on the membrane filter is counted. Confirmation tests are further carried out to identify the organism growing in the medium. This membrane filter technique is quicker, of comparable accuracy and permits the examination of larger samples. The blackening reaction is only presumptive evidence of clostridial growth. Confirmation test must be carried out for identification. There are many gram-negative bacteria that are able to reduce sulfite with iron sulfide production in this medium, but in these cases the enzymes are extra cellular and the entire medium becomes dark, rendering their enumeration impossible.

Type of specimen

Food samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (6).
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.This medium is general purpose medium and may not support the growth of fastidious organisms.

2.Further biochemical tests must be carried out 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 brownish yellow homogeneous free flowing powder

Gelling

Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Yellow coloured, slightly opalescent gel forms in Petri plates

Reaction

Reaction of 2.6% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

Cultural characteristics observed under anaerobic conditions, after an incubation at 55-56°C for 24-48 hours.

Organism	Inoculum	Growth	Recovery	Colour of colony
Clostridium botulinum ATCC 25763	50-100	luxuriant	>=50%	black
Clostridium butyricum ATCC 13732	50-100	luxuriant	>=50%	black
Clostridium sporogenes ATCC 19404 (00008*)	50-100	luxuriant	>=50%	black
Desulfotomaculum nigrificans ATCC 19998	50-100	luxuriant	>=50%	black
Escherichia coli ATCC 25922 (00013*)	50-100	good	40-50%	no blackening

Key : *Corresponding WDCM numbers.

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1.Attenborough J. and Scarr M., 1957, J. Appl. Bacteriol., 20: 460.

2.Beerens H., 1958, DSIR, Proc. 2nd Internat. Sym. Food Microbiol., 1957, HMSO, London, P. 235.

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.Mossel D. A. A., Golstein Brouwers G. W. M. V. and de Bruin A. S., 1959, J. Path. Bacteriol., 78:290.

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.

7.Tanner F. W., 1944, "The Microbiology of Foods", 2nd Ed., Garrard Press, Illinois, P. 1127.

Revision : 02 / 2019

R-2A Agar

M962

Intended Use:

Recommended for heterotrophic plate count of water samples using longer incubation periods.

Composition**

Ingredients	Gms / Litre
Acicase#	0.500
Yeast extract	0.500
Proteose peptone	0.500
Dextrose (Glucose)	0.500
Starch soluble	0.500
Dipotassium hydrogen phosphate	0.300
Magnesium sulphate	0.024
Sodium pyruvate	0.300
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Casein Acid Hydrolysate

Directions

Suspend 18.12 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 or as per validated cycle. DO NOT OVERHEAT. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

The heterotrophic plate count (HPC), formerly known as the standard plate count is a procedure for estimating the number of live heterotrophic bacteria in water and measuring changes during water treatment, in distribution systems or in swimming pools. R-2A Agar is recommended by APHA (1, 2) for estimating the heterotrophic plate count by the pour plate, spread plate or membrane filter procedure. R-2A Agar is formulated as per Reasoner and Geldreich (3). Stressed or injured organisms during water treatment are unable to grow on high nutrient media, since the faster growing organisms outgrow the former (4). Therefore the use of a low nutrient medium like R-2A Agar incubated for longer incubation periods allows these stressed organisms to grow well.

Many bacteria from natural waters which contain limited nutrients at ambient temperature, grow best on the media with less nutrient levels. They grow better at the temperatures below the routine laboratory incubation temperatures of 35 to 37°C (4).

Acicase, proteose peptone and yeast extract provide nitrogen, carbon compounds, vitamins, amino acids and minerals. Dextrose/ glucose serves as an energy source. Soluble starch aids in the recovery of injured organisms by absorbing toxic metabolic byproducts while sodium pyruvate increases the recovery of stressed cells. Magnesium sulphate is a source of divalent cations and sulphate. Dipotassium hydrogen phosphate is used to balance the pH of the medium. The number of colonies on a plate are reported as CFU (Colony Forming Units) per volume of sample.

Type of specimen

Water samples

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standard (1).

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. Longer incubation time other than specified is required for slow growing microorganisms.
2. The media is intended for water samples for recovery of stressed or injured organisms.

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 yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 1.81% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
Cultural characteristics observed *by using standard ATCC cultures after an incubation at 30-35°C for 24-72 hours. (*-In case of water samples from fields it is suggested to incubate further for upto 7 days to ascertain the absence of organisms)

Organism	Inoculum (CFU)	Growth	Recovery
Candida albicans ATCC 10231 (00054)*	50-100	good-luxuriant	>=50%
Escherichia coli ATCC 25922 (00013)*	50-100	good-luxuriant	>=50%
Salmonella Enteritidis ATCC 13076 (00030)*	50-100	good-luxuriant	>=50%
Pseudomonas aeruginosa ATCC 9027 (00026)*	50-100	good-luxuriant	>=50%
Staphylococcus aureus subsp. aureus ATCC 6538 (00032)*	50-100	good-luxuriant	>=50%
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)	50-100	good-luxuriant	>=50%
Aspergillus brasiliensis ATCC 16404 (00053)*	50-100	good-luxuriant	>=50%
Enterococcus faecalis ATCC 29212 (00087)*	50-100	good-luxuriant	>=50%
Salmonella Typhi ATCC 6539	50-100	good-luxuriant	>=50%

Key : * Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in 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 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, Wastewater, 20th Ed., American Public Health Association, Washington, D.C.
2.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.
3. Reasoner D. J. and Geldreich E. E., 1985, Appl. Environ. Microbiol., 49:1.
4. Collins V. J. and Willoughby J. G., 1962, Arch. Microbiol., 43:294.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6. 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. 1.66

Revision : 02 / 2018

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

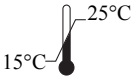
User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance 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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Antibiotic Assay Medium A with pH 7.9

ME004

Antibiotic Assay Medium A with pH 7.9 is used for microbiological assay of antibiotics in pharmaceutical and food related preparations in accordance with European Pharmacopoeia.

Composition**

Ingredients	Gms / Litre
Peptone	6.000
Pancreatic digest of casein	4.000
Yeast extract	3.000
Beef extract	1.500
Glucose monohydrate	1.000
Agar	15.000
Final pH (at 25°C)	7.9±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.40 grams in 1000 ml R/purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Advice: Recommended for the microbiological assay of Gentamicin sulphate, Kanamycin monosulphate, Kanamycin acid sulphate, Neomycin sulphate, Netilmicin sulphate, Spiramycin, Streptomycin sulphate, Tylosin,Tylosin tartarate, Vancomycin hydrochloride .

Principle And Interpretation

Antibiotic Assay media are used in the performance of antibiotic assays. Grove and Randall have elucidated those antibiotic assays and media in their comprehensive treatise on antibiotic assays (1). Schmidt and Moyer have reported the use of antibiotic assay medium for the liquid formulation used in the performance of antibiotic assay (2). This medium is recommended by EP (3) and FDA (4).

Nutrients and growth factors are supplied by the ingredients like peptone, pancreatic digest of casein, yeast extract and beef extract. Dextrose provides the carbon and energy source. Agar provides excellent medium for antibiotic diffusion and gives well-defined zones of inhibition. Higher pH provides the optimal conditions for activity of antibiotic and also supports the growth of the test organisms.

Freshly prepared plates should be used for antibiotic assays. Test organisms are inoculated in sterile seed agar pre-cooled to 40-45oC and spread evenly over the surface of solidified base agar. All conditions in the microbiological assay must be controlled carefully.

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 yellow coloured clear to slightly opalescent

Reaction
After sterilization, reaction of 3.04% w/v aqueous solution. pH : 7.9±0.1

pH
7.80-8.00

Cultural Response
ME004: Cultural characteristics observed after an incubation at specified temperature for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed & incubation temp.
When incubated anaerobically				
Micrococcus luteus ATCC 9341	50-100	good-luxuriant	>=70%	Tylosin, Tylosin tartarate (adjust the pH to 8.0±.0.1) - 32-35°C
Staphylococcus aureus ATCC 6538p	50-100	good-luxuriant	>=70%	Kanamycin monosulphate - 30-37°C, ,, Kanamycin acid sulphate-35-39°C, Netilmicin sulphate - ,,32-35°C,,
Staphylococcus epidermidis ATCC 12228	50-100	good-luxuriant	>=70%	Gentamicin sulphate - 35-39°C
Bacillus pumilis NCTC 8241	50-100	good-luxuriant	>=70%	Gentamicin sulphate - 35-39°C
Bacillus subtilis ATCC 6633	50-100	good-luxuriant	>=70%	Kanamycin monosulphate - 30-37°C, Kanamycin acid sulphate - 35-39°C, Spiramycin - 30-32°C, Streptomycin sulphate,,- 30-37°C Vancomycin hydrochloride (adjust the pH to 8.0±.0.1) -,,37-39°C
Bacillus subtilis NCTC 8236	50-100	good-luxuriant	>=70%	Streptomycin sulphate,,30-37°C

Storage and Shelf Life

Store below 30°C and use freshly prepared medium . Use before expiry date on the label.

Reference

1. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc, New York.
2. Schmidt and Moyer, 1944; J. Bact, 47:199.
3. European Pharmacopoeia, 2009, European Dept. for the Quality of Medicines.
4. Tests and Methods of Assay of Antibiotics and Antibiotic containing Drugs, FDA, CFR, 1983. Title 21, part 436, Subpart D, Washington, D.C. U.S Government printing office, paragraphs 436, 100-436, 106 pg 242-259 (April 1).

Revision : 2 / 2015

Antibiotic Assay Medium F

ME923

Antibiotic Medium F is used for microbiological assay of Amphotericin B and Nystatin using *Saccharomyces cerevisiae* ATCC 9763 and *Candida tropicalis* CIP 1433-83 in accordance with European Pharmacopoeia.

Composition**

Ingredients	Gms / Litre
Peptone	9.400
Yeast extract	4.700
Beef extract	2.400
Sodium chloride	10.000
Glucose monohydrate	10.000
Agar	23.500
pH after steilization	6.0±0.1

While assaying Amphotericin B adjust the pH to 6.1±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 59.09 grams of dehydrated medium in 1000 ml R-water/purified/distilled water. Heat to boiling to dissolve the medium completely. Dispense and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Adjust the pH of the medium, using freshly prepared buffer solution as recommended by the European Pharmacopoeia for the antibiotic assayed.

Advice : Recommended for the microbiological assay of Amphotericin B and Nystatin

Principle And Interpretation

Grove and Randall have elucidated the antibiotic assays and medias in their comprehensive treatise on antibiotic assays (1). Antibiotic assay Medium F is recommended for the microbiological assay of Nystatin and Amphotericin B using *Saccharomyces cerevisiae* and *Candida tropicalis* . This medium is formulated in accordance with the European Pharmacopoeia (2). Freshly prepared plates should be used for antibiotic assays. Test organisms are inoculated in sterile seed agar cooled to 40-45oC and spread evenly over the surface of solidified base agar. After incubation the concentration of the antibiotic being assayed is determined by measuring the zone of inhibition obtained, with that of reference standard antibiotic. All conditions in the microbiological assay must be carefully controlled. The use of standard culture media in the test is one of the important steps for good results.

Peptone, yeast extract and beef extract supply essential nutrients, minerals and growth factors for the growth of the test organisms. Glucose monohydrate in the medium provides enhanced source of carbon and energy. Osmotic equilibrium in the medium is provided by sodium chloride thereby maintaining the cell viability and integrity. Higher agar concentration provides solid substratum for growth of colonies and controls the diffusion of antibiotics.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm,comparable with 2.35% Agar gel.

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

Reaction
Reaction of 5.9% w/v aqueous solution after sterilization. pH : 6.0±0.1

pH
5.90-6.10

Cultural Response

ME923: Cultural characteristics observed after an incubation at specified temperature for 18 - 24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed	Incubation Temperature
Saccharomyces cerevisiae ATCC 9763	50-100	luxuriant	>=70%	Amphotericin B,Nystatin	35-37°C, 30-32°C,
Candida tropicalis CIP 1433-83	50-100	luxuriant	>=70%	Nystatin	30-37°C

Storage and Shelf Life

Store below 30°C in tightly closed container and use freshly prepared medium. Use before expiry date on the label.

Reference

1. Grove and Randall, 1955, Assay Methods of Antibiotics, Medical Encyclopedia, Inc. New York
2. European Pharmacopoeia 2011, European Department, for the Quality of Medicines.

Revision : 2 / 2015

Antibiotic Assay Medium B

ME1346

Antibiotic Assay Medium B is used for the microbiological assay of Colistimethate sodium using *Bordetella bronchiseptica* ATCC 4617 and *Escherichia coli* ATCC 10536 in accordance with European Pharmacopoeia .

Composition**

Ingredients	Gms / Litre
Pancreatic digest of casein	17.000
Papaic digest of soyabean	3.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	2.500
Glucose monohydrate	2.500
Agar	15.000
pH after sterilization	7.3±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 44.77 grams of dehydrated medium powder in 1000 ml R-water/purified/distilled water with 10 ml polysorbate 80. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before pouring into sterile Petriplates. Adjust the pH of the medium, using freshly prepared buffer solution as recommended by the European pharmacopoeia for the antibiotic assayed.

Principle And Interpretation

Antibiotic Assay Medium B is prepared according to European Pharmacopoeia (1) and is recommended for the assay of Colistimethate sodium using *Bordetella bronchiseptica* and *Escherichia coli* as the test organism. Combination of pancreatic digest of casein and papaic digest of soyabean provides essential nutrients for the growth of test organisms.Glucose monohydrate provides fermentable source of carbon, and enhances the growth of test organims. Phosphates in the medium enhance buffering action and sodium chloride maintains osmotic equilibrium. Freshly prepared plates should be used for antibiotic assays. Test organisms are inoculated in sterile seed agar cooled to 40-45°C and spread evenly over the surface of solidified base agar. All conditions in the microbiological assay must be controlled carefully. The use of standard culture media in the test is one of the important steps for good results.

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

pH
7.20-7.40

Cultural Response
ME1346: Cultural characteristics observed after an incubation at 35-39°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed
Bordetella bronchiseptica ATCC 4617	50-100	luxuriant	>=70%	Colistimethate sodium
Escherichia coli ATCC 10536	50-100	luxuriant	>=70%	Colistimethate sodium

Storage and Shelf Life

Store below 30°C in tightly closed container and use freshly prepared medium. Use before expiry date on the label.

Reference

1. European Pharmacopoeia, 2011, European Department, for the Quality of Medicines.

Revision : 2 / 2015

Antibiotic Assay Medium E

ME1347

For microbiological assay of Framycetin Sulphate and Neomycin sulphate using *Bacillus subtilis* and/or *Bacillus pumilus* in accordance with European Pharmacopoeia.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
Disodium hydrogen phosphate,12H_2O	26.900
Meat extract	3.000
Agar	10.000
pH after sterilization	7.9±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 28.67 grams of dehydrated medium powder in 1000 ml R-water/ purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Adjust the pH of the medium, using freshly prepared buffer solution as recommended by the European pharmacopoeia for the antibiotic assayed.

Advice : Recommended for the microbiological assay of Framycetin Sulphate and Neomycin sulphate .

Principle And Interpretation

This medium is formulated in accordance to European Pharmacopoeia (1).This medium is widely used for as seed agar in plate assay of Framycetin sulphate and Neomycin sulphate using *Bacillus subtilis* and/or *Bacillus pumilus* as test organism.

Peptone and meat extract supply nutrients essential for microbial growth. Phosphates are incorporated in the medium to provide good buffering action. The low concentration of agar facilitates proper diffusion of antibiotic in the seed agar.

Freshly prepared plates should be used for antibiotic assays. Test organisms are inoculated in sterile seed agar cooled to 40-45°C and spread evenly over the surface of solidified base agar. Zones of inhibition around the antibiotic are then measured. All conditions in the microbiological assay must be controlled carefully. The use of standard culture media in the test is one of the important steps for good results.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.0% Agar gel.

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

pH
7.80-8.00

Cultural Response
ME1347: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed
Bacillus pumilus NCTC 8241	50-100	luxuriant	>=70%	Framycetin sulphate, Neomycin sulphate

Bacillus subtilis ATCC 6633	50-100	luxuriant	>=70%	Neomycin sulphate and Framycetin sulphate

Storage and Shelf Life

Store below 30°C in tightly closed container and use freshly prepared medium . Use before expiry date on the label.

Reference

1. European Pharmacopoeia, 2011, European Department, for the Quality of Medicines.

Revision : 2 / 2015

Soybean Casein Digest Medium (Casein Soybean Digest Broth) MH011

Intended Use:

Recommended as a general-purpose medium used for cultivation of a wide variety of microorganisms and for sterility testing of moulds and lower bacteria in accordance with the harmonized method of USP/EP/BP/JP/IP.It can also be used for clinical samples.

Composition**

Ingredients	Gms / Litre
Tryptone #	17.000
Soya peptone ##	3.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	2.500
Glucose monohydrate	2.500
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Pancreatic digest of casein ## Papaic digest of soybean (soyabean)

Directions

Suspend 29.77 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/ distilled water. Heat if necessary to dissolve the medium completely. Dispense in tubes or flasks as desired. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes or as per validated cycle.

Note: If any fibres are observed in the solution, it is recommended to filter the solution through a 0.22 micron filter to eliminate the possibility of presence of fibres.

Principle And Interpretation

Soybean Casein Digest Medium is recommended as a sterility testing medium in accordance with the harmonized method of USP/EP/BP/JP/IP (1,2,3,5,7). It is used for the sensitivity testing of antimicrobial agents by the tube dilution method (8). It is also employed in diagnostic research in microbiology. This medium is used as a diluent and suspending medium for preparation of samples or test strains. It is also employed in sample preparation for testing of products, wherein incubation is carried out, only to serve sufficient resuscitation of the cell, while avoiding multiplication of the organism.

The combination of tryptone and soya peptone makes this medium nutritious by providing nitrogenous, carbonaceous compounds, long chain amino acids, vitamins and other minerals for the growth of microorganisms. Natural sugars in soybean promote growth of fastidious organism. Glucose monohydrate is the fermentable source of carbon and dipotassium hydrogen phosphate serves as the buffer in the medium. Sodium chloride maintains the osmotic balance of the medium.

This medium is recommended for sterility checking and for studying total aerobic microbial count in verification of microbiological testing procedures employed for sterility checking.

Type of specimen

Pharmaceutical samples; Clinical samples- urine, blood

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per pharmaceutical guidelines (7,2,1,5,3).

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

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 characterization is necessary to be performed on colonies from pure cultures for further identification.
2. This medium is general purpose medium and may not support the growth of fastidious organisms.

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 yellow coloured clear solution without any precipitate.

Reaction
pH of 2.98% w/v aqueous solution at 25°C (after sterilization). pH : 7.3±0.2

pH
7.10-7.50

Stability test
Light yellow coloured clear solution without any precipitation or sedimentation at room temperature for 7 days

Growth promoting properties
Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu(at 30-35°C for 18-24 hours for bacteria and 5 days for fungal). Growth promotion is carried out as per USP/EP/BP/JP.

Sterility Testing + Validation
The medium is tested with suitable strains of microrganisms inoculating <=100cfu and incubating at 20-25°C for not more than 3 days in case of bacteria and not more than 5 days in case of fungi.

Organism	Inoculum (CFU)	Growth	Incubation period	Incubation temperature
Growth promoting				
Salmonella Abony *NCTC 6017 (00029*)*	50 -100	luxuriant	18 -24 hrs	30 -35 °C
Streptococcus pneumoniae ATCC 6305	50 -100	luxuriant	18 -24 hrs	30 -35 °C
Escherichia coli NCTC 9002	50 -100	luxuriant	18 -24 hrs	30 -35 °C
Pseudomonas aeruginosa ATCC 27853 (00025)*	50 -100	luxuriant	18 -24 hrs	30 -35 °C
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003)*	50 -100	luxuriant	18 -24 hrs	30 -35 °C
Micrococcus luteus ATCC 9341	50 -100	luxuriant	18 -24 hrs	30 -35 °C
Salmonella Typhimurium *ATCC 14028 (00031*)*	50 -100	luxuriant	18 -24 hrs	30 -35 °C
Escherichia coli ATCC 8739 (00012)*	50 -100	luxuriant	18 -24 hrs	30 -35 °C
Escherichia coli ATCC 25922 (00013)*	50 -100	luxuriant	18 -24 hrs	30 -35 °C
Pseudomonas aeruginosa ATCC 9027 (00026)*	50 -100	luxuriant	18 -24 hrs	30 -35 °C
Staphylococcus aureus subsp. aureus ATCC 6538 (00032)*	50 -100	luxuriant	18 -24 hrs	30 -35 °C
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	50 -100	luxuriant	18 -24 hrs	30 -35 °C

Sterility Testing- Growth promotion+Validation				
Candida albicans ATCC 2091 (00055)*	50 -100	luxuriant	<=5 d	30 -35 °C
Salmonella Abony *NCTC 6017 (00029*)*	50 -100	luxuriant	<=3 d	20 -25 °C
Candida albicans ATCC 10231 (00054)*	50 -100	luxuriant	<=5 d	30 -35 °C
#Aspergillus brasiliensis ATCC 16404 (00053)*	50 -100	luxuriant	<=5 d	30 -35 °C
Streptococcus pneumoniae ATCC 6305	50 -100	luxuriant	<=3 d	20 -25 °C
Escherichia coli NCTC 9002	50 -100	luxuriant	<=3 d	20 -25 °C
Pseudomonas aeruginosa ATCC 27853 (00025)*	50 -100	luxuriant	<=3 d	20 -25 °C
Micrococcus luteus ATCC 9341	50 -100	luxuriant	<=3 d	20 -25 °C
Salmonella Typhimurium *ATCC 14028 (00031*)*	50 -100	luxuriant	<=3 d	20 -25 °C
Staphylococcus aureus subsp. aureus ATCC 6538 (00032)*	50 -100	luxuriant	<=3 d	20 -25 °C
Escherichia coli ATCC 8739 (00012)*	50 -100	luxuriant	<=3 d	20 -25 °C
Escherichia coli ATCC 25922 (00013)*	50 -100	luxuriant	<=3 d	20 -25 °C
Pseudomonas aeruginosa ATCC 9027 (00026)*	50 -100	luxuriant	<=3 d	20 -25 °C
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003)*	50 -100	luxuriant	<=3 d	20 -25 °C
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	50 -100	luxuriant	<=3 d	20 -25 °C

Key : (#) Formerly known as *Aspergillus niger*, (*) Corresponding WDCM numbers

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 thelabel. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,6).

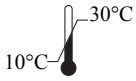
Reference

1. British Pharmacopoeia, 2019, The Stationery office British Pharmacopoeia
2. European Pharmacopoeia, 2019, European Dept. for the quality of Medicines.
3. Indian Pharmacopoeia, 2018, Govt. of India, the controller of Publication, Delhi, India.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. Japanese Pharmacopoeia, 2016.
6. 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.
7. The United States Pharmacopoeia, 2020, The United States Pharmacopoeial Convention. Rockville, MD.
8. Wright and Welch, 1959-60, Antibiotics Ann., 61.

Revision : 05 / 2021

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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Cetrimide Agar

MH024

Intended use

Recommended for the selective isolation of *Pseudomonas aeruginosa* from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP.

Composition**

Ingredients	Gms / Litre
Gelatin peptone #	20.000
Magnesium chloride	1.400
Dipotassium sulphate	10.000
Cetrimide	0.300
Agar	13.600
pH after sterilization (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Pancreatic digest of gelatin

Directions

Suspend 45.3 grams in 1000 ml purified/distilled water containing 10 ml glycerin/glycerol. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Cetrimide Agar was described by King et al (6). This media formulation is in accordance with the harmonized method of USP/EP/BP/JP/IP (1,2,3,5,9). It is used as a selective medium for the isolation of *Pseudomonas aeruginosa* from pharmaceutical products. This medium is also used for microbial limit testing for non- sterile products. Lowburry first reported the use of cetrimide as an agent for selective isolation of Pseudomonas (7). This medium is also used for determining the ability of an organism to produce fluorescein and pyocyanin. Cetrimide (N-acetyl-N,N,N-trimethylammonium bromide) is incorporated in the medium to inhibit bacteria other than *Pseudomonas aeruginosa.* This compound a cationic detergent acts as a quaternary ammonium compound, which causes nitrogen and phosphorus to be released from bacterial cells other than *Pseudomonas aeruginosa.* Magnesium chloride and potassium sulphate incorporated in the medium enhances the production of pigment pyocyanin, which is a blue-green pigment, diffusing into the medium. This improves detection of *Pseudomonas* on this medium. Presence of magnesium ions can also neutralizes EDTA, if present in the sample. Gelatin peptone provides the essential nutrients for growth of *Pseudomonas*, while glycerin serves as slow and continuous carbon source for the growing cell.

For the isolation of Pseudomonas aeruginosa, plates of Cetrimide Agar should be inoculated from non-selective medium such as Soybean Casein Digest Medium (MH011). If the count is high the test sample can be directly inoculated onto this medium. *Pseudomonas aeruginosa* colonies may appear pigmented greenish (under uv light also).

Type of specimen

Pharmaceutical samples: Clinical samples

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (1,2,3,5,9).

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

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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. This medium is a selective medium, some strains may show poor growth as cetrimide is highly toxic.
2. Further biochemical tests must be carried out for complete 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 1.36% Agar gel

Colour and Clarity of prepared medium

Light amber coloured opalescent gel with a slight precipitate forms in Petri plates

pH

7.00-7.40

Growth Promotion Test

Growth Promotion is carried out in accordance with the harmonized method of USP/EP/BP/JP/IP. Cultural response was observed after an incubation at 30-35°C for specified time. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Growth promoting properties

Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu (at 30-35°C for <=18 hours).

Inhibitory properties

No growth of the test microorganism occurs for the specified temp for not less than longest period of time specified inoculating >=100 cfu (at least 100 cfu) (at 30-35°C for >= 72 hours).

Cultural Response

Cultural characteristics observed after incubation at 30-35 °C for 18-72 hours. Recovery rate is considered as 100%for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Incubation temperature	Incubation period
Growth promoting						
Pseudomonas aeruginosa ATCC 9027 (00026*)	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	<=18 hrs
Inhibitory						
Escherichia coli ATCC 8739 (00012*)	>=10^3	inhibited	0	0 %	30 -35 °C	>=72 hrs
Additional Microbiological testing						
Pseudomonas aeruginosa ATCC 27853(00025*)	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	18 -24 hrs
Pseudomonas aeruginosa ATCC 25668 (00114*)	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	18 -24 hrs
Stenotrophomonas maltophila ATCC 13637	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
Escherichia coli ATCC 25922 (00013*)	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
Escherichia coli NCTC 9002	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs

Salmonella Typhimurium *ATCC 14028* (00031*)	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
Proteus mirabilis ATCC 29906 (00023)*	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs

Key : (*) Corresponding WDCM numbers.

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,8).

Reference

1. British Pharmacopoeia, 2016, The Stationery office British Pharmacopoeia
2. European Pharmacopoeia, 2017 European Dept. for the quality of Medicines.
3. Indian Pharmacopoeia, 2018, Govt. of India, Ministry of Health and Family Welfare, New Delhi
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition
5. Japanese Pharmacopoeia, 2016
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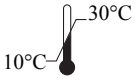
8. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. , 11th Ed., 2015, Manual of Clinical Microbiology.
9. The United States Pharmacopoeia, 2019, The United States Pharmacopeial Convention.Rockville, MD.

Revision : 04/ 2019

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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Xylose-Lysine-Deoxycholate Agar

MH031

Xylose-Lysine Deoxycholate Agar is a selective medium recommended for the isolation and enumeration of *Salmonella* Typhi and other *Salmonella* species from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP (Medium 11).

Composition**

Ingredients	Gms / Litre
Xylose	3.500
L-lysine	5.000
Lactose monohydrate	7.500
Sucrose	7.500
Sodium chloride	5.000
Yeast extract	3.000
Sodium deoxycholate	2.500
Sodium thiosulphate	6.800
Ferric ammonium citrate	0.800
Phenol red	0.080
Agar	13.500
pH after heating (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 54.8 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/ distilled water. Heat with frequent agitation until the medium boils. DO NOT HEAT IN AN AUTOCLAVE. Transfer immediately to a water bath at 50°C. After cooling, pour into sterile Petri plates. It is advisable not to prepare large volumes, which will require prolonged heating and may produce precipitate.

Note:Slight precipitation in the medium may occur,which is inheritant property of the medium,and does not affect the performance of the medium.

Principle And Interpretation

Enterobacteriaceae is a family of gram-negative, non-spore-forming bacilli that contains more than 100 species of bacteria that normally inhabit the intestines of humans and animals. Members forming part of the normal intestinal microflora are referred to as coliforms. The clinically significant genera of Enterobacteriaceae include *Cedecea, Citrobacter, Edwardsiella, Enterobacter, Escherichia, Ewingella, Hafnia, Klebsiella, Kluyvera, Proteus, Salmonella, Shigella* and *Yersinia* (1).

The Salmonellae are the most complex of all the *Enterobacteriaceae* . Human *Salmonella* infections are most commonly caused by ingestion of food, water or milk, contaminated by human or animal excreta (2). A large number of media have been developed for the selective isolation and identification of enteric bacilli including *Salmonella* .

Xylose Lysine Deoxycholate Agar is a selective as well as differential medium formulated by Taylor (3-7) for the isolation and identification of enteric pathogens especially Shigellae from stool samples. It is also used for pharmaceutical testing and non-sterile product testing for the detection (or absence) of *Salmonella* after enrichment in Rappaport Vassiliadis Salmonella Enrichment Broth (MH1491) in accordance with the harmonized method of USP/EP/BP/JP/IP (8-12).

Deoxycholate, ferric ammonium citrate and sodium thiosulphate are selective agents that inhibit gram-positive microorganisms. Essential nutrients, growth factors for growth of microorganism are provided by yeast extract. Xylose, sucrose and lactose are the fermentable sugars in this medium. Xylose is fermented by almost all the enteric bacteria except Shigellae , which enable the differentiation of Shigellae from Salmonellae.Salmonellae metabolize the xylose and decarboxylate lysine and thus change the pH to alkaline and mimic Shigellae reaction. However to prevent this reaction by lysine positive coliforms, lactose and sucrose are added in excess to produce acid and hence nonpathogenic H2S producers do not decarboxylate lysine. Sodium thiosulphate helps in reactivation of sulphur containing compounds and prevents the desication of these compounds during storage. It also forms the substrate for enzyme thiosulphate reductase, which breaks it to form H2S. Thiosulphate and ferric

ammonium citrate are the H2S indicators in the medium. Sodium thiosulphate is also inactivator of halogens, mercurial and aldehyde and can minimize its toxicity in the testing sample, if any during microbial limit tests. Sodium chloride maintains the osmotic equilibrium in this medium. Phenol red is the pH indicator.

Degradation of fermentable sugars proceed concurrently and generates acids, which cause pH indicator to give various shades of colour, causing a color change in the colonies and in the medium from red to yellow on prolonged incubation. Hydrogen sulfide production results in colonies with black centers under alkaline conditions, which can be inhibited by acid production by carbohydrate fermentation. Alkaline condition causes the color of the medium to change back to red. This medium is an ideal medium for screening samples containing mixed flora of enteric pathogens as recovery of Salmonellae and Shigellae is not conspicuous by even profuse growth of other species (13,14).

Quality Control

Appearance
Light yellow to pink homogeneous free flowing powder

Gelling
Firm, comparable with 1.35% Agar gel

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

pH
7.20-7.60

Cultural Response
Growth Promotion is carried out in accordance with the harmonized method of USP/EP/BP/JP/IP. Cultural response was observed after an incubation at 30-35°C for specified time. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Growth promoting properties
Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu(at 30-35°C for <=18 hours).

Indicative properties
Colonies are comparable in appearance and indication reaction to those previously obtained with previously tested and approved lot of medium occurs for the specified temperature for a period of time within the range specified inoculating <=100cfu (at 30-35°C for 18-72 hours).

Inhibitory properties
No growth of the test microorganism occurs for the specified temp for not less than longest period of time specified inoculating >=100cfu (at 30-35°C for >= 72 hours).

Cultural Response
MH031: Cultural characteristics observed after incubation at 30-35 °C for 18-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation temperature
Growth Promoting + Indicative						
Salmonella Typhimurium ATCC 14028	50 -100	luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
Salmonella Abony NCTC 6017	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
Additional Microbiological testing						
Escherichia coli ATCC 8739	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Escherichia coli ATCC 25922	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Escherichia coli NCTC 9002	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Proteus vulgaris ATCC 13315	50 -100	good-luxuriant	25 -100	>=50 %	grey with black centres	18 -72 hrs
Salmonella Paratyphi A ATCC 9150	50 -100	good-luxuriant	25 -100	>=50 %	red	18 -72 hrs

Salmonella Paratyphi B ATCC 8759	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
Salmonella Enteritidis ATCC 13076	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
Salmonella Typhi ATCC 6539	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
Shigella dysenteriae ATCC 13313	50 -100	good-luxuriant	25 -100	>=50 %	red	18 -72 hrs
Shigella flexneri ATCC 12002	50 -100	fair-good	15 -40	30 -40 %	red	18 -72 hrs
Shigella sonnei ATCC 25931	50 -100	fair-good	15 -40	30 -40 %	red	18 -72 hrs
Enterobacter aerogenes ATCC 13048	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Enterobacter cloacae ATCC 13047	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0	0%		>=72 hrs
Staphylococcus aureus ATCC 6538	>=10^3	inhibited	0	0%		>=72 hrs
Enterococcus faecalis ATCC 29212	>=10^3	inhibited	0	0%		>=72 hrs

Storage and Shelf Life

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

Reference

1.Pelczar M. J. Jr., Reid R. D., Chan E. C. S., 1977, Microbiology, 4th Ed., Tata McGraw-Hill Publishing Company Ltd, New Delhi.
2.Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr., 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4th Ed., J. B. Lippinccott Company.
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11.Japanese Pharmacopoeia, 2008.
12.Indian Pharmocoepoeia, 2010 Ministry of Health and Family Welfare, Govt. of India.
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Revision : 02 / 2015

Sabouraud Dextrose Broth

MH033

Intended use

Recommended for cultivation of yeasts, moulds and aciduric microorganisms from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP.

Composition**

Ingredients	Gms / Litre
Dextrose (Glucose)	20.000
Mixture of Peptone and Tryptone (1:1)#	10.000
pH after sterilization (at 25°C)	5.6±0.2

Mixture of Peptic digest of animal tissue and Pancreatic digest of casein (1:1)

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle.

Principle And Interpretation

Fungi were among the first microorganisms recognized because some of the fruiting structures, such as the mushrooms, are large enough to be seen without a microscope. Fungi can be grouped simply on the basis of morphology as either yeasts or moulds (6). Fungal diseases that occur on the skin, hair and mucous membrane are called superficial mycoses, and the organism that cause them, the dermatophytes (7). Where fungi are to be isolated, it is good practice to use a medium that favors their growth but is not optimal for the growth of bacteria.

Sabouraud Dextrose Broth is a modification of Dextrose Agar described by Sabouraud (8). It is useful for the cultivation of fungi. This medium is in accordance with the harmonized method of USP/EP/BP/JP (9,1,2,4) and is recommended for microbiological examination of non-sterile products.

Peptone and Tryptone provides nitrogenous, carbonaceous compounds, long chain amino acids and other essential for the growth of fungi. Dextrose (Glucose) acts as the energy source.

Type of specimen

Pharmaceutical samples; Clinical samples

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per pharmaceutical guidelines (9,1,2,4).
For clinical samples, follow appropriate techniques for sample collection and processing as per guidelines (3,5). 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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. For heavily contaminated samples, the medium must be supplemented with inhibitory agents for inhibiting bacterial growth with lower pH.
2. Some pathogenic fungi may produce infective spores which are easily dispersed in air, so examination should be carried out in safety cabinet
3. Further biochemical tests should be carried out 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
Cream to yellow homogeneous free flowing powder

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

pH of 3.0% w/v aqueous solution at 25°C (after sterilization).

pH
5.40-5.80

Growth Promotion Test
Growth Promotion was observed in accordance with the harmonized method of USP/EP/BP/JP after an incubation at 30-35°C for 3-5 days.

Growth promoting properties
Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating 100 cfu (at 30-35°C for 3-5 days).

Cultural Response
Cultural characteristics observed after incubation at 20-25 °C for 3-5 days.

Organism	Inoculum (CFU)	Growth	Incubation temperature	Incubation period
Candida albicans ATCC 10231 (00054)*	50 -100	luxuriant	30 -35 °C	<=3 d
Growth Promotion + Total Yeast and Mould count				
Candida albicans ATCC 10231 (00054)*	50 -100	luxuriant	20 -25 °C	<=5 d
# Aspergillus brasiliensis ATCC 16404 (00053)*	50 -100	luxuriant	20 -25 °C	<=5 d
Additional Microbiological Testing				
Saccharomyces cerevisiae ATCC 9763 (00058)*	50 -100	luxuriant	20 -25 °C	3 -5 d
Saccharomyces cerevisiae ATCC 2601	50 -100	good-luxuriant	20 -25 °C	3 -5 d
Candida albicans ATCC 2091 (00055)*	50 -100	luxuriant	20 -25 °C	3 -5 d

Key: (#) Formerly known as *Aspergillus niger* (*) Corresponding WDCM numbers

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 thelabel. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,5).

Reference

1. British Pharmacopoeia, 2016, The Stationery office British Pharmacopoeia.
2. European Pharmacopoeia, 2017, European Dept. for the quality of Medicines.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook, 2nd Edition..
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7. Pelczar M. J., Jr., Reid R. D., Chan E. C. S., 1977, Microbiology, 4th Edi, Tata McGraw-Hill Publishing Company Ltd, New Delhi
8. Sabouraud, 1892, Ann. Dermatol. Syphilol, 3:1061.
9. The United States Pharmacopoeia, 2019, The United States Pharmacopeial Convention,Rockville, MD.

Revision : 03 / 2019

IVD	In vitro diagnostic medical device
CE	CE Marking
30°C 10°C	Storage temperature
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Sabouraud Dextrose Agar

MH063

Intended Use

Recommended for the cultivation of yeasts, moulds and aciduric bacteria from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP .

Composition**

Ingredients	Gms / Litre
Dextrose (Glucose)	40.000
Mixture of Peptone and Tryptone (1:1)##	10.000
Agar	15.000
pH after sterilization(at 25°C)	5.6±0.2

Mixture of Peptic digest of animal tissue and Pancreatic digest of casein (1:1)#

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 65.0 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 or as per validated cycle. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Fungi were among the first microorganisms recognized because some of the fruiting structures, such as the mushrooms, are large enough to be seen without a microscope. Fungi can be grouped simply on the basis of morphology as either yeasts or moulds (9). Fungal diseases that occur on the skin, hair and mucous membrane are called superficial mycoses, and the organism that cause them, the dermatophytes (10). Where fungi are to be isolated, it is good practice to use a medium that favors their growth but is not optimal for the growth of bacteria.

Sabouraud Dextrose Agar is Carliers modification (3) of the formulation described by Sabouraud (11) for the cultivation of fungi (yeasts, moulds), and aciduric microorganisms. Sabouraud Dextrose Agar is recommended for microbiological examination of non-sterile products in accordance with the harmonized method of USP/EP/BP/JP (12,2,4,7). This medium is also employed in microbial limit tests in pharmaceutical testing, food, cosmetics, and clinical specimens (1)

Peptone and Tryptone provides carbonaceous, nitrogenous compounds, long chain amino acids, vitamins and other essential growth nutrients. Dextrose (Glucose) provides an energy source. High dextrose concentration and low pH favors fungal growth and inhibits contaminating bacteria from clinical specimens (8).

Some pathogenic fungi may produce infective spores, which are easily dispersed in air, so examination should be carried out in safety cabinet. For heavily contaminated samples, the plate must be supplemented with inhibitory agents for inhibiting bacterial growth. Growth of white colonies may be indicative of presence of *Candida albicans*. The total combined yeast and molds count is considered to be equal to the number of colony forming unit found using this medium, if bacterial colonies are detected they are counted as part of total yeast and mold count. In case the bacterial colonies exceeds the acceptance criterion, then antibiotics can be supplemented in this medium

Type of specimen

Pharmaceutical samples; Clinical samples-skin scrappings

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per pharmaceutical guidelines (12,2,4,7). For clinical samples, follow appropriate techniques for sample collection and processing as per 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 handing specimens and culture. Standard guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. For heavily contaminated samples, the media must be supplemented with inhibitory agents for inhibiting bacterial growth with lower pH.
2. Some pathogenic fungi may produce infective spores which are easily dispersed in air, so examination should be carried out in safety cabinet
3. Further biochemical tests should be carried out 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
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

pH
5.40-5.80

Growth Promotion Test
Growth Promotion was carried out in accordance with the harmonized method of ICH (USP/EP/BP/JP), after an incubation at 30-35 °C for 24-48 hours.Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar and fungus growth on Sabouraud Dextrose Agar

Growth Promoting Properties
Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <= 100 cfu (at 30-35°C for <=24 hours).

Indicative properties
Colonies are comparable in appearance and indication reaction to those previously obtained with previously tested and approved lot of medium occurs for the specified temperature for a period of time within the range specified inoculating <=100cfu (at 30-35°C for 24-48 hours).

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Incubation temperature	Incubation period
Growth Promotion + Indicative						
Candida albicans ATCC 10231 (00054)*	50 -100	Luxuriant (white colonies)	35 -100	>=70 %	30 -35 °C	24 -48 hrs
Growth Promotion + Total yeast and mould count						
Candida albicans ATCC 10231 (00054)*	50 -100	luxuriant	35 -100	>=70 %	20 -25 °C	<=5 d
#Aspergillus brasiliensis ATCC 16404 (00053)*	50 -100	luxuriant	35 -100	>=70 %	20 -25 °C	<=5 d
Additional Microbiological Testing						

Candida albicans ATCC 2091 (00055)*	50 -100	luxuriant	35 -100	>=70%	30 -35 °C	24 -48 hrs
Saccharomyces cerevisiae ATCC 9763 (00058)*	50 -100	luxuriant	35 -100	>=70 %	30 -35 °C	24 -48 hrs
Escherichia coli ATCC 25922 (00013)*	50 -100	good(inhibited on media with low pH)	35 -100	>=70 %	30 -35 °C	24 -48 hrs
Escherichia coli ATCC 8739 (00012)*	50 -100	good(inhibited on media with low pH)	35 -100	>=70 %	30 -35 °C	24 -48 hrs
Escherichia coli NCTC 9002	50 -100	good(inhibited on media with low pH)	35 -100	>=70 %	30 -35 °C	24 -48 hrs
Trichophyton rubrum ATCC 28191	50-100	good			20 -25 °C	<=5 d
Lactobacillus casei ATCC 334	50 -100	luxuriant	35 -100	>=70 %	30 -35 °C	24 -48 hrs

Key : (#) - Formerly known as *Aspergillus niger,* (*) - corresponding WDCM numbers

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 thelabel. 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 (5,6).

Reference

1. Bacteriological Analytical Manual, 8th Edition, Revision A, 1998. AOAC, Washington D.C.
2. British Pharmacopoeia, 2016, The Stationery office British Pharmacopoeia
3. Carlier G. I. M., 1948, Brit. J. Derm. Syph., 60:61.
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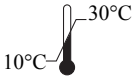
11. Sabouraud K., 1892, Ann. Dermatol. Syphilol, 3:1061
12. The United States Pharmacopoeia, 2019, The United States Pharmacopoeial Convention.,,Rockville, MD.

Revision : 03 / 2019

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

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MacConkey Agar

MH081

Intended Use

Recommended for selective isolation and differentiation of *E.coli* and other enteric bacteria from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP.

Composition**

Ingredients	Gms / Litre
Gelatin peptone #	17.000
HMC peptone ##	3.000
Lactose monohydrate	10.000
Sodium chloride	5.000
Bile salts	1.500
Neutral red	0.030
Crystal violet	0.001
Agar	13.500
pH after sterilization (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Pancreatic digest of gelatin
Equivalent to Peptones (meat and casein)

Directions

Suspend 49.53 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/distilled water. Boil for 1 minute with constant stirring. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle. Avoid overheating. Cool to 45-50°C. Mix well before pouring into sterile Petri plates. The surface of the medium should be dry when inoculated.

Principle And Interpretation

MacConkey Agar is the earliest selective and differential medium for cultivation of coliform organisms (8,9). Subsequently MacConkey Agar and Broth have been recommended for use in microbiological examination of foodstuffs (10) and for direct plating / inoculation of water samples for coliform counts (1). This medium is also accepted by the Standard Methods for the Examination of Milk and Dairy Products (12). It is recommended in pharmaceutical preparations and is in accordance with the harmonized method of USP/EP/BP/JP (2,3,6,11).

Gelatin peptone and HMC peptone provide the essential nutrients, vitamins and nitrogenous factors required for growth of microorganisms. Lactose monohydrate is the fermentable source of carbohydrate. The selective action of this medium is attributed to crystal violet and bile salts, which are inhibitory to most species of gram-positive bacteria. Sodium chloride maintains the osmotic balance in the medium.

After enrichment of *Escherichia coli* in MacConkey Broth (MH083), it is then subcultured on MacConkey Agar. Gram-negative bacteria usually grow well on the medium and are differentiated by their ability to ferment lactose. Lactose fermenting strains grow as red or pink and may be surrounded by a zone of acid precipitated bile. The red colour is due to production of acid from lactose, absorption of neutral red and a subsequent colour change of the dye when the pH of medium falls below 6.8. Lactose non-fermenting strains, such as *Shigella* and *Salmonella* are colourless and transparent and typically do not alter appearance of the medium. *Yersinia enterocolitica* may appear as small, non-lactose fermenting colonies after incubation at room temperature.

Type of specimen

Pharmaceutical samples, Clinical samples; Food and dairy samples; Water samples.

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (2,3,6,11).
For clinical samples, follow appropriate techniques for sample collection and processing as per guidelines (5,7).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (10, 12).
For water samples, follow appropriate techniques for sample collection and processing as per guidelines (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. Though the medium is recommended for selective isolation, further biochemical and serological testing must be carried out for further confirmation.
2. The surface of the medium should be dry when inoculated.

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 1.35% Agar gel.

Colour and Clarity of prepared medium

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

pH
6.90-7.30

Cultural Response
Growth Promotion is carried out in accordance with the harmonized method of ICH (USP/EP/BP/JP). Cultural response was observed after an incubation at 30-35°C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Growth promoting properties
Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating 100 cfu (at 30-35°C for <=18 hours).

Indicative properties
Colonies are comparable in appearance and indication reaction to those previously obtained with previously tested and approved lot of medium occurs for the specified temperature for a period of time within the range specified inoculating <=100 cfu (at 30-35°C for 18-72 hours).

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation period
Growth Promoting + Indicative						
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	25 -100	>=50 %	pink-red with bile precipitate	18 -72 hrs
Additional Microbiological testing						
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant	25 -100	>=50 %	pink to red with bile precipitate	18 -24 hrs
Escherichia coli NCTC 9002	50 -100	luxuriant	25 -100	>=50 %	pink to red with bile precipitate	18 -24 hrs
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50 -100	luxuriant	25 -100	>=50 %	pink to red	18 -24 hrs
Enterococcus faecalis ATCC 29212 (00087*)	50 -100	fair-good	0 - 10	<=10 %	colourless to pale pink	18 -24 hrs

Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	>=10^3	inhibited	0	0 %		>=24 hrs
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10^3	inhibited	0	0 %		>=24 hrs
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
Salmonella Paratyphi A ATCC 9150	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
Salmonella Paratyphi B ATCC 8759	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
Salmonella Typhi ATCC 6539	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
Salmonella Abony NCTC 6017 (00029*)	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
Proteus vulgaris ATCC 13315	50 -100	luxuriant	25 -100	>=50 %	colourless	18 -24 hrs
Shigella flexneri ATCC 12022 (00126*)	50 -100	fair to good	15 -40	30 -40 %	colourless	18 -24 hrs
Staphylococcus epidermidis ATCC 12228 (00036*)	>=10^3	inhibited	0	0 %		>=24 hrs
Corynebacterium diphtheriae type *gravis*	>=10^3	inhibited	0	0 %		>=24 hrs

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

Storage and Shelf Life

Store between 10- 30°C in a tightly closed container and the prepared medium at 20 - 30°C. For better performance ot is advised to store the plates 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 (5,7).

References

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MacConkey Broth

MH083

Intended use

Recommended for the selective enrichment of *E.coli* from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP.

Composition**

Ingredients	Gms / Litre
Gelatin peptone#	20.000
Lactose monohydrate	10.000
Dehydrated bile##	5.000
Bromo cresol purple	0.010
pH after sterilization (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Pancreatic digest of gelatin

Equivalent to Dehydrated Ox-bile

Directions

Suspend 34.51 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/ distilled water. Heat if necessaryto dissolve the medium completely. Dispense into test tubes with inverted Durham tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle.

Principle And Interpretation

MacConkey Broth is a modification of MacConkey Medium (7). Childs and Allen (2) demonstrated the inhibitory effect of neutral red and therefore substituted it by the less inhibitory bromocresol purple dye. BCP is more sensitive in recording pH variation in the medium. This medium is prepared in accordance with the harmonized method of USP/BP/JP (8,1,5)

Gelatin peptone provides essential growth nutrients. Lactose is the fermentable carbohydrate. Dehydrated bile inhibits gram-positive organisms. Bromocresol purple is the pH indicator in the medium, which turns yellow under acidic condition. Lactose fermenting organisms turn the medium yellow due to the acidity produced on lactose fermentation. The colour change of the dye is observed when the pH of the medium falls below 6.8. Lactose non-fermenting organisms like *Salmonella* and *Shigella* do not alter the appearance of the medium.

Transfer homogenate in Soyabean Casein Digest Medium (MH011) containing 1 gm or 1 ml of the preparation tbe examined to 100 ml MacConkey Broth Incubation is carried at 43°-45°C for 24-48 hours. For further isolation, subculture on MacConkey Agar (MH081). Growth of red generally non-mucoid colonies, sometimes surrounded by a reddish precipitation zone, indicates pressure of coliforms.

Type of specimen

Pharmaceutical samples; Clinical samples

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (1,3,5,8).

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,6).

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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Though the medium is recommended for selective isolation, further biochemical and serological testing must be carried out for further confirmation.
2. For further isolation, subculture on MacConkey Agar (MH081) is 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 with green tinge homogeneous free flowing powder

Colour and Clarity of prepared medium
Purple coloured clear to slightly opalescent solution in tubes

pH
7.10-7.50

Cultural Response
Growth Promotion is carried out in accordance with the harmonized method of USP/EP/BP/JP. For organisms not specified in pharmacopoeia, cultural response was observed after an incubation at 30-35°C for 18-48 hours.

Growth promoting properties

Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu (at 42-44°C for <= 24 hours).

Inhibitory properties
No growth of the test microorganism occurs for the specified temperature for not less than longest period of time specified inoculating >=100 cfu(at 42-44°C for >= 48 hours).

Cultural Response
Cultural characteristics observed after an incubation at 30-35°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Acid	Gas	Incubation temperature	Incubation period
Growth promoting						
Escherichia coli ATCC 8739 (00012)*	50 -100	luxuriant	positive reaction, yellow colour	positive reaction	42 -44 °C	<=24 hrs
Inhibitory						
Staphylococcus aureus subsp. aureus ATCC 6538 (00032)*	>=10^3	inhibited			42 -44 °C	>=48 hrs
Additional Microbiological testing						
Escherichia coli ATCC 25922 (00013)*	50 -100	luxuriant	positive reaction, yellow colour	positive reaction	30 -35 °C	18 -24 hrs
Escherichia coli NCTC 9002	50 -100	luxuriant	positive reaction, yellow colour	positive reaction	30 -35 °C	18 -24 hrs
# Klebsiella aerogenes ATCC 13048 (00175)*	50 -100	luxuriant	positive reaction, yellow colour	positive reaction	30 -35 °C	18 -24 hrs
Salmonella Choleraesuis ATCC 12011	50 -100	fair-good	negative reaction	negative reaction	30 -35 °C	18 -24 hrs
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	>=10^3	inhibited			30 -35 °C	>=48 hrs

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

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 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 (4,6).

Reference

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Mannitol Salt Agar

MH118

Intended Use

Recommended for selective isolation of pathogenic Staphylococci from pharmaceutical products in accordance with Microbial Limit Test by harmonized method of USP/EP/BP/JP/IP.

Composition**

Ingredients	Gms / Litre
Peptone #	5.000
Tryptone ##	5.000
HM Peptone B ###	1.000
Sodium chloride	75.000
D-Mannitol	10.000
Phenol red	0.025
Agar	15.000
pH after sterilization (at 25°C)	7.4±0.2

Peptic digest of animal tissue
Pancreatic digest of casein
Equivalent to Beef extract

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 111.02 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 or as per validated cycle. Cool to 45-50°C. Mix well and pour into sterile Petru plates.

Note : This product contains 7.5% Sodium chloride as one of its ingredients.On repeated exposure to air and absorption moisture sodium chloride has tendency to form lumps, therefore we strongly recommend storage in tightly closed containers in dry place away from bright light .

Principle And Interpretation

Staphylococci are widespread in nature, although they are mainly found on the skin, skin glands and mucous membranes of mammals and birds. The coagulase-positive species i.e. Staphylococcus aureus is well documented as a human opportunistic pathogen. The ability to clot plasma continues to be the most widely used and accepted criterion for the identification of pathogenic staphylococci associated with acute infections (13). Staphylococci have the unique ability of growing on a high salt containing media (11). Isolation of coagulase-positive staphylococci on Phenol Red Mannitol Agar supplemented with 7.5%NaCl was studied by Chapman (3). The resulting Mannitol Salt Agar Base is recommended for the isolation of coagulase-positive staphylococci from cosmetics, milk, food and other specimens (13,1,4,6,14). The additional property of lipase activity of *Staphylococcus aureus* can be detected by the addition of the Egg Yolk Emulsion (FD045). The lipase activity can be visualized as yellow opaque zones around the colonies (7). It is also used in the performance of microbial limit tests for the selective isolation of *Staphylococcus.* The formulation is in accordance with the harmonization of USP/EP/BP/JP/IP (15,5,2,10,8).

The medium contains HM peptone B, tryptone and peptone which makes it very nutritious as they provide carbon, nitrogen compounds, long chain amino acids, vitamins and other essential growth factors and trace nutrients. Many other bacteria except Staphylococci are inhibited by 7.5% sodium chloride. Mannitol is the fermentable carbohydrate fermentation of which leads to acid production, detected by phenol red indicator.

S.aureus ferment mannitol and produce yellow coloured colonies surrounded by yellow zones. Coagulase-negative strains of *S.aureus* are usually mannitol non-fermenters and therefore produce pink to red colonies surrounded by red-purple zones. Presumptive coagulase-positive yellow colonies of *S.aureus* should be confirmed by performing the coagulase test [tube or slide (13)]. Lipase activity of *S.aureus* can be detected by supplementing the medium with egg yolk emulsion

A possible *S.aureus* must be confirmed by the coagulase test. Also the organism should be subcultured to a less inhibitory medium not containing excess salt to avoid the possible interference of salt with coagulase testing or other diagnostic tests (e.g. Nutrient Broth)(M002) (12). Few strains of *S.aureus* may exhibit delayed mannitol fermentation. Negative results should therefore be re-incubated for an additional 24 hours before being discarded (12).

Type of specimen

Pharmaceutical samples; Clinical samples

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (15,5,2,10,8).

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,6).

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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. This medium is a selective medium, some strains of *Staphylococcus aureus* may exhibit a delayed fermentation of mannitol.
2. Certain other bacteria are also mannitol fermenting other than *Staphylococcus*, therefore further biochemical testing is required for 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

Light yellow to pink homogeneous free flowing powder

Gelling

Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Red coloured clear to slightly opalescent gel forms in Petri plates

pH

7.20-7.60

Growth Promotion Test

Growth Promotion was carried out in accordance with the harmonized method of USP/EP/BPJP/IP, after an incubation at 30-35°C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Growth promoting properties

Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu(at 30-35°C for <=18 hours).

Indicative properties

Colonies are comparable in appearance and indication reaction to those previously obtained with previously tested and approved lot of medium occurs for the specified temperature for a period of time within the range specified inoculating <=100cfu (at 30-35°C for 18-72 hours).

Inhibitory properties

No growth of the test microorganism occurs for the specified temp for not less than longest period of time specified inoculating >=100cfu (at 30-35°C for >= 72 hours).

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation temperature
Growth Promoting + Indicative						
Staphylococcus aureus subsp. aureus ATCC 6538 (00032)*	50 -100	luxuriant	25 -100	>=50 %	yellow/white colonies surrounded by yellow zone	18 -72 hrs
Inhibitory						
Escherichia coli ATCC 8739 (00012)*	>=10^3	inhibited	0	0 %		>=72 hrs
Additional Microbiological testing						
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	50 -100	luxuriant	25 -100	>=50 %	yellow/white colonies surrounded by yellow zone	18 -72 hrs
Staphylococcus epidermidis ATCC 12228 (00036)*	50 -100	fair - good	15 -40	30 -40 %	red	18 -72 hrs
Staphylococcus epidermidis ATCC 14990 (00132)*	50 -100	fair - good	15 -40	30 -40 %	red	18 -72 hrs
Proteus mirabilis ATCC 12453	50 -100	none-poor	0 -10	0 -10 %	yellow	18 -72 hrs
Escherichia coli ATCC 25922 (00013)*	>=10^3	inhibited	0	0%		>=72 hrs
Escherichia coli NCTC 9002	>=10^3	inhibited	0	0%		>=72 hrs
# Klebsiella aerogenes ATCC 13048	>=10^3	inhibited	0	0%		>=72 hrs

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

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,13).

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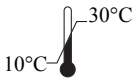

Storage temperature


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Columbia Agar

MH144

Intended use

Recommended for detection of *Clostridium sporogenes* from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP (Medium 15).

Composition**

Ingredients	Gms / Litre
Tryptone #	10.000
HM extract ##	5.000
HM hydrolysate ###	3.000
Yeast extract	5.000
Maize starch	1.000
Sodium chloride	5.000
Agar	15.000

If necessary adjust the pH so that after sterilization it is 7.3±0.2

* pH can also be measured after sterilization at 25°C

**Formula adjusted, standardized to suit performance parameters

Pancreatic digest of casein
Meat peptic digest
Heart pancreatic digest

Directions

Suspend 44.0 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 or as per validated cycle. Cool to 45-50°C, if required add the rehydrated contents of 1 vial of Gentamicin Selective Supplement (FD252). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Columbia Blood Agar Base used as a general-purpose nutritious medium was devised by Ellner et al from Columbia University, which was further enriched by the addition of sheep blood (2). It can also be used for the isolation of organisms by addition of various supplements. Columbia Agar is prepared in accordance with the microbial limit testing harmonized methodology of USP/EP/BP/JP/IP (8,1,3,6,4). This medium is recommended to check the presence of *Clostridium* in non-sterile products like food, dietary, nutritional supplements related products. The genus *Clostridium* belongs to the family *Clostridiaceae* in the class Clostridia.

The product to be examined is initially enriched in Reinforced medium for clostridia . This medium contains 0.05% Agar and cysteine, which creates anaerobic conditions, thereby allowing anaerobic organisms to grow. The enriched sample is then subcultured on Columbia Agar. Columbia Agar is used as a base for media containing blood and for selective media formulations in which different combinations of antimicrobial agents are used as additives. This medium is highly nutritious as it contains tryptone, HM extract and HM hydrolysate and yeast extract provides carbonaceous and nitrogenous substances, long chain amino acids, vitamins of B complex group and other essential nutrients for the luxuriant growth of fastidious as well as non-fastidious organisms. Sodium chloride maintains osmotic balance of medium. Maize starch acts as an energy source and also neutralizes toxic metabolites if produced. It is used in detection of Clostridia from pharmaceutical products. Gentamicin (FD252) inhibits a number of contaminating gram-negative organisms and *Staphylococcus* species.

Clostridia grows under anaerobic conditions as gram positive rods giving a catalase negative test. Further confirmation is carried out by identification tests.

Type of specimen
Pharmaceutical samples; Clinical samples

Specimen Collection and Handling
For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (8,1,3,6,4). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,7). 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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations
1. Some *Clostridium* species may show poor growth due to nutritional variations.
2. Further biochemical tests must be carried out for complete 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
Light amber coloured clear to slightly opalescent gel forms in Petri plates.

pH
7.10-7.50

Growth Promotion Test
Growth Promotion was carried out in accordance with the harmonized method of USP/EP/BP/JP, and growth was observed under anaerobic conditions after an incubation at 30-35°C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Casein Soybean Digest Agar (Soybean Casein Digest Agar).

Growth promoting properties
Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu under anaerobic conditions (at 30-35°C for <=48 hours).

Cultural Response

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Incubation temperature	Incubation period
Growth Promoting						
Clostridium sporogenes ATCC 19404 (00008)*	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	<=48 hrs
Clostridium sporogenes ATCC 11437	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	<=48 hrs
Bacteroides vulgatus ATCC 8482	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	<=48 hrs
Additional Microbiological testing						
Clostridium perfringens ATCC 13124 (00007)*	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	<=48 hrs
Bacteroides fragilis ATCC 23745	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	<=48 hrs

Key : (*) Corresponding WDCM numbers

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,7).

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IVD — In vitro diagnostic medical device

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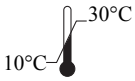

Storage temperature

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Enterobacteria Enrichment Broth, Mossel

MH287

Intended use

Recommended for selective enrichment of *Enterobacteriaceae* from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP .

Composition**

Ingredients	Gms / Litre
Gelatin peptone #	10.000
Glucose monohydrate	5.000
Dehydrated bile ##	20.000
Disodium hydrogen phosphate, dihydrate	8.000
Potassium dihydrogen phosphate	2.000
Brilliant green	0.015
pH after heating (at 25°C)	7.2±0.2

Pancreatic digest of gelatin

Dehydrated ox-bile

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 42.93 grams (the equivalent weight of dehydrated medium per litre)in 1000 ml purified/distilled water. Dispense into tubes or flasks as desired. Heat in free flowing steam or boiling water (100°C) for 30 minutes and cool immediately. DO NOT AUTOCLAVE.

Principle And Interpretation

The family *Enterobacteriaceae* consists of *Salmonella, Shigella* and other enteric pathogens. These organisms find entry into the food system through faecally contaminated water. Majority of these organisms may be eliminated under the stringent food processing parameters. But some of these organisms may become sub lethally injured during the changes in pH, exposure to steam or heat and other unfavourable conditions (7). Therefore the important aspect of food monitoring depends upon the identification and enumeration of these injured cells, after resuscitation. EE Broth Mossel, formulated by Mossel et al (5) is recommended as an enrichment medium for bile tolerant gram-negative bacteria in the biological examination of foods (5), animal feed stuffs (1). This medium is prepared in accordance with the harmonized method of USP/EP/BP/JP/IP(12,2,1,4,11). Gelatin peptone and glucose monohydrate allows the growth of most of the members of *Enterobacteriaceae.* Brilliant green and dehydrated bile are the inhibitory agents for gram-positive bacteria. Phosphates act as a good buffering agent and neutralizes acids produced by lactose fermenters that otherwise would adversely affect the growth of the organism. Lactose negative, anaerogenic lactose-positive or late lactose fermenting *Enterobacteriaceae* are often missed by the standard Coli-aerogenes test. To overcome this problem, lactose is replaced by glucose in this medium. Phosphates form the buffering system of the medium. The cells damaged while drying or low pH are resuscitated in well-aerated Soybean Casein Digest Broth (MH011) for 2 hours at 25°C prior to enrichment in EE Broth. The resuscitation procedure is recommended for dried foods (6), animal feeds (9) and semi-preserved foods (8). EE Broth is an enrichment broth and should be used in conjunction with Violet Red Bile Glucose Agar (MH581). A loopful of the enriched sample from EE Broth. is subcultured onto Violet Red Bile Glucose Agar (MH581) after an initial incubation at 30-35°C for 24 hours. Typical pink colonies from MH581 are subcultured for biochemical confirmation by oxidase and fermentation reactions (12). Decimal dilutions of the food homogenate are used if the expected counts are high or else initial suspension is used. EE Broth, Mossel (MH287)

Type of specimen

Pharmaceutical samples; Clinical samples

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (12,2,1,4,11).
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,10).
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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Further isolation has to be carried out 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 greenish yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Emerald green coloured, clear solution without any precipitate

pH
7.00-7.40

Growth Promotion Test
Growth Promotion is carried out in accordance with the harmonized method of USP/EP/BP/JP/IP. Cultural response was observed after an incubation at 30-35°C for specified time.

Growth promoting properties
Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu (at 30-35°C for <=24 hours).

Inhibitory properties
No growth of the test microorganism occurs for the specified temp for not less than longest period of time specified inoculating >= 100cfu (at 30-35°C for >= 48 hours).

Cultural Response
Cultural characteristics observed after incubation at 30-35 °C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Acid	Incubation temperature	Incubation period
Growth Promoting					
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	positive reaction, yellow colour	30 -35 °C	<=24 hrs
Pseudomonas aeruginosa ATCC 9027 (00026*)	50 -100	luxuriant	-	30 -35 °C	<=24 hrs
Inhibitory					
Staphylococcus aureus subsp.aureus ATCC 6538 (00032*)	>=10^3	inhibited		30 -35 °C	>=48 hrs
Additional Microbiological testing					
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant	positive reaction, yellow colour	30 -35 °C	24 -48 hrs

Escherichia coli NCTC 9002	50 -100	luxuriant	positive reaction, yellow colour	30 -35 °C	24 -48 hrs
Pseudomonas aeruginosa ATCC 27853 (00025*)	50 -100	luxuriant	-	30 -35 °C	24 -48 hrs
# Klebsiella aerogenes ATCC 13048 (00175*)	50 -100	luxuriant	positive reaction, yellow colour	30 -35 °C	24 -48 hrs
Proteus mirabilis ATCC 25933	50 -100	luxuriant	positive reaction, yellow colour	30 -35 °C	24 -48 hrs
Salmonella Enteritidis *ATCC* 13076 (00030*)	50 -100	luxuriant	positive reaction, yellow colour	30 -35 °C	24 -48 hrs
Shigella boydii ATCC 12030	50 -100	luxuriant	negative reaction	30 -35 °C	24 -48 hrs
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10^3	inhibited		30 -35 °C	>=48 hrs

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

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 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,10).

Reference

1. British Pharmacopoeia, 2016, The Stationery office British Pharmacopoeia
2. European Pharmacopoeia, 2017, European Dept. for the quality of Medicines.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition
4. Japanese Pharmacopoeia, 2016.
5. Mossel D. A. A., Vissar M. and Cornellisen A. M. R., 1963, J. Appl.Bacteriol., 26(3):444.
6. Mossel D.A.A. and Ratto M.A., 1970, Appl. Microbiol., 20:273.
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8. Mossel D.A.A., Ratto M.A., 1973, J. Fd. Technol., 8 : 97.
9. Mossel D.A.A. and Shennan J.L. and Clare V., 1973, J. Sci. Fd. Agric., 24 : 499.
10. Murray P. R, Baron E, J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology,
11. The Indian Pharmacopoeia 2018, Govt. of India, 2019. The Controller of Publication, Delhi.
12. The United States Pharmacopoeia, 2019, The United States Pharmacopeial Convention. Rockville, MD.
13. Van Schothurst M. et al, 1966, Vet Med., 13(3):273.

Revision : 05 / 2019

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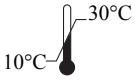

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Soybean-Casein Digest Agar (Casein Soyabean Digest Agar) MH290

Intended use

Recommended as a general purpose medium used for cultivation of a wide variety of microorganisms from pharmaceutical products in accordance with harmonized method of USP/EP/BP/JP/IP (Medium 2).

Composition**

Ingredients	Gms / Litre
Tryptone #	15.000
Soya peptone ##	5.000
Sodium chloride	5.000
Agar	15.000
pH after sterilization (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Pancreatic digest of casein

Papaic digest of soyabean (soybean)

Directions

Suspend 40 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 or as per validated cycle. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Various pharmacopoeias recommend Soybean Casein Digest Agar as sterility testing medium. It is also used in validation of sterility checking procedure in accordance with the microbial limit testing harmonized methodology of USP/ EP/BP/JP/IP (7,2,1,5,3). This medium is used in microbial limit test and antimicrobial preservative- effective test. Gunn et al (5) used this medium for the growth of fastidious organisms and study of haemolytic reaction after addition of 5% v/v blood.

The combination of tryptone and soya peptone makes these media nutritious by providing amino acids and long chain peptides for the growth of microorganisms. Natural sugars of soy enhance growth of microorganism. Sodium chloride maintains the osmotic balance in the medium. Agar is the solidifying agent.

The total aerobic count is considered to be equal to the number of colony forming units found on this medium, if colonies of fungi are detected on this medium they are counted along with total aerobic count.

Type of specimen

Pharmaceutical samples; Clinical samples- blood

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per pharmaceutical guidelines (7,2,1,5,3). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,6). 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. Biochemical characterization is necessary to be performed on colonies from pure cultures for further identification.
2. This medium is general purpose medium and may not support the growth of fastidious organisms.

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 yellow coloured clear to slightly opalescent gel forms in Petri plates

pH
7.10-7.50

Growth Promotion Test
Growth Promotion was carried out in accordance with the harmonized method of USP/EP/BP/JP, and growth was observed after an incubation at 30-35°C for 18-24 hours. Recovery rate is considered 100% for bacteria growth on Blood Agar and fungus growth on Sabouraud Dextrose Agar.

Growth promoting properties
Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu (at 30-35°C for 18 hours).

Cultural Response

Organism	Inoculum (CFU)	Observed Lot value (CFU)	Recovery	Incubation period
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003)*	50 -100	35 -100	>=70 %	18 -24 hrs
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	50 -100	35 -100	>=70 %	18 -24 hrs
Staphylococcus aureus subap. aureus ATCC 6538 (00032)*	50 -100	35 -100	>=70 %	18 -24 hrs
Escherichia coli ATCC 25922 (00013)*	50 -100	35 -100	>=70 %	18 -24 hrs
Escherichia coli ATCC . 8739 (00012)*	50 -100	35 -100	>=70 %	18 -24 hrs
Escherichia coli NCTC 9002	50 -100	35 -100	>=70 %	18 -24 hrs
Pseudomonas aeruginosa ATCC 27853 (00025)*	50 -100	35 -100	>=70 %	18 -24 hrs
Pseudomonas aeruginosa ATCC 9027 (00026)*	50 -100	35 -100	>=70 %	18 -24 hrs
Salmonella Abony *NCTC 6017 (00029*)*	50 -100	35 -100	>=70 %	18 -24 hrs
Micrococcus luteus ATCC 9341	50 -100	35 -100	>=70 %	18 -24 hrs
Streptococcus pneumoniae ATCC 6305	50 -100	35 -100	>=70 %	18 -24 hrs
Salmonella Typhimurium *ATCC 14028 (00031*)*	50 -100	35 -100	>=70 %	18 -24 hrs

Candida albicans ATCC 10231 (00054)*	50 -100	35 -100	>=70 %	<=5 d
Candida albicans ATCC 2091 (00055)*	50 -100	35 -100	>=70 %	<=5 d
#Aspergillus brasiliensis ATCC 16404 (00053)*	50 -100	25 -70	50-70 %	<=5 d

Key : (#) Formerly known as *Aspergillus niger*, (*) Corresponding WDCM numbers

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 thelabel. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,6).

Reference

1. British Pharmacopoeia, 2016, The Stationery office British Pharmacopoeia
2. European Pharmacopoeia, 2017, European Dept. for the quality of Medicines.
3. Indian Pharmacopoeia, 2018, Govt. of India, the controller of Publication, Delhi, India.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. Japanese Pharmacopoeia, 2016.
6. 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.
7. The United States Pharmacopoeia, 2019, The United States Pharmacopoeial Convention. Rockville, MD.

Revision : 04 / 2019

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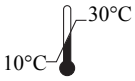

Storage temperature

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Reinforced Medium for Clostridia

MH443

Intended use

Recommended for the enrichment of Clostridia from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
HM Peptone B#	10.000
Yeast extract	3.000
Glucose monohydrate	5.000
Sodium chloride	5.000
Soluble starch	1.000
Cysteine hydrochloride	0.500
Sodium acetate	3.000
Agar	0.500

If necessary adjust the pH so that after sterilization it is 6.8±0.2

*pH can also be measured after sterilization at 25°C

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 37.54 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle.

Principle And Interpretation

Reinforced Medium for Clostridia was formulated by Hirsch and Grinsted (4). This media is prepared in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP (10,2,3,8,5). It is recommended for sterility checking of non-sterile products, nutritional and dietary supplements. It can be used to initiate growth from small inocula and to obtain the highest viable count of clostridia. Barnes and Ingram used the broth medium for diluting an inoculum of vegetative cells of Clostridium perfringens (1, 6). It can be used in studies of spore forming anaerobes, especially *Clostridium butyricum* in cheese, for enumeration of Clostridia in tube dilution counts or for preparation of plates for isolation (6). Other spore forming anaerobes, Streptococci and Lactobacilli also grow in these media. These are enriched but non-selective media.

Peptone, yeast extract and HM peptone B carbonaceous and nitrogenous substances, long chain amino acids, vitamins and other necessary nutrients for the growth of clostridia. Glucose monohydrate is a fermentable carbohydrate in the medium while sodium chloride maintains osmotic equilibrium. Cysteine hydrochloride acts as reducing agent. Small amount of soluble starch removes toxic metabolites from the medium. Sodium acetate also acts as a good buffering agent. Small quantity of agar keeps the medium semi solid and helps in maintaining anaerobic conditions.

Type of specimen

Pharmaceutical samples, Clinical samples

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per pharmaceutical guidelines (10,2,3,8,5). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,9).

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. Some *Clostridium* species may show poor growth due to nutritional variations.
2. Further biochemical tests must be carried out for complete 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

Colour and Clarity of prepared medium
Light yellow coloured clear solution in tubes.

pH
6.60-7.00

Growth Promotion Test
Growth promotion was carried out in accordance with the harmonized method of USP/EP/BP/JP/IP, and growth was observed under anaerobic conditions after an incubation at 30-35°C for <=48 hours

Growth promoting properties

Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating not more than 100 cfu under anaerobic conditions (at 30-35°C for <=48 hours).

Cultural Response
Cultural characteristics observed in an anaerobic atmosphere, after an incubation at 30-35°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Incubation temperature	Incubation period
Growth Promoting				
Clostridium sporogenes ATCC 11437	50 -100	good - luxuriant	30 -35 °C	<=48 hrs
Clostridium sporogenes ATCC 19404 (00008)*	50 -100	good - luxuriant	30 -35 °C	<=48 hrs
Bacteroides vulgatus ATCC 8482	50 -100	good - luxuriant	30 -35 °C	<=48 hrs
Additional Microbiological testing				
Bacteroides fragilis ATCC 23745	50 -100	good - luxuriant	30 -35 °C	24 -48 hrs
Clostridium sporogenes ATCC 13124 (00007)*	50 -100	good - luxuriant	30 -35 °C	24 -48 hrs

Key : (*) Corresponding WDCM numbers

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 thelabel. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,9).

Reference

1. Barnes and Ingram, 1956, J. Appl. Bact., 19:11.
2. British Pharmacopoeia, 2016, The Stationery office British Pharmacopoeia.
3. European Pharmacopoeia, 2017, European Dept. for the quality of Medicines.
4. Hirsch and Grinsted, 1954, J. Dairy Res., 21:101.
5. Indian Pharmacopoeia, 2018 Ministry of Health and Family Welfare, Govt. of India.
6. Indicator Bacteria, Dept. of HEW, PHS Publication, 1142, Washington.
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
8. Japanese Pharmacopoeia, 2016.
9. 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.
10. The United States Pharmacopoeia, 2019, The United States Pharmacopoeial Convention. Rockville, MD.

Revision : 03 / 2019

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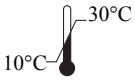

Storage temperature

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Violet Red Bile Glucose Agar

MH581

Intended use

Recommended for detection and enumeration of *Enterobacteriaceae* from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP .

Composition**

Ingredients	Gms / Litre
Yeast extract	3.000
Gelatin peptone #	7.000
Bile salts	1.500
Sodium chloride	5.000
Glucose monohydrate	10.000
Agar	15.000
Neutral red	0.030
Crystal violet	0.002
pH after heating (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Pancreatic digest of gelatin

Directions

Suspend 40.62 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified /distilled water. Heat to boiling to dissolve the medium completely. DO NOT HEAT IN AN AUTOCLAVE. Cool to 45 - 50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Violet Red Bile Glucose Agar is a selective medium recommended for detection and enumeration of *Enterobacteriaceae* especially the bile tolerant gram negative bacteria in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP (7,1,2,3,4) from non-sterile products and pharmaceutical preparations.

Gelatin peptone and yeast extract provide nitrogenous, carbonaceous compounds, long chain amino acids, vitamins and other nutrients essential for bacterial metabolism. This media is selective due to presence of the inhibitors; bile salts positive organisms especially Staphylococci. Neutral red indicator helps to detect glucose fermentation. Glucose fermenting and crystal violet. Crystal violet inhibits gram-strains produce red colonies with pink-red halos in the presence of neutral red. Sodium chloride maintains the osmotic equilibrium in the medium. The red colour is due to absorption of neutral red and a subsequent colour change of the dye when the pH of medium falls below 6.8.

Type of specimen

Pharmaceutical samples, Clinical samples

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (7,1,2,3,4).

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. 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. Though the medium is for selective isolation of *Enterobacteriaceae*, further biochemical and serological testing must be carried out for further confirmation.
2. Over incubation may result in reverting of reaction.

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 pinkish beige homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel.

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

pH
7.20-7.60

Growth Promotion Test
Growth Promotion is carried out in accordance with the harmonized method of USP/EP/BP/JP. Cultural response was observed after an incubation at 30-35°C for 18-24 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Growth promoting properties
Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu (at 30-35°C for <=18 hours).

Indicative properties
Colonies are comparable in appearance and indication reaction to those previously obtained with previously tested and approved lot of medium occurs for the specified temperature for a period of time within the range specified inoculating <= 100 cfu (at 30-35°C for 18-24 hours).

Cultural Response

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation temperature
Growth Promoting + Indicative						
Escherichia coli ATCC 8739 (00012)*	50 -100	luxuriant	25 -100	>=50 %	pink-red with bile precipitate	18 -24 hrs
Pseudomonas aeruginosa ATCC 9027 (00026)*	50 -100	luxuriant	25 -100	>=50 %	pink to red	18 -24 hrs
Additional Microbiological Testing						
Escherichia coli NCTC 9002	50 -100	good-luxuriant	25 -100	>=50 %	pink-red with bile precipitate	18 -24 hrs
Escherichia coli ATCC 25922 (00013)*	50 -100	good-luxuriant	25 -100	>=50 %	pink-red with bile precipitate	18 -24 hrs
Salmonella Enteritidis ATCC 13076 (00030)*	50 -100	good-luxuriant	25 -100	>=50 %	light pink	18 -24 hrs
# Klebsiella aerogenes ATCC 13048 (00175)*	50 -100	good-luxuriant	25 -100	>=50 %	pink-red	18 -24 hrs
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	>=10^3	inhibited	0	0%		>=24 hrs
Staphylococcus aureus subsp. aureus ATCC ATCC 6538 (00032)*	>=10^3	inhibited	0	0%		>=24 hrs

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

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 (5,6).

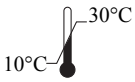
Reference

1. British Pharmacop eia, 2017, The Stationery office British Pharmacopoeia
2. European Pharmacopoeia, 2016, European Dept. for the quality of Medicines.
3. Japanese Pharmacopoeia, 2016.
4. Indian Pharmacopoeia, 2018 Ministry of Health and Family Welfare, Govt. of India.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6. 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.
7. The United States Pharmacopoeia, 2019 The United States Pharmacopoeial Convention. Rockville, MD.

Revision : 03 / 2019

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

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Buffered Sodium Chloride-Peptone Solution pH 7.0

MH1275

Intended use

Recommended as a diluent for carrying out microbial limit testing by harmonized methodology of pharmaceutical products in accordance with USP/EP/BP/JP/IP.

Composition**

Ingredients	Gms / Litre
Potassium dihydrogen phosphate	3.600
Disodium hydrogen phosphate dihydrate	7.200
Sodium chloride	4.300
HMC Peptone #	1.000
Final pH (at 25°C)	7.00

**Formula adjusted, standardized to suit performance parameters

Peptone (meat or casein)

Directions

Suspend 14.64 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified /distilled water. Heat if necessary to dissolve the medium completely. For preparation of nonfatty products insoluble in water, add 0.1% w/v Polysorbate 80 to assist the suspension of poorly wettable substances. Dispense in tubes or flasks or as desired and sterilize by autoclaving at 15 lbs pressure 121°C for 15 minutes or as per validated cycle.

Principle And Interpretation

The composition of this medium is in accordance with the harmonized methodology of USP/EP/BP/JP/IP (7,1,2,5,3). This medium is recommended for preparation of stable test strain suspension employed for validating the microbiological testing procedures of non-sterile products. The standardized stable suspensions are used so that the suitability of this test to detect microorganism in presence of product can be established. Non-fatty products insoluble in water and water-soluble products are diluted/dissolved using this solution.

HMC Peptone serves as nutrient source and maintains the cell viability. Phosphates in the medium act as good buffering agents. Sodium chloride maintains the osmotic balance and cell integrity. Polysorbates reduce surface tension and also inactivate phenolic compound, if present in the test sample.

Preparation of test strain is recommended in Buffered Sodium chloride-Peptone solution pH 7.0 (MH1275) at 30-35°C wherein there is no multiplication of organisms or there is no decrease in count for upto 4 hours.

Type of specimen

Pharmaceutical samples, Clinical samples -faeces, blood

Specimen Collection and Handling

For pharmaceutical samples follow appropriate techniques for handling specimens as per established guidelines (7,1,2,5,3).

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,6).

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 specimens. Safety guidelines may be referred in individual safety data sheets

Limitations :

1. This medium contains less nutrients and is not recommended for the growth of microrganisms

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

Colour and Clarity of prepared medium

Colourless to pale yellow clear solution w/o any precipitate

pH

7.00

Growth Promotion Test

Growth Promotion is carried out in accordance with the harmonized method of ICH(USP/EP/BP/JP/IP).

Cultural response

Cultural characteristics observed after recovery on Soybean Casein Digest Agar after an incubation at 30-35°C for 18-24 hours for bacteria and Sabouraud Dextrose Agar at 30-35°C for 24-48 hours .

Organism	Inoculum (CFU)	Recovery within 2 hours of incubation	Recovery within 4 hours of incubation	Recovery within 24 hours of incubation
Preparation of test strain				
Escherichia coli ATCC 8739 (00012*)	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Escherichia coli ATCC 25922 (00013*)	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Escherichia coli NCTC 9002	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Pseudomonas aeruginosa ATCC 9027 (00026*)	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Pseudomonas aeruginosa ATCC 27853 (00025*)	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Salmonella Abony *NCTC* 6017 (00029*)	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Bacillus subtilis subsp. *spizizennii* ATCC 6633 (00003*)	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at

				2-8°C)
Micrococcus luteus ATCC 9341	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Candida albicans ATCC 10231 (00054)*	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Candida albicans ATCC 2091 (00055)*	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)

Key : (*) Corresponding WDCM Numbers

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 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 must be decontaminated and disposed of in accordance with current laboratory techniques (4,6).

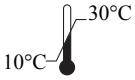
Reference

1. British Pharmacopoeia, 2016 The Stationery office British Pharmacopoeia
2. European Pharmacopoeia, 2017, European Dept. for the quality of Medicines.
3. Indian Pharmacopoeia, 2018, Govt. of India, the controller of Publication, Delhi, India.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. Japanese Pharmacopoeia, 2016.
6. 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.
7. The United States Pharmacopoeia, 2019, The United States Pharmacopoeial Convention. Rockville, MD.

Revision : 05 / 2019

IVD — In vitro diagnostic medical device

CE Marking



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Rappaport Vassiliadis Salmonella Enrichment Broth MH1491

Intended use

Rappaport Vassiliadis Salmonella Enrichment Broth is recommended for selective enrichment of *Salmonella* species from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP.

Composition**

Ingredients	Gms / Litre
Soya peptone	4.500
Sodium chloride	8.000
Dipotassium hydrogen phosphate	0.400
Potassium dihydrogen phosphate	0.600
Magnesium chloride, hexahydrate	29.000
Malachite green	0.036
pH after sterilization (at 25°C)	5.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 27.11 grams of dehydrated medium(the equivalent weight of dehydrated medium per litre) in 1000 ml purified/ distilled water. Heat if necessary to dissolve the medium completely. Dispense as desired into tubes and sterilize by autoclaving at 115°C or as per validated cycle

Principle And Interpretation

Rappaport Vassiliadis Salmonella Enrichment Medium is designed according to the revised formulation by Van Schothorst et al (10) and is recommended for the selective enrichment of Salmonellae from pharmaceutical products. This medium can also be used in direct enrichment of samples containing low inoculum. Present medium is a modification of the Rappaport Vassiliadis Enrichment Broth described by Van Schothorst and Renauld (11). It is prepared in accordance with the harmonized methodology of USP/EP/BP/JP/IP (9,1,2,5,3) has been found to be superior to other *Salmonella* selective medias. Addition of magnesium chloride to the medium was reported by Peterz et al (8).

Salmonella species can be isolated from human faeces without pre-enrichment by using this medium. *Salmonella* generally survive at little high osmotic pressure, grow at slightly low pH and are resistant to malachite green compared to other bacteria. These characteristics are exploited in this medium for selective enrichment of Salmonella. Magnesium chloride present in the medium raises the osmotic pressure. Natural sugars of soya peptone provide essential growth nutrients and enhance the growth of *Salmonella* (4). Phosphate buffers the medium to maintain constant pH. Sodium chloride maintains the osmotic balance. Malachite green inhibits many gram-positive bacteria, while selectively enriches *Salmonella.*

The relatively lower concentration of nutrition, also aids selective enrichment of *Salmonella*. This medium was reported to be superior to *Salmonella* selective medium like Tetrathionate Broth and Selenite enrichment broth and to Tetrathionate-Brilliant Green Broth for the detection of Salmonellae in milk samples. The enriched culture of Rappaport Vasiliadis Salmonella Enrichment Broth (MH1491) can be further subcultured and isolated on Xylose Lysine Deoxycholate Agar (MH031).

Type of specimen

Pharmaceutical samples, Clinical samples: faeces, blood

Specimen Collection and Handling

For pharmaceutical samples follow appropriate techniques for handling specimens as per established guidelines (9,1,2,5,3).

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,6).

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 specimens. Safety guidelines may be referred in individual safety data sheets

Limitations :

Overheating may destroy the selectivity of medium.

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 light blue homogeneous free flowing powder

Colour and Clarity of prepared medium

Greenish blue coloured clear to slightly opalescent solution with a slight precipitate in tubes.

pH

5.00-5.40

Cultural Response

Growth Promotion is carried out in accordance with harmonized method of USP/BP/EP/JP/IP. Cultural response was observed after an incubation at 30-35°C for specified time. Recovery is carried out using Xylose Lysine Deoxycholate Agar (MH031), after enrichment in Rappaport Vassiliadis Salmonella Enrichment Broth.

Growth promoting properties

Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu (at 30-35°C for <=18 hours).

Inhibitory properties

No growth of the test microorganism occurs for the specified temperature for not less than longest period of time specified inoculating >=100 cfu (at least 100 cfu) (at 30-35°C for >= 24 hours).

Cultural Response

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation temperature
Growth promoting						
Salmonella Typhimurium *ATCC 14028* (00031*)	50 -100	luxuriant	>=35	>=70 %	red with black centers	<=18 hrs
Salmonella Abony *NCTC 6017* (00029*)	50 -100	luxuriant	>=35	>=70 %	red with black centers	<=18 hrs
Inhibitory						
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)	>=10^3	inhibited	0	0 %		>=24 hrs
Additional Microbiological testing						
Escherichia coli ATCC 25922 (00013*)	50 -100	none-poor	0 -10	0 -10 %	yellow	18 -24 hrs
Escherichia coli ATCC 8739 (00012*)	50 -100	none-poor	0 -10	0 -10 %	yellow	18 -24 hrs
Salmonella Enteritidis *ATCC 13076* (00030*)	50 -100	luxuriant	>=35	>=70 %	red with black centre	18 -24 hrs
Salmonella Paratyphi B *ATCC 8759*	50 -100	luxuriant	>=35	>=70 %	red with black centre	18 -24 hrs
Staphylococcus aureus subsp. aureusATCC 25923 (00034*)	>=10^3	inhibited	0	0 %		>=24 hrs

Pseudomonas aeruginosa ATCC 9027 (00026*)	>=10^3	inhibited	0	0%		>=24 hrs
Pseudomonas aeruginosa ATCC 27853 (00025*)	>=10^3	inhibited	0	0 %		>=24 hrs
Enterococcus faecalis ATCC 29212 (00087*)	>=10^3	inhibited	0	0 %		>=24 hrs
E.coli +S.Typhimurium (mixed culture)						
Salmonella Typhimurium *ATCC 14028* (00031*)	50 -100	luxuriant	>=35	>=70 %	red with black centre	18 -72 hrs

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. Product performance is best if used within stated expiry period.

Disposal

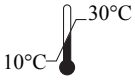
User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,6).

Reference

1. British Pharmacopoeia,2016, The Stationery office British Pharmacopoeia
2. European Pharmacopoeia, 2017, European Dept. for the quality of Medicines.
3. Indian Pharmacopoeia, 2018, Govt. of India, Ministry of Health and Family Welfare, New Delhi
4. Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 2nd Edition.
5. Japanese Pharmacopoeia, 2016.
6. 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.
7. McGibbon L., Quail E. and Fricker C.R. 1984, Inter. J. Food Microbiol . 1:171
8. Peterz M., Wiberg C. and Norberg P., 1989, J. Appl. Bact., 66:523
9. The United States Pharmacopoeia,2019 The United States Pharmacopoeial Convention. Rockville, MD.
10. Van Schothorst M., Renauld A. and VanBeek C., 1987, Food Microbiol., 4:1
11. Van Schothorst M. and Renauld A., 1983, J. Appl. Bact., 54:20

IVD — In vitro diagnostic medical device

CE Marking



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*Buffer solution, pH 7.0 ± 0.02

R063

Buffer solution, pH 7.0 ± 0.02 is used to establish and maintain an ion activity within narrow range. It is most commonly used to establish hydrogen-ion activity for the calibration of pH meters, in analytical procedures. It is also used to maintain stability of various dosage forms.

Composition**

Ingredients	
Sodium dihydrogen phosphate	1.20gm
Disodium hydrogen phosphate	0.885gm
Distilled water	1000.00ml

**Formula adjusted, standardized to suit performance parameters

Principle And Interpretation

Buffer is defined as a solution which resists changes in the activity of an ion on addition of substances that are expected to change the activity of that ion. Buffer capacity refers to the amount of material that may be added to solution without causing a significant change in ion activity. Buffered solutions are systems in which the ion is in equilibrium with substances capable of removing or releasing the ion. For successful completion of many pharmacopeial tests and assay requires adjustment or maintenance of a specified pH by addition of buffer solutions .In pH measurements standard buffer solutions are required for reference purposes.

Quality Control

Appearance
Colourless liquid

Clarity
Clear with no insoluble particles.

Results
The buffer solution gives a pH value of 7.0 ± 0.02 at 25°C

Storage and Shelf Life

Store between 2°C to 8°C in tightly closed container. Use before expiry date on label.

Reference

1)U.S. Pharmacopeia ,USP 37, NF32.

2)Delloyd's Lab Tech resources reagent and solution: Preparation of pH buffer solutions.

Revision : 1 / 2015

CE

*Buffer solution, pH 4.0 ± 0.02

R064

Buffer solution, pH 4.0 ± 0.02 is used to establish and maintain an ion activity within narrow range. It is most commonly used to establish hydrogen-ion activity for the calibration of pH meters, in analytical procedures. It is also used to maintain stability of various dosage forms.

Composition**

Ingredients

Disodium hydrogen phosphate,12H_2O	8.954gm
Potassium dihydrogen phosphate	3.4023gm
Distilled water	1000.00ml

**Formula adjusted, standardized to suit performance parameters

Principle And Interpretation

Buffer is defined as a solution which resists changes in the activity of an ion on addition of substances that are expected to change the activity of that ion. Buffer capacity refers to the amount of material that may be added to solution without causing a significant change in ion activity. Buffered solutions are systems in which the ion is in equilibrium with substances capable of removing or releasing the ion. For successful completion of many pharmacopeial tests and assay requires adjustment or maintenance of a specified pH by addition of buffer solutions .In pH measurements standard buffer solutions are required for reference purposes.

Quality Control

Appearance
Colourless liquid

Clarity
Clear with no insoluble particles.

Result
The buffer solution gives a pH value of 4.0 ± 0.02 at 25°C.

Storage and Shelf Life

Store between 2°C to 8°C in tightly closed container.Use before expiry date on label.

Reference

1)U.S. Pharmacopeia USP 37,NF32.
2)Delloyd's Lab Tech resources reagent and solution: Preparation of pH buffer solutions.

Revision : 1 / 2015

CE

Peptone, Bacteriological

RM001

It contains high tryptophan content used as culture media ingredient in variety of media. It can also be used for commercial production of enzymes, vaccines, antibiotics,steroids and other products.

Principle And Interpretation

Peptone, Bacteriological is prepared by enzymatic digestion of selected fresh meat. Being highly nutritious it supports good growth of wide variety of microorganisms and can be used for identification of bacteria by performing various biochemical tests. As peptones confer nutritional benefit, especially at low dilution rates, for the recombinant cell lines it have been recently used as medium additives for the production of a recombinant therapeutic protein in high density perfusion cultures of mammalian cells .

Quality Control

Appearance
Light yellow to brownish yellow homogenous free flowing powder ,having Characteristic odour but not putrescent.

Solubility
Freely soluble in distilled/purified water, insoluble in alcohol.

Clarity
2% w/v aqueous solution remains clear and neutral without any haziness after autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

Reaction
Reaction of 2% w/v aqueous solution at 25ºC.

pH
6.10- 7.10

Microbial Load:

Total aerobic microbial count (cfu/gm)

By plate method when incubated at 30-35°C for not less than 3 days.
Bacterial Count : <= 2000 CFU/gram

Total Yeast and mould count (cfu/gm)

By plate method when incubated at 20-25°C for not less than 5 days.
Yeast & mould Count : <= 100 CFU/gram

Test for Pathogens
1. E.coli-Negative in 10 gms of sample2. Salmonella species-Negative in 10 gms of sample3. Pseudomonas aeruginosa-Negative in 10 gms of sample4. Staphylococcus aureus- Negative in 10 gms of sample5. C.albicans- Negative in 10 gms of sample6. Clostridia- Negative in 10 gms of sample

Degree of digestion
As per method specified in USP 32,NF26. a. Absence of undigested protein b. Presence of proteoses c. Presence of tryptophan

Nitrite test
As per method specified in USP 32,NF26 Negative:No development of pink or red colour.

Microbial Content
As per method specified in USP 32,NF26 <=Total of 50 microorganisms or clumps in 10 consecutive fields.

Bacteriological Testing
Bacteriological tests are carried out as per USP 32,NF26 where respective medium is prepared by using peptone under test.

Test for fermentable carbohydrate
Medium :2% peptone w/phenol red broth w/durhams tube.After inoculation with test culture and incubation for 24 hours at 35-37°C

Escherichia coli ATCC 25922	Acid production ,(Positive test)
Streptococcus liquefaciens	No acid production,(Negative test)

Production of acetyl methyl carbinol

Medium :0.1% peptone and 0.5% of dextrose in water.After inoculation with test culture and incubation for 24 hours at 35-37°C.

Enterobacter aerogenes ATCC 13048	Formation of pink colour (Positive test).
Escherichia coli ATCC 25922	No formation of pink colour (Negative test).

Production of H2S

Medium :1% peptone in water.After inoculation with test culture and incubation for 24 hours at 35-37°C .

Salmonella Typhi ATCC 6539	The lead acetate test paper shows brownish blackening (lead sulphide)

Production of Indole

Medium : 0.1% peptone in water.After inoculation with test culture and incubation for 24 hours at 35-37°C.

Escherichia coli ATCC 25922	Appearance of distinct pink to red colour ring (Positive test).
Enterobacter aerogenes ATCC 13048	No formation of pink to red coloured ring (Negative test).

Cultural response

Cultural response observed after incubation at 35-37°C for 24 hours by using 2% peptone,0.5% sodium chloride and 1.5% agar in water,pH 7.2-7.4

Cultural Response

Organism	**Growth**
Cultural response	
Escherichia coli ATCC 25922	Luxuriant
Pseudomonas aeruginosa ATCC 27853	Luxuriant
Enterobacter aerogenes ATCC 13048	luxuriant
Salmonella Typhi ATCC 6539	Luxuriant
Staphylococcus aureus ATCC 25923	luxuriant
Streptomyces albus ATCC 3004	luxuriant
Streptococcus pyogenes ATCC 19615	luxuriant w/ beta haemolysis (With addition of sterile 5% sheep blood to above medium, after an incubation at 35-37°C for 48 hours.
Neisseria gonorrhoeae ATCC 19424	luxuriant w/ beta haemolysis (With addition of sterile 10% sheep blood to above medium heated to 80-90°C until blood has turned to chocolate brown and incubated in 10% CO2 atmosphere at 35-37°C for 48 hours).

Chemical Analysis

Total Nitrogen	>= 13.50%
AminoNitrogen	>= 3.00%
Sodium chloride	<= 5.0%
Loss on drying	<= 5.0%
Residue on ignition	<= 15%

Storage and Shelf Life

Store below 30°C. Use before expiry date on the label.

L-Histidine

(From non-animal source)
Cell Culture Tested

Product Code: TC076

Product Description :

Molecular Weight: 155.2
Molecular Formula: $C_6H_9N_3O_2$
CAS No.: 71-00-1
Synonym:(S)-a-amino-1H-imidazole-4-propanoic acid, glyoxaline-5-alanine, His, H

L-Histidine is positively charged hydrophilic, essential α-amino acid coded by codons CAU and CAC. It is chemically basic in nature. It carries positively charged imidazole functional group.

It is used as a major component in wide range of cell culture media including classical and serum-free media. It plays many important roles in cell culture. Some of them are mentioned below:

1. Protein synthesis and protein folding:
Like all other amino acids, L-Histidine also acts as a substrate for protein synthesis during translation process. Histidine's side chain allows it to act as both a base and an acid, both donating and accepting protons, which can be of considerable importance in its role as part of proteins. Hence it is a proteinogenic amino acid.

2. Nucleic acid synthesis:
Biosynthesis of Histidine is inherently linked to the pathways of nucleotide formation. Thus it participates in synthesis of nucleic acids also.

3. As a catalyzer:
Because of presence of imidazole ring, Histidine is a nucleophile and a good acid/base catalyzer. Histidine residues are found in enzyme active sites.

4. Precursor for synthesis of carnosine:
Histidine acts as a precursor for synthesis of carnosine, a dipeptide of amino acids beta-alanine and histidine. Carnosine exerts anti-oxidant effect and protects cellular proteins by preventing oxidation of sugars. It also binds with potentially harmfulcarbonyl groups that attack and bind with proteins imbedded in cell membrane.

Directions :

Preparation instructions:
L-Histidine is soluble in water (100mg/ml) and 0.5M HCl (50mg/ml)

Quality Control:

Appearance
White to offwhite crystalline powder.

Solubility
Clear colorless solution at 1gm in 100ml of water .

pH of 2% solution water
7.00 -8.50

Specific rotation α_D^{20}
+11.8° to +12.8°

Chloride (Cl)
NMT 0.02%

Ammonium (NH4)
NMT 0.1%

Sulphate (SO4)
NMT 0.02%

Iron (Fe)
NMT 0.001%

Heavy metals
NMT 0.001%

Arsenic (As)
NMT 0.0001%

Other amino acids
NMT 0.5%

Assay
NLT 98.5%

Cell Culture Test
Passes

Storage and Shelf Life:

Store at 10-30°C away from bright light
Shelf life is 36 months.
Use before expiry date given on the product label.

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