Lauryl Sulphate Broth (Lauryl Tryptose Broth)

M080

Intended use

Recommended for detection and enumeration of coliform bacteria in water, waste water, dairy products ,other food and clinical samples.

Composition**

Ingredients	Gms / Litre
Tryptose	20.000
Lactose	5.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	2.750
Potassium dihydrogen phosphate	2.750
Sodium lauryl sulphate (SLS)	0.100
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 35.60 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Distribute into tubes containing inverted Durhams tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. For inoculum of 1 ml or less, use single strength medium. For inocula of 10 ml or more, double strength or proportionate medium should be prepared.

Principle And Interpretation

Coliforms are considered to be members of *Enterobacteriaceae*, which grow in the presence of bile salts and produce acid and gas from lactose within 48 hours at 37°C (1). These bacteria can also be defined as, members of *Enterobacteriaceae* capable of growing at 37°C, that normally possess β-galactosidase (2). Lauryl Sulphate Broth is used for the detection of coliforms in water, dairy products and other foods, as recommended by APHA (3,4,5). It can also be used for the presumptive detection of coliforms in water, effluent or sewage by the MPN test (6). Lauryl Sulphate Broth was developed by Mallmann and Darby (7). Cowls (6) demonstrated that inclusion of sodium lauryl sulphate makes the medium selective for coliform bacteria. It was later investigated that Lauryl Sulphate Broth gave a higher colon index than the confirmatory standard methods media and also that gas production in Lauryl Sulphate Broth not only acts as a presumptive test but also as a confirmatory test for the presence of coliforms, in the routine testing of water (7). Lauryl Sulphate Broth is also recommended by the ISO Committee for the detection of coliforms (8).

Lauryl Sulphate Broth is designed to obtain rich growth and substantial amount of gas from small inocula of coliform organisms. Aerobic spore-bearers are completely inhibited in this medium. Tryptose provides essential growth substances, such as nitrogen and carbon compounds, sulphate and trace ingredients. The potassium phosphates provide buffering system, while sodium chloride maintains osmotic equilibrium. Sodium lauryl sulphate inhibits organisms other than coliforms.For inoculum of 1 ml or less, use single strength medium. For inocula of 10 ml or more, double strength or proportionate medium should be prepared. After inoculation, incubate the tubes at 37°C for 24 to 48 hours. For every tube showing fermentation (primary fermentation), inoculate two tubes of Lauryl Tryptose Broth from the tube showing primary fermentation and incubate these tubes at 37°C and 44°C respectively. If there is fermentation in the tube incubated at 44°C after 8 to 24 hours, perform indole test by adding Kovacs reagent. A positive indole test in a broth tube showing gas production at 44°C indicates the presence of *Escherichia coli*. If no fermentation occurs in the tube incubated at 37°C after 24 hours, the primary fermentation is assumed to be due to organisms other than coliforms. Broth becomes cloudy if stored at 2-8°C, but it gets cleared at room temperature. Refer appropriate references for standard procedures (1,6,8).

Type of specimen

Food and dairy samples; Water samples, Clinical samples- faeces

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (9,10).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (4,5).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(1,3). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations :

1. Due to poor nutritional variations, some strains may show poor growth.

2. Further 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

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured, clear solution without any precipitate

Reaction

Reaction of 3.56% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Gas Production	Indole production (44°C)
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	positive reaction	positive reaction, red ring at the interface of the medium
# Klebsiella aerogenes ATCC 13048 (00175*)	50-100	luxuriant	positive reaction	negative reaction, no colour development / cloudy ring
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited		
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	luxuriant	negative reaction	negative reaction, no colour development / cloudy ring
Staphylococcus aureus subsp aureus ATCC 25923 (00034)*	>=10^4	inhibited		

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 inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

Reference

Department of Environment, Department of Health and Social Security, Public Health Laboratory Service, 1982, Methods for the Examination of Water and Associated Materials, The Bacteriological Examination of Drinking Water Supplies, 1982, Her Majestys Stationary Office, London.

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3.Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

4.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.

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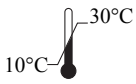
IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

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MacConkey Agar w/ 0.15% Bile salts, CV and NaCl

M081

Intended Use:

Recommended for the selective isolation and differentiation of coliform organisms and other enteric pathogens from clinical and non-clinical samples.

Composition**

Ingredients	Gms / Litre
Gelatin peptone	17.000
Tryptone	1.500
Peptone	1.500
Lactose	10.000
Bile salts	1.500
Sodium chloride	5.000
Neutral red	0.030
Crystal violet	0.001
Agar	15.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 51.53 grams in 1000 ml purified/ distilled water. Heat to boiling with gentle swirling to dissolve the agar completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Avoid overheating. Cool to 45 - 50°C and pour into sterile Petri plates. The surface of the medium should be dry when inoculated.

Principle And Interpretation

MacConkey agars are slightly selective and differential plating media mainly used for the detection and isolation of gram-negative organisms from clinical (10), dairy (14), food (5,11), water (1), pharmaceutical (3,12) and industrial sources (15). It is also recommended for the selection and recovery of the *Enterobacteriaceae* and related enteric gram-negative bacilli. USP recommends this medium for use in the performance of Microbial Limit Tests (12).

These agar media are selective since the concentration of bile salts, which inhibit gram-positive microorganisms, is low in comparison with other enteric plating media. The medium M081, which corresponds with, that recommended by APHA can be used for the direct plating of water samples for coliform bacilli, for the examination of food samples for food poisoning organisms (11) and for the isolation of *Salmonella* and *Shigella* species in cheese (14). Other than that this medium is also used for count of coli-aerogenes bacteria in cattle and sheep faeces (9), the count of coli-aerogenes and non-lactose fermenters in poultry carcasses (9), bacterial counts on irradiated canned minced chicken (13) and the recognition of coli-aerogenes bacteria during investigations on the genus *Aeromonas* (4).

MacConkey Agar is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of clinical specimens (7,8). The original medium contains protein, bile salts, sodium chloride and two dyes. The selective action of this medium is attributed to crystal violet and bile salts, which are inhibitory to most species of gram-positive bacteria. 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 colonies 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, transparent and typically do not alter appearance of the medium.

Peptone, Tryptone and gelatin peptone are sources of nitrogen, carbon, long chain amino acids and other nutrients. Lactose is a fermentable carbohydrate,Sodium chloride maintains the osmotic equilibrium. Bile salts and crystal violet are selective agents that inhibit growth of gram-positive organisms. Neutral red is the pH indicator dye.

Type of specimen

Clinical - faeces, urine and other pathological material, foodstuffs and dairy samples, water samples, pharmaceutical samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,10).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5,11,14.).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(1)
For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines.(3,12) 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

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

Reaction
Reaction of 5.15% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH
6.90-7.30
Cultural Response
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.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Corynebacterium diphtheriae type gravis	>=10^4	inhibited	0%	
Shigella flexneri ATCC 12022 (00126*)	50 -100	fair to good	30 -40 %	colourless
Salmonella Paratyphi A *ATCC 9150*	50 -100	luxuriant	>=50 %	colourless
Salmonella Abony *NCTC 6017 (00029*)*	50 -100	luxuriant	>=50 %	colourless
Proteus vulgaris ATCC 13315	50 -100	luxuriant	>=50 %	colourless
Salmonella Typhi *ATCC 6539*	50 -100	luxuriant	>=50 %	colourless
Staphylococcus epidermidis ATCC 12228 (00036)*	>=10^4	inhibited	0%	
Escherichia coli ATCC 8739 (00012)*	50 -100	luxuriant	>=50 %	pink-red with bile precipitate

Staphylococcus aureus subsp.aureus ATCC 6538 (00032*)	>=10^4	inhibited	0%	
Salmonella Paratyphi B *ATCC 8759*	50 -100	luxuriant	>=50 %	colourless
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant	>=50 %	pink to red with bile precipitate
Escherichia coli NCTC 9002	50 -100	luxuriant	>=50 %	pink to red with bile precipitate
# Klebsiella aerogenes ATCC 13048 (00175*)	50 -100	luxuriant	>=50 %	pink to red
Salmonella Typhimurium *ATCC 14028 (00031*)*	50 -100	luxuriant	>=50 %	colourless
Enterococcus faecalis ATCC 29212 (00087*)	50 -100	none - poor	<=10 %	colourless to pale pink
Salmonella Enteritidis *ATCC 13076* (00030*)	50 -100	luxuriant	>=50 %	colourless
Staphylococcus aureus subsp.aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%	

Key :- * Corresponding WDCM numbers

 # Formerly known as Enterobacter aerogenes

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 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 (6,10).

Reference

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
2. Barnes Ella M. and Shrimpton D. H., 1957, J. Appl. Bacteriol., 20(2),273-285.
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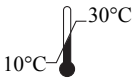
Revision : 05/ 2019

IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
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Pseudomonas Agar Base

M085

Intended use :

Recommended for selective isolation of *Pseudomonas* species

Composition**

Ingredients	Gms / Litre
Tryptone	10.000
Gelatin peptone	16.000
Potassium sulphate	10.000
Magnesium chloride, anhydrous	1.400
Agar	11.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 24.2 grams in 500 ml purified/distilled water containing 5 ml glycerol. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add sterile rehydrated contents of either CetriNix Supplement (FD029) or CFC Supplement (FD036) as desired. Mix well and pour into sterile Petri plates. Note : Do not keep the molten agar for longer than 4 hours.

Principle And Interpretation

Pseudomonas Agar Base is a modification of Kings A medium (5) which contains magnesium chloride and potassium sulphate to enhance pigment production. Goto and Enomoto (2) formulated CetriNix supplement for the selective isolation of *Pseudomonas aeruginosa* from clinical specimens. Lowbury and Collins (6) studied cetrimide as a selective agent. CetriNix supplement suppresses *Klebsiella, Proteus* and *Providencia* species.

Tryptone and gelatin peptone supplies nitrogenous and carbonaceous compounds, long chain amino acids, and other essential growth nutrients.

C-F-C Supplement was formulated by Mead and Adams (7) making the medium specific for isolation of *Pseudomonas* from chilled foods and processing plants, environmental samples and water. This medium is recommended for enumeration of *Pseudomonas* species from meat and meat products. It can also be used for clinical samples.

Examine inoculated plates after 24 hours and 48 hours using both white and UV light. The presence of blue-green or brown pigmentation may be considered as presumptive evidence of *Pseudomonas aeruginosa* . *Alteromonas* species may form brown or pink colonies on the medium.

Type of specimen

Clinical samples - pus, urine, body fluids, Food samples; Water samples.

Specimen Collection and Handling

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

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

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

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

Warning and Precautions

In Vitro diagnostic use. Read the label before opening the container. The media should be handled by trained personnel only. 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. Due to nutritional variation, some strains may show poor growth.

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 temmperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.1% Agar gel.

Colour and Clarity of prepared medium

Yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 4.84% w/v aqueous solution containing 1% v/v glycerol at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

Cultural characteristics observed after an incubation for 40-48 hours. Recovery rate is considered as 100% for growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth (at 34-38°C with FD029)	Recovery 34-38°C with FD029)	Growth (at 24-26°C with FD036)	Recovery (at 24-26°C with FD036)	Colour/ Fluorescence under uv
Proteus vulgaris ATCC 13315	>=10^4	inhibited	0%	-	-	-
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	good-luxuriant	>=50%	-	-	blue-green /positive
Pseudomonas aeruginosa ATCC 9027 (00026*)	50-100	good-luxuriant	>=50%	-	-	blue-green /positive
Pseudomonas aeruginosa ATCC 10145 (00024*)	50-100	good-luxuriant	>=50%	-	-	blue-green /positive
Pseudomonas cepacia ATCC 10661	50-100	-	-	good-luxuriant	>=50%	
Pseudomonas fluorescens ATCC 13525 (00115*)	50-100	-	-	good-luxuriant	>=50%	
Pseudomonas fragi ATCC 4973 (00116*)	50-100	-	-	good-luxuriant	>=50%	
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%	-	-	-
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	inhibited	0%	-	-	-
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	inhibited	0%	
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	0%	inhibited	0%	

Cultural characteristics observed after an incubation for 40-48 hours. Recovery rate is considered by comparing with previously approved lot of the same medium.

Organism	Inoculum (CFU)	Growth (at 34-38°C with FD029)	Recovery 34-38°C with FD029)	Growth (at 24-26°C with FD036)	Recovery (at 24-26°C with FD036)	Colour/ Fluorescence under uv
Proteus vulgaris ATCC 13315	>=10^4	inhibited	0%	-	-	-
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	good-luxuriant	>=70%	-	-	blue-green /positive
Pseudomonas aeruginosa ATCC 9027 (00026*)	50-100	good-luxuriant	>=70%	-	-	blue-green /positive
Pseudomonas aeruginosa ATCC 10145 (00024*)	50-100	good-luxuriant	>=70%	-	-	blue-green /positive
Pseudomonas cepacia ATCC 10661	50-100	-	-	good-luxuriant	>=70%	
Pseudomonas fluorescens ATCC 13525 (00115*)	50-100	-	-	good-luxuriant	>=70%	
Pseudomonas fragi ATCC 4973 (00116*)	50-100	-	-	good-luxuriant	>=70%	
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%	-	-	-
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	inhibited	0%	-	-	-
Escherichia coli ATCC 25922 (00012*)	>=10^4	inhibited	0%	inhibited	0%	
Escherichia coli ATCC 8739 (00013*)	>=10^4	inhibited	0%	inhibited	0%	

Key : * - Corresponding WDCM numbers

Storage and Shelf Life

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

References

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
2. Goto S. and Entomoto S., 1970, Jap. J. Microbiol., 14:65.
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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.

Revision : 04 / 2019

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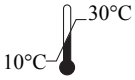	Storage temperature
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Plate Count Agar (Standard Methods Agar)

M091

Intended use

Recommended for the determination of plate counts of microorganisms in food, water, waste water and also from clinical samples.

Composition**

Ingredients	Gms / Litre
Tryptone	5.000
Yeast extract	2.500
Dextrose (Glucose)	1.000
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 23.5 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.

Principle And Interpretation

Plate Count Agar is formulated as described by Buchbinder et al (2) which is recommended by APHA (1,6,7) and FDA (3). Tryptone provides nitrogenous and carbonaceous compounds, long chain amino acids, and other essential nutrients. Yeast extract supplies Vitamin B complex. APHA recommends the use of pour plate technique. The samples are diluted and appropriate dilutions are added in Petri plates. Sterile molten agar is added to these plates and plates are rotated gently to ensure uniform mixing of the sample with agar. The poured plate count method is preferred to the surface inoculation method, since it gives higher results. Plate Count Agar is also suitable for enumerating bacterial count of sterile rooms.

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 (4.5).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,7).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(1).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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

Limitations :

1. 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

Reaction
Reaction of 2.35% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

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

Organism	Inoculum (CFU)	Growth	Recovery
Bacillus subtilis subsp. spizizenni ATCC 6633 (00003*)	50-100	luxuriant	>=70%
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=70%
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=70%
Lactobacillus casei ATCC 9595	50-100	luxuriant	>=70%
Staphylococcus aureus subsp. *aureus ATCC 25923* (00034*)	50-100	luxuriant	>=70%
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	>=70%

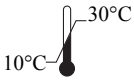
Key : *Corresponding WDCM numbers.

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 inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

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

Reference
1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
2. Buchbinder L., Baris Y., Aldd E., Reynolds E., Dilon E., Pessin V., Pincas L. and Strauss A., 1951, Publ. Hlth. Rep., 66:327.
3. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, DC.
4. Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 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. 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. 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
	Storage temperature
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Simmons Citrate Agar

M099

Intended Use:

Recommended for differentiation the members of *Enterobacteriaceae* on the basis of citrate utilization from clinical and non clinical samples. .

Composition**

Ingredients	Gms / Litre
Magnesium sulphate	0.200
Ammonium dihydrogen phosphate	1.000
Dipotassium phosphate	1.000
Sodium citrate	2.000
Sodium chloride	5.000
Bromothymol blue	0.080
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 24.28 grams in 1000 ml purified/ distilled water. Heat, to boiling, to dissolve the medium completely. Mix well and distribute in tubes or flasks. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Precaution: Before using water, ensure pH of water is 6.5 to 7.0.Initial colour of the medium may deviate from expected colour, if the above precaution is ignored.

Principle And Interpretation

These media are used for the differentiation between *Enterobacteriaceae* and the members of aerogenes group on the basis of citrate utilization as sole carbon source. Initially the citrate medium was developed by Koser (6) containing ammonium salt as the only nitrogen source and citrate as the only carbon source for differentiating *Escherichia coli* and *Enterobacter aerogenes* by IMViC tests. Later on Simmons (9) modified Kosers formulation by adding agar and bromothymol blue (7). It is recommended by APHA (3).

Ammonium dihydrogen phosphate and sodium citrate serve as the sole nitrogen and carbon source respectively. Microorganisms also use inorganic ammonium salts as their sole nitrogen source. Metabolism of these salts causes the medium to become alkaline, indicated by a change in colour of the pH indicator from green to blue. Bromothymol blue is the pH indicator. The medium should be freshly prepared because in dry conditions, changes in colour may appear even before inoculation, especially at the bottom of the slant.

Type of specimen

Isolated microorganism from clinical and non clinical samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,8,10).

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.Before using water, ensure pH of water is 6.5 to 7.0.Initial colour of the medium may deviate from expected colour, if the above precaution is ignored.
2. The pH affects the performance of the medium and must be correctly monitored.

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
Forest green coloured slightly opalescent gel forms in tubes as slants

Reaction
Reaction of 2.43% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

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

Organism	Inoculum (CFU)	Growth	Citrate utilisation
# *Klebsiella aerogenes ATCC 13048 (00175*)*	50-100	good-luxuriant	positive reaction, blue colour
Escherichia coli ATCC 25922 (00013)*	>=10^4	inhibited	
Salmonella Typhi *ATCC 6539*	50-100	fair-good	negative reaction, green colour
Salmonella Typhimurium *ATCC 14028 (00031*)*	50-100	good-luxuriant	positive reaction, blue colour
Shigella dysenteriae ATCC 13313	>=10^4	inhibited	
Salmonella Choleraesuis ATCC 12011	50-100	good-luxuriant	positive reaction, blue colour
Salmonella Enteritidis *ATCC 13076 (00030*)*	50-100	good-luxuriant	positive reaction, blue colour

Key: * Corresponding WDCM numbers
Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

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

Disposal

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

Reference

1. 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. Eaton A. D., Clesceri L. S., Rice E. W., and Greenberg A W., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, Washington, D.C.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. Koser, 1923, J. Bact., 8:493.
7. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
8. 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.
9. Simmons, 1926, J. Infect. Dis., 39:209.
10. 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 : 03 / 2019

IVD	In vitro diagnostic medical device
CE	CE Marking
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SS Agar (Salmonella Shigella Agar)

M108

Intended Use:

Recommended for the isolation of *Salmonella* and some *Shigella* species from pathological specimens, suspected foodstuffs etc.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
HM peptone B #	5.000
Lactose	10.000
Bile salts mixture	8.500
Sodium citrate	10.000
Sodium thiosulphate	8.500
Ferric citrate	1.000
Brilliant green	0.00033
Neutral red	0.025
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 63.02 grams in 1000 ml purified /distilled water. Boil with frequent agitation to dissolve the medium completely. DO NOT AUTOCLAVE OR OVERHEAT. Overheating may destroy selectivity of the medium. Cool to about 50°C. Mix and pour into sterile Petri plates.

Principle And Interpretation

SS Agar medium is recommended as differential and selective medium for the isolation of *Salmonella* and *Shigella* species from pathological specimens (4) and suspected foodstuffs (1,6, 8, 9) and for microbial limit test (7). SS Agar is a moderately selective medium in which gram-positive bacteria are inhibited by bile salts, brilliant green and sodium citrate.

Peptone, HM peptone B provides nitrogen and carbon source, long chain amino acids, vitamins and essential growth nutrients. Lactose is the fermentable carbohydrate. Brilliant green, bile salts and thiosulphate selectively inhibit gram-positive and coliform organisms. Sodium thiosulphate is reduced by certain species of enteric organisms to sulphite and H2S gas and this reductive enzyme process is attributed by thiosulphate reductase. Production of H2S gas is detected as an insoluble black precipitate of ferrous sulphide, formed upon reaction of H2S with ferric ions or ferric citrate, indicated in the center of the colonies.

The high selectivity of Salmonella Shigella Agar allows the use of large inocula directly from faeces, rectal swabs or other materials suspected of containing pathogenic enteric bacilli. On fermentation of lactose by few lactose-fermenting normal intestinal flora, acid is produced which is indicated by change of colour from yellow to red by the pH indicator-neutral red. Thus these organisms grow as red pigmented colonies. Lactose non-fermenting organisms grow as translucent colourless colonies with or without black centers. Growth of *Salmonella* species appears as colourless colonies with black centers resulting from H 2S production. *Shigella* species also grow as colourless colonies which do not produce H2S.

Type of specimen

Clinical: faeces, blood, rectal swabs; Suspected food stuffs.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,3,4).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,6,8,9).
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.The medium is highly selective and may be toxic to certain *Salmonella* or *Shigella* species. Hence it is recommended to use to inoculate plates of less inhibitory media parallel to SS Agar, such as Hektoen Enteric Agar (M467) or Deoxycholate Citrate Agar (M065) for easier isolation of *Shigella* species (6).

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

Reddish orange coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 6.3% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	fair	20-30%	cream pink
Escherichia coli ATCC 25922 (00013*)	50-100	fair	20-30%	pink with bile precipitate
Salmonella Choleraesuis ATCC 12011	50-100	good-luxuriant	>=50%	colourless with black centre
Salmonella Typhi *ATCC 6539*	50-100	good-luxuriant	>=50%	colourless with black centre
Enterococcus faecalis ATCC 29212 (00087*)	50-100	none-poor	<=10%	colourless
Proteus mirabilis ATCC 25933	50-100	fair-good	30-40%	colourless, may have black centre
Shigella flexneri ATCC 12022 (00126*)	50-100	good	40-50%	colourless
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	good-luxuriant	>=50%	colourless with black centre
Salmonella Enteritidis *ATCC* 13076 (00030*)	50-100	good-luxuriant	>=50%	colourless with black centre

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

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 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 (2,3).

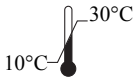
Reference

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. Lennette and others (Eds.), 1985, Manual of Clinical Microbiology, 4th ed., ASM, Washington, D.C.
5. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
6. 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.
7. The United States Pharmacopoeia, 2006, USP29/NF24, The United States Pharmacopoeial Convention. Rockville, MD.
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.
9. Williams S., (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed.,AOAC,Washington, D.C.

Revision : 03/ 2018

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

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Urea Agar Base (Christensen)(Autoclavable)

M112

Intended Use:

Urea Agar Base with the addition of Urea is recommended for the detection of urease production, particularly by members of the genus *Proteus*.

Composition**

Ingredients	Gms / Litre
Peptone	1.000
Dextrose (Glucose)	1.000
Sodium chloride	5.000
Disodium hydrogen phosphate	1.200
Potassium dihydrogen phosphate	0.800
Phenol red	0.012
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 24.01 grams in 950 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 10 lbs pressure (115°C) for 20 minutes. Cool to 45-50°C and aseptically add 50 ml of sterile 40% Urea Solution (FD048) and mix well. Dispense into sterile tubes and allow to set in the slanting position. Do not overheat or reheat the medium as urea decomposes very easily.

Principle And Interpretation

Urea Agar is used to detect urease production. Urea Agar described by Christensen (3,7) detected urease activity by all rapidly urease-positive *Proteus* organisms and also by other members of *Enterobacteriaceae* (3) that exhibited a delayed urease reaction (8). This was accomplished by :

a) adding glucose to the medium.

b) decreasing the peptone concentration and

c) decreasing the buffering system, as a less buffered medium detects even smaller amount of alkali (4).

Peptone is the source of essential nutrients. Dextrose is the energy source. Sodium chloride maintains the osmotic equilibrium of the medium whereas phosphates serve to buffer the medium. Urea is hydrolyzed to liberate ammonia. Phenol red indicator detects the alkalinity generated by visible colour change from orange to pink.

Prolonged incubation may cause alkaline reaction in the medium. A medium without urea serves as negative control to rule out false positive results. Also, all urea test media rely on the alkalinity formation and so they are not specific for determining the absolute rate of urease activity (8). The utilization of proteins may raise the pH to alkalinity due to protein hydrolysis and excess of amino acids liberation results in false positive reaction.

Type of specimen

Isolated microorganism from clinical, food 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(1,9,10).
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.Prolonged incubation may cause alkaline reaction in the medium.
2.Also, all urea test media rely on the alkalinity formation and so they are not specific for determining the absolute rate of urease activity (8).
3. The utilization of proteins may raise the pH to alkalinity due to protein hydrolysis and excess of amino acids liberation results in false positive 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 light pink homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

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

Reaction
Reaction of 2.4% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
Cultural characteristics observed on addition of sterile 40% Urea Solution (FD048) after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Urease
Escherichia coli ATCC 25922 (00013*)	50-100	negative reaction, no change
# Klebsiella aerogenes ATCC 13048 (00175*)	50-100	negative reaction, no change
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	positive reaction, cerise colour
Proteus mirabilis ATCC 25933	50-100	positive reaction, cerise colour
Proteus vulgaris ATCC 13315	50-100	positive reaction, cerise colour
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	negative reaction, no change

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

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 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.Christensen W. B., 1946, J. Bacteriol., 52:461.

4.Farmer J. J. III, McWhorter A. C., Huntley G. A., Catignani J., J. Clin. Microbiol. 1975: 1 (1): 106-107.

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. MacFaddin J. F, 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williamsand Wilkins, Baltimore, Md.

8. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Williams and Wilkins, Baltimore.Md.

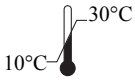
9. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

10. 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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Mannitol Salt Agar

M118

Intended Use:

Recommended for the isolation of pathogenic Staphylococci from clinical and non-clinical samples.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
HM peptone B #	1.000
Sodium chloride	75.000
D-Mannitol	10.000
Phenol red	0.025
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 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. Cool to 45-50°C. If desired, add 5% v/v Egg Yolk Emulsion (FD045). Mix well and pour into sterile Petri 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 (10). Staphylococci have the unique ability of growing on a high salt containing media (8). Isolation of coagulase-positive staphylococci on Phenol Red Mannitol Agar supplemented with 7.5%NaCl was studied by Chapman (2). The resulting Mannitol Salt Agar Base is recommended for the isolation of coagulase-positive staphylococci from cosmetics, milk, food and other specimens (10, 5,3,12,11). 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 (4). HM peptone B and proteose peptone supply essential growth factors and trace nutrients to the growing bacteria. Sodium chloride serves as an inhibitory agent against bacteria other than staphylococci. 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] (10). 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) (9). 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 (9).

Type of specimen

Clinical samples: pus, urine; Food and dairy samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,12,13).
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. A possible *S.aureus* must be confirmed by the coagulase test.
2. 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) (9).
3. 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 (9).

Performance and Evaluation

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

Quality Control

Appearance
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
Reaction
Reaction of 11.1% 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 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Incubation temperature
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	50 -100	luxuriant	>=50 %	yellow/white colonies surrounded by yellow zone	18 -72 hrs
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	0 %		>=72 hrs
Staphylococcus aureus subsp. *aureus ATCC 25923* (00034*)	50 -100	luxuriant	>=50 %	yellow/white colonies surrounded by yellow zone	18 -72 hrs

Staphylococcus epidermidis ATCC 14990 (00132*)	50 -100	fair-good	30 -40 %	red	18 -72 hrs
Proteus mirabilis ATCC 12453	50 -100	none-poor	0 -10 %	yellow	18 -72 hrs
Escherichia coli ATCC 25922 (00013*)	>=10^4	Inhibited	0%		>=72 hrs
Escherichia coli NCTC 9002	>=10^4	Inhibited	0%		>=72 hrs
# Klebsiella aerogenes ATCC 13048 (00175*)	>=10^4	Inhibited	0%		>=72 hrs

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

Storage and Shelf Life

Store between 10- 30°C in a tightly closed container and the prepared medium at 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. 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 (6,7).

Reference

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

2. Chapman G. H., 1945, J. Bacteriol., 50:201.

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

7. 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.

8. Koch P. K., 1942, Zentralbl. Bakteriol. Parasitenkd. Abt. I Orig.149:122.

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

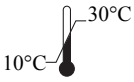
10. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed.,American Society for Microbiology, Washington, D.C.

11. Silverton R. E. and Anderson M. J., 1961, Handbook of Medical Laboratory Formulae, Butterworths, London.

12. 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.

13. 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 : 03 / 2019

IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
	Do not use if package is damaged
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Pseudomonas Agar (For Fluorescein) M120

Intended Use:
Recommended for detection of fluorescein production by *Pseudomonas* species.

Composition**

Ingredients	Gms / Litre
Tryptone	10.000
Proteose peptone	10.000
Dipotassium hydrogen phosphate	1.500
Magnesium sulphate	1.500
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions
Suspend 38 grams in 1000 ml purified / distilled water containing 10 ml glycerol. 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
Pseudomonas Agar (For Fluorescein) is based on the formula described by King et al (3) and as modified in the U.S. Pharmacopeia (5) for the detection of fluorescein production a water soluble, chloroform insoluble fluorescent pigment by *Pseudomonas* species (4). The medium enhances the elaboration of fluorescein by *Pseudomonas* and inhibits the pyocyanin formation. The fluorescein pigment diffuses from the colonies of *Pseudomonas* into the agar and shows yellow fluorescent colouration. Some *Pseudomonas* strains produce small amounts of pyocyanin resulting in a yellow-green colouration.

Tryptone and proteose peptone provide the essential nitrogenous nutrients, carbon, sulphur and trace elements for the growth of *Pseudomonas*. Dipotassium hydrogen phosphate buffers the medium while magnesium sulphate provides necessary cations for the activation of fluorescein production. Salt concentration exceeding 2% affects pigment production. UV illumination may be bactericidal, so make sure that there is good growth before placing culture under UV light (4).

A pyocyanin-producing *Pseudomonas* strain will usually also produce fluorescein. It must, therefore, be differentiated from other simple fluorescent *Pseudomonads* by other means. Temperature can be a determining factor as most other fluorescent strains will not grow at 35°C. Rather, they grow at 25-30°C (4).

Type of specimen
Pharmaceutical samples

Specimen Collection and Handling:
For pharmaceutical samples follow appropriate techniques for handling specimens as per established guidelines (4).
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.

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 3.8% w/v aqueous solution (containing 1% v/v glycerol) at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
Cultural characteristics observed with added 1% glycerol after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Pseudomonas aeruginosa ATCC 17934	50-100	luxuriant	>=70%	greenish yellow
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	luxuriant	>=70%	greenish yellow
Pseudomonas aeruginosa ATCC 9027 (00026*)	50-100	luxuriant	>=70%	greenish yellow

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 (1,2).

Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. 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.
3. King, Ward and Raney, 1954, J. Lab. Clin. Med., 44 : 301.
4. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
5. The United States Pharmacopoeia, 2006, USP29/NF24, The United States Pharmacopeial Convention, Rockville, MD.

Revision : 03/ 2019

Brilliant Green Bile Broth 2%

M121

Intended Use:

Recommended for detection and confirmation of coliform bacteria in water, waste water, food, milk and dairy products.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Lactose	10.000
Bile#	20.000
Brilliant green	0.0133
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Oxgall

Directions

Suspend 40.01 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Distribute in fermentation tubes containing inverted Durhams tubes and 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. For preparation of double strength it is recommended to heat the dissolved broth (80.02 grams per litre) at 100°C for 30 minutes.

Principle And Interpretation

Brilliant Green Bile Broth 2% is one of the most widely used medium for the detection of coliform bacteria in water, wastewater, foods, and milk and dairy products. This medium is formulated as per APHA (3,9,10) for the presumptive identification and confirmation of coliform bacteria (7,8). This medium is also recommended by the ISO Committee for enumeration of coliforms by most probable number technique (4).

Peptone serves as a source of essential nutrients. Lactose is the fermentable carbohydrate. Bile inhibits gram-positive bacteria whereas the gram-negative bacteria are inhibited by brilliant green. Production of gas from lactose fermentation is detected by incorporating inverted Durham's tube, which indicates the positive evidence of faecal coliform since non faecal coliforms growing in this medium do not produce gas. Further gas production in EC broth (M127) at 45°C used as a confirmation of faecal coliform. Gram-positive spore formers may produce gas if the bile or brilliant green inhibition is weakened by reaction with food material.

During examination of water samples, growth from presumptive positive tubes showing gas in Lactose Broth (M026) or Lauryl Tryptose Broth (M080) is inoculated in Brilliant Green Bile Broth 2% (M121). Gas formation within 48 ± 2 hours confirms the presumptive test (3).

Type of specimen

Food and dairy samples; Water samples

Specimen Collection and Handling

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 :

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. Do not autoclave double-strength broth.
2. Gram-positive sporing organisms may produce gas if the bile/brilliant green inhibition is attenuated by foodmaterial.

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 pale green homogeneous free flowing powder

Colour and Clarity of prepared medium

Emerald green coloured, clear solution without any precipitate.

Reaction

Reaction of 4.0% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Gas
Bacillus cereus ATCC 10876	>=10^4	inhibited	
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	positive reaction
# Klebsiella aerogenes ATCC 13048 (00175*)	50-100	good-luxuriant	positive reaction
Enterococcus faecalis ATCC 29212 (00087*)	50-100	none-poor	negative reaction
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10^4	inhibited	

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

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle 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 (5,6).

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.
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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. McCrady and Langerin, 1932, J. Dairy Science, 15:321.

8. McCrady, 1937, Am. J. Publ. Health, 27:1243.
9. Richardson G., (Ed.), 1985, Standard Methods for the Examination of Dairy Products, 15th Ed, APHA, Washington, D.C.
10. 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.
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 : 03/ 2019

EC Broth

M127

Intended Use:

Recommended for the selective enumeration of presumptive *Escherichia coli* by MPN technique from water samples and from clinical samples.

Composition**

Ingredients	Gms / Litre
Tryptone	20.000
Lactose	5.000
Bile salts mixture	1.500
Dipotassium hydrogen phosphate	4.000
Potassium dihydrogen phosphate	1.500
Sodium chloride	5.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37.0 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense in test tubes containing inverted Durhams tube. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Adjust the concentration of medium in accordance with sample size.

Principle And Interpretation

EC Medium is used for detection of coliforms during bacteriological examination of water, milk and foods. It was originally described by Hajna and Perry (1). This medium was later used by Fishbein and Surkiewicz to carry out *Escherichia coli* confirmatory tests (2). It is also used in MPN methods (3) and is often used for confirmation of coliforms. The procedure employing EC Medium provides information regarding the source of the coliform group (fecal or non-fecal) when used as a confirmatory test (4). EC Broth should not be used for the direct isolation of coliforms since prior enrichment in a presumptive medium for optimal recovery of faecal coliforms is required. Tryptone provides nitrogenous and carbonaceous compounds, long chain amino acids and other essential growth nutrients. Lactose is the fermentable sugar. Bile salts mixture inhibit gram-positive bacteria especially bacilli and faecal Streptococci. Phosphates control the pH during fermentation of lactose. Gas production in a fermentation tube within 24 hour or less is a presumptive evidence of the presence of coliform bacteria. This medium can be used at 37°C for the detection of coliform organisms or at 44.5°C for the isolation of *Escherichia coli* from water and shellfish) or 45.5°C for foods.

When using sample more than 10 ml, the medium must be reconstituted at a concentration equivalent to that specified on the directions, once the sample is added, the working procedure is as follows:

Transfer a loopful of culture from all the tubes of Lauryl Sulphate Broth (M080) showing gas formation within 24 hours and from all the tubes showing xbacterial growth within 48 hours to EC Broth tubes. Within 30 minutes from the inoculum, place the tubes in a water bath and incubate at 44°C for 24 hours. Consider the growth showing gas production as positive.

Calculate the density of the faecal coliform organisms by using MPN tables. False-negative reactions in recovering coliforms from water supplies can occur due to low pH, refrigeration and use of bactericidal or bacteriostatic agents (5).

Gas formation at 44.5°C or 45.5°C (and 37°C) *Escherichia coli*, possibly also other coliforms. Gas formation at 37°C Coliform bacteria without *Escherichia coli.*

Type of specimen

Clinical - faeces ; Food samples; Water sample.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (8,9,10).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (3).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

1.For identification, organisms must be in pure culture.
2.Morphological, biochemical and/or serological tests should be performed for final 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
Yellow coloured, clear solution without any precipitate

Reaction
Reaction of 3.7% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH
6.70-7.10

Cultural Response
Cultural characteristics observed after an incubation at 44.5°C ± 0.2 for 24 hours.

Organism	Inoculum (CFU)	Growth	Gas
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	good-luxuriant	positive reaction
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	fair to good	negative reaction
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)	>=10^4	inhibited	
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	positive reaction
# *Klebsiella aerogenes ATCC 13048* (00175*)	>=10^4	inhibited	

Key *- Corresponding WDCM Numbers ; # - Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle 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 (6,7).

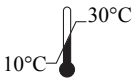
Reference

1. Hajna A. A. and Perry C. A., 1943, Am. J. Public Health, 33:550.
2. Fishbein M. and Surkiewicz B. F., 1964, Appl. Microbiol., 12:127.
3. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
4. Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
5. Ray B., 1986, J. Food Prot., 49:651. 6. 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.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
7. 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.
8. Marshall, (Ed.), 1993, Standard Methods for the Examination of Dairy Products, 16th Ed., American Public Health Association, Washington, D.C.
9. 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.
10. 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 : 05/2022

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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Columbia Blood Agar Base

M144

Intended Use:

Recommended for preparation of blood agar, chocolate agar and for preparation of various selective and identification media and isolation of organisms from clinical and non clinical samples.

Composition**

Ingredients	Gms / Litre
Peptone, special	23.000
Corn starch	1.000
Sodium chloride	5.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 44.0 grams of 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 before adding heat sensitive compounds.

For Blood Agar: Add 5% v/v sterile defibrinated sheep blood to sterile cool base.

For Chocolate Agar: Add 10% v/v sterile defibrinated sheep blood to sterile cool base. Heat to 80°C for 10 minutes with constant agitation.

The medium can be made selective by adding different antimicrobials to sterile base.

For *Brucella* species: Add rehydrated contents of 1 vial of Brucella Selective Supplement (FD005) to 500 ml sterile molten base.

For *Campylobacter* species: Add rehydrated contents of 1 vial of Campylobacter Supplement- I (Blaser-Wang) (FD006) or Campylobacter Supplement- II, (Butzler) (FD007) or Campylobacter Supplement- III (Skirrow) (FD008) or Campylobacter Selective Supplement (FD090) or Campylobacter Supplement- VI (Butzler) (FD106) to 500 ml sterile molten base along with rehydrated contents of 1 vial of Campylobacter Growth Supplement (FD009) and 5-7% v/v horse or sheep blood.

For *Gardnerella* species: Add rehydrated contents of 1 vial of G.Vaginalis Selective Supplement (FD056) to 500 ml sterile molten base.

For Cocci: Add rehydrated contents of 1 vial of Staph-Strepto Supplement (FD030) or Strepto Supplement (FD031) or Streptococcus Selective Supplement (FD119) to 500 ml sterile molten base.

Principle And Interpretation

Columbia Blood Agar Base was devised by Ellner et al (1). This medium contains special peptone which supports rapid and luxuriant growth of fastidious and non-fastidious organisms. Also, this medium promotes typical colonial morphology; better pigment production and more sharply defined haemolytic reactions. Fildes found that Nutrient Agar supplemented with a digest of sheep blood supplied both of these factors and the medium would support the growth of *H. influenzae* (2,3). The inclusion of bacitracin makes the enriched Columbia Agar Medium selective for the isolation of *Haemophilus* species from clinical specimens, especially from upper respiratory tract (4). Columbia Agar Base is used as the base for the media containing blood and for selective media formulations in which different combinations of antimicrobial agents are used as additives.

Corn starch serves as an energy source and also neutralizes toxic metabolites. Sheep blood permits the detection of haemolysis and also provides heme (X factor) which is required for the growth of many bacteria. However it is devoid of V factor (Nicotinamide adenine dinucleotide) and hence *Haemophilus influenzae* which needs both the X and V factors, will not grow on this medium.

Columbia Agar Base with added sterile serum provides an efficient medium for *Corynebacterium diphtheriae* virulence test medium. After following the established technique for *C. diphtheriae*, lines of toxin-antitoxin precipitation are clearly visible in 48 hours. Many pathogens require carbon dioxide; therefore, plates may be incubated in an atmosphere containing approximately 3-10% CO_2.

Precaution: *Brucella* cultures are highly infective and must be handled carefully; incubate in 5-10% CO_2. *Campylobacter* species are best grown at 42°C in a micro aerophillic atmosphere. Plates with *Gardenerella* supplements plates should be incubated at 35°C for 48 hours containing 7% CO_2 (2).

Type of specimen

Clinical samples : respiratory exudates, pus.

Specimen Collection and Handling

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

Warning and Precautions

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

Limitations

1. Certain fastidious organisms like *Haemophilus influenzae* may not grow on the medium, blood supplementation may be required.
2. As this medium have a relatively high carbohydrate content, beta-hemolytic Streptococci may exhibit a greenish hemolytic reaction which may be mistaken for the alpha haemolysis.
3. Biochemical characterization is required on colonies of pure culture 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
Basal medium: Light amber coloured clear to slightly opalescent gel.
After addition of 5%w/v sterile defibrinated blood : Cherry red coloured opaque gel forms in Petri plates.

Reaction
Reaction of 4.4% w/v aqueous solution at 25°C. pH : 7.3±0.2
pH
7.10-7.50
Cultural Response
Cultural characteristics observed with added 5% w/v sterile defibrinatedblood, after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
Neisseria meningitidis ATCC 13090	50-100	luxuriant	>=70%	none
Staphylococcus aureus subsp. *aureus ATCC 25923* (00034*)	50-100	luxuriant	>=70%	beta / gamma
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	luxuriant	>=70%	gamma
Staphylococcus aureus subsp. *aureus ATCC 6538* (00032*)	50-100	luxuriant	>=70%	beta / gamma
Streptococcus pneumoniae ATCC 6303	50-100	luxuriant	>=70%	alpha
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	>=70%	beta
Clostridium sporogenes ATCC 19404 (00008*)	50-100	luxuriant	>=50 %	
Clostridium sporogenes ATCC 11437	50-100	good-luxuriant	>=50 %	
Clostridium perfringens ATCC 13124 (00007*)	50-100	luxuriant	>=50 %	
Clostridium perfringens ATCC 12934	50-100	luxuriant	>=50 %	

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

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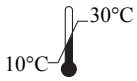
Revision : 03/2022

IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
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Acetamide Broth (Twin Pack)

M148

Intended Use:

Recommended for confirmation of *Pseudomonas aeruginosa* in water samples.

Composition**

Ingredients	Gms / Litre
Part A	-
Acetamide	10.000
Part B	-
Sodium chloride	5.000
Dipotassium hydrogen phosphate	1.390
Potassium dihydrogen phosphate	0.730
Magnesium sulphate	0.500
Phenol red	0.012
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 7.63 grams of part B in 1000 ml purified / distilled water. Add 10.0 grams of Part A. Heat if necessary to dissolve the medium completely. Dispense in 10ml amounts in tubes or as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C.

Principle And Interpretation

Acetamide Broth is formulated as per the recommendation of Standard Methods for the Examination of Water and Wastewater (3). Acetamide is utilized by a wide variety of non-fermenting organisms (4,12). The media contains inorganic salts and acetamide a sole carbon and nitrogen source. However very few organisms growing in the medium metabolize acetamide by the process of deamination (acrylamidase activity) (9,10). This unique ability is useful in identification of various non-fermenting gram-negative organisms (2,5,11). This ability is shown by *Pseudomonas aeruginosa, Pseudomonas aciovorans* Group III *(Achromobacter xylosoxidans)* and *Alcaligenes odorans* (8).

Acetamide deamination leads to the liberation of ammonia, which thereby increases the pH of the medium, leading to a subsequent colour change of the phenol red indicator from yellow orange to purplish red. Some strains require upto seven days to exhibit a positive reaction as they deaminate acrylamide slowly. However, only about 40% of apyocyanogenic strains of *Pseudomonas aeruginosa* exhibit a positive reaction. It is therefore, not advisable to rely on this test as the only criterion for identification. Phosphates in the media serve as buffering agents, Magnesium sulphate is a source of ions that stimulate metabolism whereas Acetamide serves as the sole nitrogen and carbon source. Sodium chloride maintains osmotic equilibrium. Phenol red is the pH indicator.

Type of specimen

Water samples

Specimen Collection and Handling

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

Warning and Precautions

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. Only about 40% of apyocyanogenic strains of *Pseudomonas aeruginosa* exhibit a positive reaction. It is therefore, not advisable to rely on this test as the only criterion 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

Part A : Colourless deliquescent crystals Part B : Light yellow to light pink homogeneous free flowing powder

Colour and Clarity of prepared medium

Orange coloured clear solution in tubes

Reaction

Reaction of the medium (Mixture of 1% w/v Part A and 0.76% w/v of Part B) at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 4-7 days.

Organism	Inoculum (CFU)	Growth	Deamination
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	good - luxuriant	positive reaction, purplish red colour (within 7days)
Stenotrophomonas maltophilia ATCC 13637	50-100	good - luxuriant	negative reaction,no purplish red colour (after 7 days)

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. 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 (6,7).

Reference

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Mueller Hinton Agar

M173

Intended Use:

Recommended for determination of susceptibility of microorganisms to antimicrobial agents isolated from clinical samples.

Composition**

Ingredients	Gms / Litre
HM infusion B from #	300.000
Acicase ##	17.500
Starch	1.500
Agar	17.000
Final pH (at 25°C)	7.3±0.1

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef infusion from

- Equivalent to Casein acid hydrolysate

Directions

Suspend 38.0 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. Mix well and pour into sterile Petri plates. Note: The performance of this batch has been tested and standardised as per the current CLSI (formerly, NCCLS) document M6-protocols for Evaluating Dehydrated Mueller Hinton Agar.

Principle And Interpretation

The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria* species (6). Other media were subsequently developed that replaced the use of Mueller Hinton Agar for the cultivation of pathogenic *Neisseria* species, but it became widely used in the determination of sulfonamide resistance of gonococci and other organisms. Mueller Hinton Agar is now used as a test medium for antimicrobial susceptibility testing (9). Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agar gel as described in CLSI Approved Standard (3). Mueller Hinton Agar has been selected by the CLSI for several reasons:

i. It demonstrates good batch-to-batch reproducibility for susceptible testing.

ii. It is low in sulfonamide, trimethoprim and tetracycline inhibitors.

iii. It supports the growth of most non-fastidious bacterial pathogens and

iv. Many data and much experience regarding its performance have been recorded (7).

Kirby-Bauer et al recommended this medium for performing antibiotic susceptibility tests using a single disc of high concentration (4). WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility (11). Mueller Hinton Agar with 5% sheep blood and Mueller Hinton Agar with Hemoglobin have been recommended for antimicrobial susceptibility testing of *Streptococcus pneumoniae* and *Haemophilus influenzae*.

HM infusion B from and acicase provide nitrogenous compounds, carbon, sulphur and other essential nutrients. Starch acts as a protective colloid against toxic substances present in the medium. Starch hydrolysis yields dextrose, which serves as a source of energy. These ingredients are selected for low thymine and thymidine content as determined by MIC values for

Enterococcus faecalis with sulfamethoxazole trimethoprim (SXT).

The Kirby-Bauer procedure is based on agar diffusion of antimicrobial substances impregnated on paper discs. This method employs disc with a single concentration of antimicrobial agent and the zone diameters observed are correlated with minimum inhibitory concentration (MIC) values (2,6,9). A standardized suspension of the organism is swabbed over the entire surface

of the medium. Paper discs impregnated with specific amounts of antimicrobial agents are then placed on the surface of the medium, incubated and zones of inhibition around each disc are measured. The susceptibility is determined by comparing with CLSI standards (7). The various factors, which influence disc diffusion susceptibility tests, are agar depth, disc potency, inoculum concentration, pH of the medium and beta-lactamase production by test organisms (7,8).

Mueller Hinton Agar is not appropriate for assay by disc diffusion method with slow growing organisms, anaerobes and capnophiles. With slow growing organisms, increased incubation may cause deterioration of diffusing antibiotic and produce unprecise readings (5).

Type of specimen

Clinical samples : Isolated microorganisms from urine , stool, blood etc.

Specimen Collection and Handling

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

Warning and Precautions

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

Limitations

1. This medium is recommended for susceptibility testing of pure cultures only.
2. Inoculum density may affect the zone size. Heavy inoculum may result in smaller zones or too less inoculum may result in bigger zones.
3. Fastidious organisms may not grow on this medium and may require supplementation of blood.
4. Fastidious anaerobes may not grow on this medium.
5. As antimicrobial susceptibility is carried with antibiotic disc, proper storage of the disc is desired which may affect the potency of the disc.
6. Under certain circumstances, the in vitro results of antibiotic susceptibility may not show the same in vivo.

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 1.7% agar gel.
Colour and Clarity of prepared medium
Light amber coloured clear to slight opalscent gel froms in Petri plates.
Reaction
Reaction of 3.8% w/v aqueous solution at 25°C. pH : 7.3±0.1
pH
7.20-7.40
Cultural Response
Cultural characteristics observed after incubation at 30-35°C for 18 -24 hours for bacterial cultures. For testing S.pneumoniae : The medium was supplemented with 5% Sheep blood and incubated at 35°C for 16-18 hours at 5% CO2 For testing *H.influenaze* : The medium was supplemented with 5g/l of Yeast extract & 2 vials /l of Haemophilus Growth Supplement (FD117 containing 15 mg/l of Haematin + 15 mg/l of NAD) and incubated at 35°C for 20-24 hours at 5% CO2
Antibiotic Sensitivity test
Various discs were tested for standard ATCC strains and zone of inhibition were measured after an incubation 30-35°C for 18 hours. (As per the latest CLSI Protocol M6 & Standards as per the current CLSI M100)
Thymine/Thymidine Content
The zones for these discs are indicative of the Thymine/Thymidine content of the medium.
Divalent Cation Content

$ The zones for these discs are indicative of the Divalent Cation content of the medium

Organism	Growth	Standard Zone	Zone of inhibition Observed
***Escherichia coli* ATCC 25922 (00013*)**	luxuriant		
Cephalothin CEP 30mcg		29-37 mm	29 -37 mm
Chloramphenicol C 30 mcg		21-27 mm	21 -27 mm
Co-Trimoxazole COT 25 mcg #		23-29 mm	23 -29 mm
Cefotaxime CTX 30 mcg		29-35 mm	29 -35 mm
Gentamicin GEN 10 mcg		19-26 mm	19 -26 mm
Sulphafurazole SF 300 mcg		15-23 mm	15 -23 mm
***Staphylococcus aureus* subsp. *aureus* ATCC 25923 (00034*)**	luxuriant		
Co-Trimoxazole COT 25 mcg #		# 20 mm (Clear zone)	>=20 mm
Cefoxitin CX 30 mcg		23-29 mm	23 -29 mm
Erythromycin E 15 mcg		22-30 mm	22 -30 mm
Linezolid LZ 30 mcg		25-32 mm	25 -32 mm
Oxacillin OX 1mcg		18-24 mm	18 -24 mm
Pristinomycin RP 15 mcg		21-28 mm	21 -28 mm
Tetracycline TE 30 mcg $		18-25 mm	18 -25 mm
Ciprofloxacin CIP 5mcg		22-30 mm	22 -30 mm
***Pseudomonas aeruginosa* ATCC 27853 (00025*)**	luxuriant		
Ceftazidime CAZ 30 mcg		22-29 mm	22 -29 mm
Ciprofloxacin CIP 5mcg		30-40 mm	30 -40 mm
Tobramycin TOB 10 mcg $		19-25 mm	19 -25 mm
Amikacin AK 30 mcg $		18-26 mm	18 -26 mm
Aztreonam AT 3mcg		23-29 mm	23 -29 mm
Cephotaxime CTX 30 mcg		18-22 mm	18 -22 mm
Gentamicin GEN 10 mcg $		16-21 mm	16 -21 mm
Imipenem IPM 10 mcg		20-28 mm	20 -28 mm
Piperacillin PI 100 mcg		12-18 mm	25 -33 mm
***Escherichia coli* ATCC 35218**	luxuriant		
Amoxyclav AMC 30 mcg		18-24 mm	18 -24 mm
Piperacillin/Tazobactam PIT 100/10 mcg		24-30 mm	24 -30 mm
Ticarcillin TI 75 mcg		6 mm	6 -6 mm
Ticarcillin/Clavulanic acid TCC 75/10mcg		20-28 mm	20 -28 mm
Ampicillin AMP 10 mcg		16-22 mm	16 -22 mm
Ampicillin/Sulbactam A/S 10/10 mcg		29-37 mm	29 -37 mm
***Enterococcus faecalis* ATCC 29212 (00087*)**	luxuriant		
Trimethoprim TR 5 mcg #		# 20 mm	>=20 mm
Vancomycin VA 30 mcg		17-21 mm	17 -21 mm
***Staphylococcus aureus* subsp. *aureus* ATCC 43300 (MRSA) (00211*)**	luxuriant		
Oxacillin OX 1 mcg		Very Hazy to No Zone	No zone

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).

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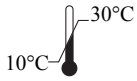
Revision : 04 / 2018

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

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Bordet Gengou Agar Base

M175

Intended use

Recommended for the detection and isolation of *Bordetella pertussis* and *Bordetella parapertussis*.

Composition**

Ingredients	Gms / Litre
Potato infusion from	125.000
Peptone	10.000
Sodium chloride	5.500
Agar	20.000
Final pH (at 25°C)	6.7±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.00 grams in 1000 ml purified / distilled water containing 10 ml glycerol. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 15-20% sterile, fresh defibrinated blood (sheep, rabbit, human or horse). For selectivity aseptically add rehydrated contents of 2 vials of Bordetella Selective supplement (FD004). Mix thoroughly, taking care to avoid incorporation of air bubbles and pour into sterile Petri plates.

Principle And Interpretation

Bordet Gengou Agar Media were originally formulated by Bordet and Gengou (1) for cultivation of *Bordetella* species. *Bordetella pertussis* is the causative agent of whooping cough and with the help of cough-plate technique, *B. pertussis* can be isolated from pharyngeal extracts, nasopharyngeal secretions and pre-nasal swabs. Kendrick and Eldering (6) modified the original media by replacing 50% human or rabbit blood with 15% sheep blood to make the medium more enriched for detection of *B. pertussis* by the virtue of its haemolytic reaction. Enrichment of the basal media with 25% human blood aids in the detection of Mycobacterium species from small sputum inocula and in Streptomycin sensitivity testing (8).

The medium is highly nutritious thus supports luxuriant growth of *Bordetella* species and can also be used for mass cultivation of *B.pertussis* for vaccine production (2) and for maintaining stock cultures (1).

Potato infusion and peptone serve as carbon and nitrogen source, amino acids while glycerol and blood enrichment provides additional nutrients. Sodium chloride maintains osmotic equilibrium. Incubation should be carried out in a moist chamber (60% humidity) at 37°C for upto 7 days. Medium should not be over dried before use. After 40 hours *B.pertussis* colonies appear smooth, raised, glistening with a zone of haemolysis. Some strains of *Bordetella* are not haemolytic. For confirmation, serodiagnosis and biochemical test should be performed. This medium can be made more selective for Bordetella, by using antibiotics like penicillin (3), methicillin (2), cephalexin (6) of which, cephalexin was found to be superior. Cephalexin suppresses unwanted nasopharyngeal growth and significantly increases the isolation rate of Bordetella species. Cephalexin is used at a concentration of 40 mg/liter (FD004). Amphotericin B (10 µg/ml) can be added as an antifungal agent to the medium.

For isolation of *B.pertussis* from specimens, use standard procedures. Incubate the plates in a moist chamber at 35-37°C for 7 days and examine daily with or without dissecting microscope (oblique illumination) to detect the presence of B. pertussis . Sometimes the accompanying mold colonies can mask the *B.pertussis* colonies. Use sterile scalpel or needle to remove the portion of the agar that contains spreading colonies of moulds. *B.pertussis* colonies may not be visible without the aid of a microscope for 2-4 days. After 7 days of incubation plates may be discarded as negative. Some *Haemophilus* species will grow on *Bordetella* isolation media and cross-react with *B.pertussis* antisera. It may be prudent to rule out X and V factor dependence.

Type of specimen

Clinical samples -Pharyngeal extracts, nasopharyngeal secretions and pre-nasal swabs.

Specimen Collection and Handling:

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

Warning and Precautions :

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

Limitations :

1. Some *Haemophilus* species will grow on *Bordetella* isolation media and cross-react with *B. pertussis* antisera.
2. *B. pertussis* colonies may not be visible without the aid of a microscope for 2-4 days.

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 2.0% agar gel.

Colour and Clarity of prepared medium
Basal Medium : Light yellow coloured clear to slightly opalescent gel. After addition of glycerol and 15% v/v sterile defibrinated blood: Cherry red coloured opaque gel forms in Petri plates.

Reaction
Reaction of 4% w/v aqueous solution at 25°C. pH : 6.7±0.2

pH
6.50-6.90

Cultural Response
Cultural characteristics observed with added Glycerol and 15% v/v sterile defibrinated blood and Bordetella Selective Supplement (FD004), after an incubation at 35-37°C for 3-4 days.

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
Bordetella bronchiseptica ATCC 4617	50-100	good-luxuriant	>=50%	gamma
Bordetella parapertussis ATCC 15311	50-100	good-luxuriant	>=50%	gamma
Bordetella pertussis ATCC 8467	50-100	good-luxuriant	>=50%	beta
Staphylococcus aureus subsp.aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%	

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. 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 (4,5).

Reference

1. Bordet and Gengou, 1906, Ann. Inst. Pasteur, 20:731.
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3. Flemming A., 1932, J. Path. Bacteriol., 35:831.
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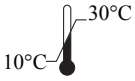
Revision : 02 / 2019

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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SIM Medium

M181

Intended use

Recommended for determination of hydrogen sulphide production, indole formation and motility of enteric bacilli from clinical and non-clinical samples.

Composition**

Ingredients	Gms / Litre
HM Peptone B#	3.000
Peptone	30.000
Peptonized iron	0.200
Sodium thiosulphate	0.025
Agar	3.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 36.23 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubes to cool in an upright position.

Principle And Interpretation

SIM Medium is used to differentiate enteric bacilli particularly *Salmonella* and *Shigella* on the basis of sulphide production, indole formation and motility (1,5). Jordan and Victorson (3) reported that *Salmonella* Paratyphi A and Paratyphi B can be distinguished on the basis of H_2S production using lead acetate. Sulkin and Willett (7) used Triple Sugar Iron Agar with 1% agar for motility along with H_2S production and carbohydrate fermentation. Sosa (6) described a peptone medium with low agar for motility and indole determination.

SIM Medium enables determination of three characteristics by which enteric bacteria can be differentiated. Peptonized iron and sodium thiosulphate are the indicators of H_2S production. This H_2S reacts with peptonized iron to form black precipitate of ferrous sulphide (6,7). Motile organisms intensify the H_2S reaction. Motile organisms grow away from line of inoculation showing diffused growth while non-motile organisms grow along the stab line. Motility detection is possible due to the semisolid nature of the medium. Growth radiating out from the central stab line indicates that the test organism is motile. Peptone and HM peptone B provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential nutrients. Tryptophan from peptone, is degraded by specific bacteria to produce indole (1). The indole is detected by the addition of chemical reagents following the incubation period.

Inoculate fresh culture with a single stab using straight needle through the center of the medium. Following incubation, observe for motility (diffuse growth outward from the stab line or turbidity throughout the medium) and for H_2S production (blackening of the medium). To detect indole production, add three or four drops of Kovacs reagent (1) and observe for development of red color (positive reaction). Determine motility and H_2S production prior to determination of indole production.

Type of specimen

Isolated Microorganisms

Specimen Collection and Handling

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.

Performance and Evaluation

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

Quality Control

Appearance

Cream to beige homogeneous free flowing powder

Gelling

Semisolid, comparable with 0.3% Agar gel.

Colour and Clarity of prepared medium

Medium amber coloured slightly opalescent gel forms in tubes as butts

Reaction

Reaction of 3.6% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Motility	Indole production(on addition of Kovac's	H_2S
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	positive, growth away from stabline causing turbidity	positive reaction, red ring at the interface of the medium	negative reaction
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	luxuriant	positive, growth away from stabline causing turbidity	negative reaction	positive reaction, blackening of medium
Shigella flexneri ATCC 12022 (00126*)	50-100	luxuriant	negative, growth along the stabline, surrounding medium remains clear	negative reaction	negative reaction
Salmonella Paratyphi A *ATCC 9150*	50-100	luxuriant	positive, growth away from stabline causing turbidity	negative reaction	Negative reaction
Salmonella Paratyphi B *ATCC 8739*	50-100	luxuriant	positive, growth away from stabline causing turbidity	Negative reaction	Positive reaction, blackening of medium
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	negative, growth along the stabline, surrounding medium remains clear	Negative reaction	Negative reaction

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 (2,4).

Reference

1. Ewing W. H., 1986, Edwards and Ewings Identification of Enterobacteriaceae, 4th Ed., Elsevier Science Publishing Co., Inc. New York.

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

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Revision : 02 / 2018

Dey-Engley Neutralizing Agar (D/E Agar Disinfectant Testing) M186

Intended use

Dey-Engley Neutralizing Agar is used in disinfectant testing, where neutralization of the chemical is important for determining its bactericidal activity.

Composition**

Ingredients	Gms / Litre
Tryptone	5.000
Yeast extract	2.500
Dextrose(Glucose)	10.000
Sodium thiosulphate	6.000
Sodium thioglycollate	1.000
Sodium bisulphite	2.500
Lecithin	7.000
Polysorbate 80	5.000
Bromocresol purple	0.020
Agar	15.000
Final pH (at 25°C)	7.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 54.02 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. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Dey-Engley Neutralizing Agar is formulated as per the procedure described by Engley and Dey (3). A strongly bacteriostatic substance inhibits the growth and reproduction of bacteria without killing them. These bacteria hold the ability to cause infection under favorable conditions. Dey-Engley Neutralizing Agar neutralizes a broad spectrum of antiseptics and disinfectants including quaternary ammonium compounds, phenolic, iodine and chlorine preparations, mercurial, formaldehyde and glutaraldehyde. (3).

Tryptone provide nitrogen and carbon source, long chain amino acids, vitamins and other essential nutrients. Dextrose is an energy source. Yeast extract is also a rich source of vitamin B-complex. The present formulation incorporate neutralizing substances for almost all the active products used as antiseptics and disinfectants. Sodium bisulfite neutralizes aldehydes; sodium thioglycollate neutralizes mercurial; sodium thiosulfate neutralizes iodine and chlorine (3); lecithin neutralizes quaternary ammonium compounds; and polysorbate 80, a non-ionic surface-active agent, neutralizes substituted phenolic (2,4,7,8). Bromocresol purple is an indicator for dextrose utilization. Due to the high concentration of lecithin in the broth medium, turbidity cannot be used to detect growth. Therefore, bromocresol purple and dextrose are added to the medium. Those organisms that ferment dextrose will turn the medium from purple to yellow. (3).
For Agar Medium: Dey -Engley Neutralizing Agar medium can be over-filled, producing a meniscus or dome-shaped surface that can be pressed onto a surface for sampling its microbial burden. Incubate the plates, by covering the lids, at an appropriate temperature. The presence of microorganism is determined by the appearance of colonies on the surface of agar medium. Neutralization Test: Growth in Neutralizing Broth and no growth in Neutralizing Broth Base indicate neutralization of disinfectant. To check bactericidal activity, both broth tubes are inoculated on D/E Neutralizing Agar. Positive growth from negative tubes of Neutralizing Broth Base indicates bacteriostatic substance while negative growth indicates a bactericidal disinfectant. All positive tubes should show growth on Dey-Engley Neutralizing Agar. The control disinfectants used in test procedure are 2% chlorine, 2% formaldehyde, 1% glutaraldehyde, 2% iodine, 2% phenol, 1/750 quaternary ammonium compounds, 1/1000 mercurial etc.

Type of specimen

Food and dairy samples; Environmental samples, cosmetic, pharmaceutical .

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,8,9). 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. Due to nutritional variations, some strains may show poor growth

Performance and Evaluation

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

Quality Control

Appearance

Light yellow to bluish grey homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Purple to reddish purple coloured, opalescent gel (may have particulate precipitate) forms in Petri plates.

Reaction

Reaction of 5.4% w/v aqueous solution at 25°C. pH : 7.6±0.2

pH

7.40-7.80

Cultural Response

Cultural characteristics observed after an incubation at 35 - 37°C for 40 - 48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=70%
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	luxuriant	>=70%
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	>=70%
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	luxuriant	>=70%
Bacillus subtilis subsp. spizizenniii ATCC 6633 (00003*)	50-100	luxuriant	>=70%
Candida albicans ATCC 10231 (00054*)	50-100	luxuriant	>=70%
Aspergillus brasiliensis ATCC 16404 (00053*)	50-100	luxuriant	>=50%

* - 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.
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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 : 05 / 2019

TCBS Agar

M189

Intended Use:

Recommended for the selective isolation and cultivation of *Vibrio cholerae* and other enteropathogenic *Vibrio's* causing food poisoning from clinical and food specimen.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
Yeast extract	5.000
Sodium thiosulphate	10.000
Sodium citrate	10.000
Bile	8.000
Sucrose	20.000
Sodium chloride	10.000
Ferric citrate	1.000
Bromo thymol blue	0.040
Thymol blue	0.040
Agar	15.000
Final pH (at 25°C)	8.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 89.08 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

TCBS Agar was developed by Kobayashi et al (7), who modified the selective medium of Nakanishi (11). Although this medium was originally designed for the isolation of *V.cholerae* and *V. parahaemolyticus*, most *Vibrio's* grow to healthy large colonies with many different colonial morphologies. TCBS Agar is also recommended by APHA for the selective isolation of *V. cholerae* and *V. parahaemolyticus* (2,12). Enrichment in Alkaline Peptone Water (M618), followed by isolation on TCBS Agar is routinely used for isolation of *V.cholerae* (3,4,10).

Proteose peptone and yeast extract provide nitrogenous compounds, vitamin B complex and other essential growth nutrients. Bile, a derivative of bile salts and sodium citrate inhibit gram-positive bacteria and coliforms (5). Sodium thiosulphate serves as a good source of sulphur, which in combination with ferric citrate detects the production of hydrogen sulphide. For the metabolism of *Vibrio's*, sucrose is added as a fermentable carbohydrate. *Vibrio* that is able to utilize sucrose will from yellow colonies. Bromothymol blue and thymol blue are the pH indicators. The alkaline pH of the medium improves the recovery of *V.cholerae*. Strains of *V. cholerae* produce yellow colonies on TCBS Agar because of fermentation of sucrose. *V. alginolyticus* also produce yellow colonies. *V.parahaemolyticus* is a sucrose non-fermenting organism and therefore produces blue-green colonies, as does *V.vulnificus*.

Type of specimen

Clinical : faeces; Food samples; Water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,10).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,12,13).
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. The medium should be inoculated heavily with faecal specimens because growth of few species may be inhibited on the medium due to fermentation of sucrose and accumulation of acids.
2. However, occasional isolates of *Pseudomonas* and *Aeromonas* may also form blue green colonies on TCBS Agar (8).
3. *Proteus* species that are sucrose-fermenters may form yellow colonies (8).
4. TCBS Agar is not a suitable medium for oxidase testing of *Vibrio* species (9).
5. A few strains of *V. cholerae* may appear green or colourless on TCBS Agar due to delayed sucrose fermentation (8).
6. TCBS Agar is highly selective for *Vibrio* species. Any H_2S negative colony of TCBS Agar can be considered presumptive positive for *Vibrio.*
7. Further biochemical and serological 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

Light yellow to light tan homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Bluish green coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 8.9% w/v aqueous solution at 25°C. pH : 8.6±0.2

pH

8.40-8.80

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Shigella flexneri ATCC 12022 (00126*)	>=10^4	inhibited	0%	
Vibrio cholerae ATCC 15748	50-100	good-luxuriant	>=50%	yellow
Vibrio fluvialis ATCC 33809 (00137*)	50-100	good-luxuriant	>=50%	yellow
Vibrio parahaemolyticus ATCC 17802 (00037*)	50-100	good-luxuriant	>=50%	bluish green
Vibrio vulnificus ATCC 29307 (00187*)	50-100	fair-good	>=30%	greenish yellow
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%	
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Proteus vulgaris ATCC 13315	>=10^4	inhibited	0%	

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 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 (6,10).

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. Forbes B. A., Sahm A. S. and Weissfeld D. F., 1998, Bailey & Scotts Diagnostic Microbiology, 10th Ed., Mosby, Inc. St. Louis, Mo.

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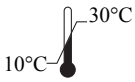
11. Nakanishi Y., 1963, Modern Media 9: 246.

12. 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.

13. 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/ 2019

IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
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