



Technical Data

Urea 40% (5 ml per vial)

FD048

Filter sterilized urea solution recommended for detection of urease activity.

Composition

Per vial sufficient for 100 ml medium

Ingredients

Urea

Distilled water

Final pH (at 25°C)

Concentration

2g

5ml

8.0±0.2

Directions:

Warm up the refrigerated Urea Solution to room temperature and aseptically add 5 ml in 95 ml sterile, molten, cooled (45-50°C)

Urea Broth Base [M111](#) / Urea Agar Base (Christensen) [M112](#) / [M112S](#) / [M112I](#) / Urea HiVeg™ Agar Base (Christensen) [MV112](#) / MIU Medium Base [M1076](#) / Hemmes Medium Base [M775](#) or 25 ml in 975 ml Kohn Two Tube Medium No. 1 Base [M142](#) / Kohn Two Tube HiVeg™ Medium No.1 Base [MV142](#) or to Yersinia Identification Broth Base [M1221](#) as desired. Mix well and dispense in sterile tubes.

Storage and Shelf Life

Store at 2-8°C. Use before expiry period on the label.

Revision : 1 / 2012

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Technical Data

Nalidixic Selective Supplement

FD130

An antibiotic supplement recommended for the selective isolation of *Pseudomonas aeruginosa* from clinical specimens.

Composition

Per vial sufficient for 1000 ml medium

*Ingredients

Nalidixic acid

Concentration

15mg

Directions:

Rehydrate the content of 1 vial aseptically with 5 ml of sterile distilled water. Mix well and aseptically add to 1000 ml of sterile, molten, cooled (45-50°C) Cetrimide Agar Base [M024](#) / Cetrimide HiVeg™ Agar Base [MV024](#). Mix well and pour into sterile petri plates.

Storage and Shelf Life

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

Revision : 1 / 2012

* Not For Medicinal Use

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Technical Data

Brucella Selective Supplement, Modified

FD161

An antibiotic supplement recommended for the selective isolation of *Brucella* species from milk.

Composition

Per vial sufficient for 500 ml medium

*Ingredients	Concentration
Polymyxin B sulphate	2500IU
Bacitracin	12500IU
Nystatin	50000IU
Natamycin	50mg
Naildixic acid	2,500mg
Vancomycin	10mg

Directions:

Rehydrate the contents of 1 vial aseptically with 10 ml of 50% methanol. Shake to form a uniform suspension. Add the contents to 500 ml of sterile, molten, cooled (45-50°C) media such as, Blood Agar Base No.2 [M834](#) / [M834A](#) / Blood Agar Base No.2, HiVeg™ [MV834](#) / [MV834A](#) / Columbia Blood Agar Base [M144](#) / [M144A](#) / Columbia Blood Agar Base HiVeg™ [MV144](#) / [MV144A](#) with 5-10% v/v inactivated horse serum [RM1239](#) and 1% w/v sterile dextrose or Brucella Agar Base [M074](#) / Brucella HiVeg™ Agar Base [MV074](#) / Brucella Broth Base [M348](#) / Brucella HiVeg™ Broth Base [MV348](#) / Brucella Selective Medium Base [M822](#) with 5-10% v/v inactivated horse serum [RM1239](#). Mix well and pour into sterile petri plates / tubes.

Storage and Shelf Life

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

Revision : 02 / 2016

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Technical Data

IMRV/RV Selective Supplement

FD193

An antibiotic supplement recommended for isolation of *Salmonella* from food stuffs and other materials.

Composition

Per vial sufficient for 500ml / 1000ml medium

*Ingredients

Novobiocin

Concentration

10mg

Directions:

Rehydrate the contents of one vial aseptically with 5 ml of sterile distilled water and aseptically add it to 1000 ml sterile, molten, cooled (45-50°C) Semisolid IMRV Medium Base [M1427](#) / Semisolid IMRV HiVeg™ Medium Base [MV1427](#) / Modified Semisolid RV Medium Base [M1482](#) & 500 ml of Semisolid RV Medium Base [M1428](#) / Semisolid RV HiVeg™ Medium Base [MV1428](#) . Mix well and pour into sterile petri plates.

Storage and Shelf Life

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

Revision : 1 / 2012

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Technical Data

MKTT Novobiocin Supplement

FD203

A selective supplement for enrichment and isolation of *Salmonella* species.

Composition

Per vial sufficient for 1000 ml medium

*Ingredients

Novobiocin

Concentration

40mg

Directions:

Rehydrate contents of 1 vial aseptically with 5 ml of sterile distilled water and aseptically add to sterile, cooled (45-50°C) Mueller Kauffman Tetrathionate Novobiocin Broth Base [M1496I](#) . Mix well and dispense as desired.

Storage and Shelf Life

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

Revision : 1 / 2012

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Durham Tubes

GW163

Durham Tubes are very small size test tubes made from Neutral Glass. The Durham Tube is inverted tube inside the fermentation tube which actually captures the gas produced by microorganisms.

Application : Chemical laboratory, Biological laboratory , Microbiology & Diagnostic sectors , Industrial laboratory and various other laboratories.

Product Name	Product Code	Description	Size (mm)
Durham Tubes	GW163	These tube are made of neutral Glass. and they are autoclavable.	Length = 25-27 Diameter = 6-7

Product features :

- Neutral Glass.
- Glass is very clear and transparent.
- The edges are properly polished and hence smooth.
- The bottoms of these Durham tubes are rounded.
- Pack Size : 1 X 100 Nos.

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Technical Data

Brilliant Green Agar Base, Modified

M016

Intended Use:

Recommended for selective isolation of Salmonellae other than *Salmonella* Typhi from faeces and other materials.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
Yeast extract	3.000
Lactose	10.000
Sucrose	10.000
Sodium chloride	5.000
Phenol red	0.080
Brilliant green	0.0125
Agar	20.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 29.0 grams in 500 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. AVOID OVERHEATING. Cool to 45-50°C. For more selectivity, aseptically add rehydrated contents of 1 vial of Sulpha Supplement (FD068). Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Salmonella species cause many types of infections, from mild self-limiting gastroenteritis to life threatening typhoid fever. The most common form of *Salmonella* disease is self-limiting gastroenteritis with fever lasting less than 2 days and diarrhoea lasting less than 7 days. Brilliant Green Agar Base, Modified, as a primary plating medium for isolation of *Salmonella* species was first described by Kristensen et. al. (8) and further modified by Kauffmann (7). Brilliant Green Agar is also recommended by APHA (9,10) FDA (2) and described in EP, BP and IP (4,11,12).

This medium contains brilliant green, which inhibits growth of majority of Gram-negative and Gram-positive bacteria. *Salmonella* Typhi, *Shigella* species *Escherichia coli*, *Pseudomonas* species, *Staphylococcus aureus* are mostly inhibited. Clinical specimens can be directly plated on this medium. However, being highly selective, it is recommended that this medium should be used along with a less inhibitory medium to increase the chances of recovery. Often cultures enriched in Selenite or Tetrathionate Broth is plated on Brilliant Green Agar along with Bismuth Sulphite Agar, SS Agar, MacConkey Agar.

The medium contains proteose peptone and yeast extract as sources of carbon, nitrogen, vitamins, amino acids and essential nutrients. The two sugars namely lactose and sucrose serve as energy sources. Fermentation of lactose and/or sucrose in the medium results in the formation of acidic pH which is detected by phenol red indicator. Sodium chloride maintains the osmotic equilibrium. Brilliant green helps to inhibit the contaminating microflora. The medium can further supplemented with sulphaacetamide (1g/l) and sodium mandelate (0.25g/l) to inhibit contaminating microorganisms when the sample is suspected to contain large number of competing organisms along with *Salmonella* species.

Non-lactose fermenting bacteria develop white to pinkish red colonies within 18 - 24 hours of incubation.

Type of specimen

Clinical : blood, faeces; Foodstuffs & dairy samples; Water samples; Pharmaceutical 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 (1,3,9,13).

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. Though this medium is selective for *Salmonella* other species of *Enterobacteriaceae* may grow.
2. *Salmonella* Typhi and *Shigella* species may not grow on this medium.
3. Moreover *Proteus*, *Pseudomonas* and *Citrobacter* species may mimic enteric pathogens by producing small red colonies.
4. Further confirmation has to be carried out on presumptive *Salmonella* isolates.

Performance and Evaluation

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

Quality Control

Appearance

Light yellow to light pink homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium

Greenish brown clear to slightly opalescent gel forms in Petriplates

Reaction

Reaction of 5.8% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH

6.70-7.10

Cultural Response

Cultural response was carried out after an incubation at 30-35°C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
<i>Escherichia coli</i> ATCC 25922 (00013*)	50 -100	none-poor	0 -10 %	yellowish green
<i>Escherichia coli</i> ATCC 8739 (00012*)	50 -100	none-poor	0 -10 %	yellowish green
<i>Escherichia coli</i> NCTC 9002	50 -100	none-poor	0 -10 %	yellowish green
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	>=10 ⁴	inhibited	0%	
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	>=10 ⁴	inhibited	0%	
<i>Salmonella</i> Typhi ATCC 6539	50 -100	fair-good	30 -40 %	reddish pink
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50 -100	good-luxuriant	>=50 %	pinkish white
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	>=50 %	pinkish white
<i>Salmonella</i> Abony NCTC 6017 (00029*)	50 -100	good-luxuriant	>=50 %	pinkish white

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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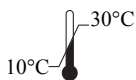
Revision : 03 / 2019



In vitro diagnostic medical device



CE Marking



Storage temperature



Do not use if package is damaged



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Xylose-Lysine Deoxycholate Agar (XLD Agar)

M031

Intended use

Recommended for the isolation and enumeration of *Salmonella* Typhi and other *Salmonella* species from clinical and non-clinical samples.

Composition**

Ingredients	Gms / Litre
Yeast extract	3.000
L-Lysine	5.000
Lactose	7.500
Sucrose	7.500
Xylose	3.500
Sodium chloride	5.000
Sodium deoxycholate	2.500
Sodium thiosulphate	6.800
Ferric ammonium citrate	0.800
Phenol red	0.080
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 56.68 grams in 1000 ml purified / distilled water. Heat with frequent agitation until the medium boils. DO NOT AUTOCLAVE OR OVERHEAT. Transfer immediately to a water bath at 50°C. After cooling, pour into sterile Petri plates. It is advisable not to prepare large volumes that will require prolonged heating, thereby producing precipitate. Note : Slight precipitation in the medium may occur, which is inheritant property of the medium, and does not affect the performance of the medium.

Principle And Interpretation

XLD Agar has been recommended for the identification of *Enterobacteriaceae* (3) and for the microbiological testing. XLD Agar was formulated by Taylor (13-17) for the isolation and differentiation of enteric pathogens including *Salmonella* Typhi from other *Salmonella* species of foods, water and dairy products (2,12,20,21). XLD Agar exhibits increased selectivity and sensitivity as compared to other plating media e.g. SS Agar (M108), EMB Agar (M022) and Bismuth Sulphite Agar (M027) (14,16,18, and 4,9,11,19). The media formulation does not allow the overgrowth of other organisms over *Salmonella* and *Shigella* (7). Samples suspected of containing enteric pathogens, along with other mixed flora, are initially enriched in Modified Semisolid RV Medium Base (M1482) (1).

The medium contains yeast extract, which provides nitrogen and vitamins required for growth. Though the sugars xylose, lactose and sucrose provide sources of fermentable carbohydrates, xylose is mainly incorporated into the medium since it is not fermented by *Shigellae* but practically by all enterics. This helps in the differentiation of *Shigella* species. Sodium chloride maintains the osmotic balance of the medium. Lysine is included to differentiate the *Salmonella* group from the non-pathogens. *Salmonellae* rapidly ferment xylose and exhaust the supply. Subsequently lysine is decarboxylated by the enzyme lysine decarboxylase to form amines with reversion to an alkaline pH that mimics the *Shigella* reaction. However, to prevent this reaction by lysine-positive coliforms, lactose and sucrose are added to produce acid in excess. Degradation of xylose, lactose and sucrose to acid causes phenol red indicator to change its colour to yellow. Bacteria that decarboxylate lysine to cadaverine can be recognized by the appearance of a red colouration around the colonies due to an increase in pH. These reactions can proceed simultaneously or successively, and this may cause the pH indicator to exhibit various shades of colour or it may change its colour from yellow to red on prolonged incubation. To add to the differentiating ability of the formulation, an H₂S indicator system, consisting of sodium thiosulphate and ferric ammonium citrate, is included for the visualization of hydrogen sulphide produced, resulting in the formation of colonies with black centers. The non-pathogenic H₂S producers do not decarboxylase lysine; therefore, the acid reaction produced by them prevents the blackening of the colonies (13).

XLD Agar is both selective and differential medium. It utilizes sodium deoxycholate as the selective agent and therefore it is inhibitory to gram-positive microorganisms.

Type of specimen

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

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,8).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (12,20).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(15)

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

Warning and Precautions :

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

Limitations :

1. Slight precipitation in the medium may occur, which is inheritant property of the medium, and does not affect the performance of the medium.
2. This medium is general purpose medium and may not support the growth of fastidious organisms.
3. Some *Proteus* strains may give red to yellow colouration with most colonies developing black centers, giving rise to false positive reactions.
4. Non-enterics like *Pseudomonas* and *Providencia* may exhibit red colonies.
5. *S. Paratyphi A*, *S. Choleraesuis*, *S. Pullorum* and *S. Gallinarum* may form red colonies without H₂S, thus resembling *Shigella* species.

Performance and Evaluation

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

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Red coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.67% w/v aqueous solution at 25°C . pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural response was observed after an incubation at 35-37°C for specified time. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation period
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	25 -100	≥50 %	red with black centres	18 -72 hrs
<i>Salmonella</i> Abony NCTC 6017 (00029*)	50 -100	good-luxuriant	25 -100	≥50 %	red with black centres	18 -72 hrs

<i>Escherichia coli</i> ATCC 8739 (00012*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
<i>Escherichia coli</i> ATCC 25922 (00013*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
<i>Escherichia coli</i> NCTC 9002	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
<i>Proteus vulgaris</i> ATCC 13315	50 -100	good-luxuriant	25 -100	>=50 %	grey with black centres	18 -72 hrs
<i>Salmonella</i> Paratyphi A ATCC 9150	50 -100	good-luxuriant	25 -100	>=50 %	red	18 -72 hrs
<i>Salmonella</i> Paratyphi B ATCC 8759	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
<i>Salmonella</i> Typhi ATCC 6539	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
<i>Shigella dysenteriae</i> ATCC 13313	50 -100	good-luxuriant	25 -100	>=50 %	red	18 -72 hrs
<i>Shigella flexneri</i> ATCC 12022 (00126*)	50 -100	fair-good	15 -40	30 -40 %	red	18 -72 hrs
<i>Shigella sonnei</i> ATCC 25931	50 -100	fair-good	15 -40	30 -40 %	red	18 -72 hrs
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
<i>Enterobacter cloacae</i> ATCC 13047 (00083*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	>=10 ⁴	inhibited	0	0%		>=72 hrs
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	>=10 ⁴	inhibited	0	0%		>=72 hrs
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	>=10 ⁴	inhibited	0	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 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 (6,8).

Reference

1. Aspinall S. T., Hindle M. A. and Hutchinson D. N., 1992, Eur. J. Clin. Microbiol., Inf. Dis. 11, 936-939.
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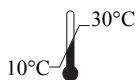
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Lysine Decarboxylase Broth without Peptone

M376I

Lysine Decarboxylase Broth w/o Peptone are used for differentiating *Salmonella* Arizonae from the Bethesda Ballerup group of *Enterobacteriaceae*.

Composition**

Ingredients	Gms / Litre
L-Lysine hydrochloride	5.000
Yeast extract	3.000
Dextrose	1.000
Bromocresol purple	0.015
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 9.01 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense 5 ml amount into screw-capped test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the tubed medium in an upright position and overlay with 2-3 ml of sterile mineral oil.

Principle And Interpretation

Decarboxylase media were first described by Moeller (1-3) for detecting lysine and ornithine decarboxylase and arginine dihydrolase. Falkow developed a lysine decarboxylase medium for the identification and differentiation of *Salmonella* and *Shigella* (4). Falkows Medium was further modified by Taylor (5) by deleting peptone from the formulation (M376I), thus eliminating false positives caused by *Citrobacter freundii* and its paracolons. Taylor's modification has same advantage of Falkow's formulation over Moeller; it does not require the special conditions of anaerobic culture and low pH.

During the initial stages of incubation, fermentation of dextrose by the organisms, with acid production results in a colour change of the indicator to yellow. On further incubation, if L-Lysine is decarboxylated to cadaverine, there will be an alkaline reaction and the indicator colour will then revert back to purple. If the colour remains yellow, the decarboxylase reaction is negative.

Yeast extract provide essential growth nutrients. Dextrose is the fermentable carbohydrate and bromo cresol purple is the pH indicator. Dextrose non-utilizers will not show any change in the medium colour. Use light inocula and do not read the tests under 24 hours incubation as some organisms require longer incubation time of upto 4 days.

Inoculate 25 grams of the test sample into Buffered Peptone Water (M614S). After incubation at 35-37°C for 16-20 hours, inoculate into RVS Broth (M1491) and Fluid Selenite Cystine Broth (M1533I) and incubate at 35-37°C for 24-48 hours. From the second enrichment, streak a loopful on Brilliant Green Agar Base w/ phosphates (M971S). Presumptive *Salmonella* so isolated on M971S are further confirmed by performing biochemical testing using the following media i.e. Nutrient Agar, pH 7.0 (M561A), Triple Sugar Iron Agar (M021S), Urea Agar Base, Christensen (M112I), Lysine Decarboxylase Broth w/o peptone (M376I), VP test, Indole test.

Quality Control

Appearance

Light yellow to greenish yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Purple coloured clear solution without any precipitate

Reaction

Reaction of 0.9% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Please refer disclaimer Overleaf.

M376I: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours .

Organism	Inoculum (CFU)	Lysine decarboxylation
<i>Citrobacter freundii</i> ATCC 8090	50-100	variable reaction
<i>Escherichia coli</i> ATCC 25922	50-100	variable reaction
<i>Enterobacter aerogenes</i> ATCC 13048	50-100	positive reaction, purple colour
<i>Klebsiella pneumoniae</i> ATCC 13883	50-100	positive reaction, purple colour
<i>Proteus mirabilis</i> ATCC 25933	50-100	negative reaction, yellow colour
<i>Proteus vulgaris</i> ATCC 13315	50-100	negative reaction, yellow colour
<i>Salmonella Arizonae</i> ATCC13314	50-100	Positive reaction, purple colour
<i>Salmonella Paratyphi A</i> ATCC 9150	50-100	negative reaction, yellow colour
<i>Salmonella Typhi</i> ATCC 6539	50-100	positive reaction, purple colour
<i>Serratia marcescens</i> ATCC 8100	50-100	positive reaction, purple colour
<i>Shigella dysenteriae</i> ATCC 13313	50-100	negative reaction, yellow colour

Storage and Shelf Life

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

Reference

1. Moeller V., 1954, Acta. Pathol. Microbiol. Scand., 34:102.
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3. Moeller V., 1955, Acta. Pathol. Microbiol. Scand., 36:158.
4. Falkow, 1958, Am. J. Clin. Pathol., 29:598.
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Buffered Peptone Water

M614

Intended use

Recommended as a pre-enrichment medium used for increasing the recovery of injured *Salmonella* species from food prior to selective enrichment and isolation and also from clinical samples.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
Sodium chloride	5.000
Disodium hydrogen phosphate	3.500
Potassium hydrogen phosphate	1.500
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 20.0 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense in 50 ml amounts into tubes or flasks or as desired. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. If desired aseptically add rehydrated contents of one vial of EC O157:H7 Selective Supplement (FD247) to 1000 ml of medium for enrichment of *Escherichia coli* O157:H7.

Principle And Interpretation

Buffered Peptone Water is a pre-enrichment medium designed to help recovery of sub-lethally damaged Salmonellae before transfer to a selective medium. This pre-enrichment medium is free from inhibitors and is well buffered and provides conditions for resuscitation of the cells that have been injured by processes of food preservation. It was noted by Edel and Kampelmacher (1) that sub-lethal injury to *Salmonella* may occur due to food preservation techniques involving heat, desiccation, high osmotic pressure, preservatives or pH changes. Buffered Peptone Water during the pre-enrichment period helps in recovery of injured cells that may be sensitive to low pH (2). This is particularly important for vegetable specimens, which have low buffering capacity. This medium can be used for testing dry poultry feed (3). In a survey involving isolation of Salmonellae from meat that had been artificially contaminated with sub-lethally injured organisms, pre-enrichment in Buffered Peptone Water at 37°C for 18 hours before selection in Tetrathionate Brilliant Green Bile Broth (M1255) showed superior results compared with direct selection method. Lactose Broth is frequently used as a pre-enrichment medium but it may be detrimental to recovery of Salmonellae (4).

The media contain proteose peptone as a source of carbon, nitrogen, vitamins and minerals. Sodium chloride maintains the osmotic balance and phosphates buffer the medium. The broth is rich in nutrients and produces high resuscitation rates for sub lethally injured bacteria and supports intense growth. The phosphate buffer system prevents bacterial damage due to changes in the pH of the medium.

Inoculate 10 grams specimen in 50 ml of these media and incubate at 35-37°C for 18 hours. Transfer 10 ml from this medium to 100 ml of Tetrathionate Broth (M032) and incubate at 43°C for 24 - 48 hours and then subculture on selective plating media.

Examine the plates for characteristic *Salmonella* colonies.

Type of specimen

Clinical samples - Stool samples for primary enrichment, Food and dairy samples

Specimen Collection and Handling

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

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (7,8,9).

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 nutritional variations some strains may show poor growth. 2. Further enrichment and isolation 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 2.0% 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 18-24 hours. (Recovery is carried out using XLD Agar, M031).

Organism	Inoculum (CFU)	Growth	Recovery
<i>Salmonella</i> Enteritidis ATCC 50-100 13076 (00030*)		good-luxuriant	≥50%
<i>Salmonella</i> Typhi ATCC 6539	50-100	good-luxuriant	≥50%
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50-100	good-luxuriant	≥50%
<i>Escherichia coli</i> 0157:H7 NCTC 12900 (00014*)	50-100	good-luxuriant [Recovery on Tryptone soya Agar(M290)]	≥50%

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Product performance is best if used within stated expiry period.

Disposal

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

Reference

1. Edel and Kampelmacher, 1973, Bull. W.H.O., 48:167.
2. Sadowski, 1977, J. Food Technol., 12:85.
3. Juven, Cox, Bailey, Thomson, Charles and Schutze, 1984, J. Food Prot., 47:299.
4. Angelotti, 1963, Academic Press, New York, N.Y.

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

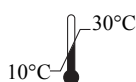
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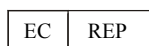
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Brucella Selective Medium Base

M822

Intended Use:

Recommended for the isolation and identification of *Brucella* species.

Composition**

Ingredients	Gms / Litre
HM infusion B from #	500.000
Tryptose	10.000
Sodium chloride	5.000
Gelatin	1.000
Dextrose (Glucose)	2.500
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef heart, infusion from

Directions

Suspend 21.75 grams in 500 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add sterile 10% v/v Sheep blood. Also add rehydrated contents of one vial of Brucella Selective Supplement (FD005). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Brucellosis is a zoonotic disease with a domestic animal reservoir. It is an occupational disease of veterinarians, microbiologists, farmers etc. The route of infections is genital, nasopharyngeal, gastrointestinal, conjunctival, respiratory and through abraded skin (6,7). Brucellosis in humans has a variable incubation period, an insidious or abrupt onset and no pathognomic symptoms or signs. Brucella Agar was designed for cultivating *Brucella* species from diagnostic specimens. With the incorporation of blood or other nutritious substances, it facilitates the cultivation of variety of fastidious anaerobic organisms (2). However, Brucella Medium is supplemented with antibiotics to prevent overgrowth of other accompanying organisms. Brucella Agar Base w/ 1.0 % Dextrose was originally developed by Jones and Morgan (5) for preparations of serum-dextrose-antibiotic medium used for the isolation and cultivation of *Brucella* species.

The medium contains HM infusion B and tryptose, which facilitates cultivation of variety of fastidious anaerobic organisms; by providing essential nutrients. Gelatin serves as a source of nutrients. Glucose serves as source of energy. Addition of antibiotics (as FD) makes the medium highly selective for *Brucella* species. Ethyl violet and circulin, which were recommended initially, are no longer used (1).

Type of specimen

Clinical samples: faeces

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4). 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. All presumptive anaerobic organisms must be identified by confirmatory test

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 On addition of 10% v/v sterile sheep blood cherry red coloured opalescent gel forms in Petri plates

Reaction

Reaction of 4.35% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural characteristics observed in presence of 10% Carbon dioxide (CO₂) atmosphere with added sterile 10% v/v sheep blood and Brucella Selective Supplement(FD005), after an incubation at 35-37°C for 24-48 hours

Organism

Growth

Brucella melitensis ATCC 4309 luxuriant

Brucella suis ATCC 4314 luxuriant

Escherichia coli ATCC 25922 (00013*) inhibited

Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*) inhibited

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. Alton G. G. and Jones L. M., 1967, Lab Technique in Brucellosis, WHO, Geneva.
2. Atlas R. M., 1997, Handbook of Microbiological Media, 2nd Edi., Parks L.C. (Ed.), CRC Press, New York.
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6. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Tenover F. C., Tenover K. C., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
7. Young E. J., 1983, Human Brucellosis, Rev. Infect. Dis., 5:821-842

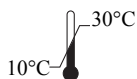
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Technical Data

Modified Semisolid Rappaport Vassiliadis Medium Base (MSRC) M14281

Intended Use

Recommended for selective enrichment and isolation of *Salmonella* from food stuffs and environmental samples from the food production area. The composition and performance criteria of this medium are as per the specifications laid down in ISO 6579-1:2017.

Composition**

Ingredients	Gms / Litre
Biopeptone #	4.600
Acicase ##	4.600
Sodium chloride	7.300
Potassium dihydrogen phosphate	1.500
Magnesium chloride, hexahydrate	40.00
Malachite green oxalate	0.040
Agar	2.700
Final pH (after sterilization)	5.10- 5.40

**Formula adjusted, standardized to suit performance parameters

Equivalent to Enzymatic digest of animal and plant tissue

Equivalent to Acid hydrolysate of casein

Directions

Suspend 39.47 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified / distilled water. Heat with stirring to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 47-50°C and aseptically add 1 vial of rehydrated content of IMRV/RV Selective Supplement (FD193). Mix well and dispense into sterile Petri plates.

Note: The motility of *Salmonella* can be drastically reduced when the agar surface becomes too dry. Hence the plates should be well dried before use. If visible moisture occurs on the lid of the plates or the surface of agar, it must be removed. While incubation, incubate the plates aerobically in an upright position for no longer than 24 hours at 42°C.

Principle And Interpretation

Semisolid Rappaport Vassiliadis Medium Base is based on the formulation described by DeSmedt et al (1) for the detection of motile *Salmonella* species from food and environmental specimens. Modified Semisolid Rappaport Vassiliadis Medium Base is recommended by ISO 6579 (2) for detection of *Salmonella* from foodstuffs and the area of food production and food handling. This medium detects more *Salmonella* positive samples than the routinely used enrichment procedures (2, 3, 4).

Bio peptone and Acicase provides the nitrogenous and carbonaceous substances, long chain amino acids, vitamins and other essential growth nutrients. The motility of other microorganisms is largely inhibited by the selective agents (magnesium chloride, malachite green and novobiocin). Sodium chloride maintains osmotic balance. Phosphate buffers the medium.

The working of medium is based on the ability of *Salmonella* species to migrate in the selective medium competing with the other motile organisms, thus producing opaque halos of growth. The motile bacteria will show a halo or zone of growth originating from inoculation spot.

Type of specimen

Food and animal feeding samples, environmental samples in the area of food production and food handling. Samples from primary production stage such as animal faeces, dust and swabs.

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. The medium is intended for the detection of motile *Salmonella* and is not appropriate for the detection of non-motile *Salmonella* strains.

Quality Control

Appearance

Light yellow to light blue homogeneous free flowing powder

Gelling

Semisolid, comparable with 0.27% Agar gel.

Colour and Clarity of prepared medium

Blue coloured clear to slightly opalescent semisolid gel forms in Petri plates.

Reaction

Reaction of 3.95% w/v aqueous solution at 25°C. pH : 5.10-5.40

pH

5.10-5.40

Cultural Response

Cultural characteristics observed after an incubation at 41.5°C for 24 hours with added IMRV/RV Selective Supplement (FD193) when one drop of culture is inoculated in the centre of the medium plate. (Motility is checked by inoculating a drop of culture in the centre of the medium plate).

Organism	Inoculum (CFU)	Growth	Motility
<i>Salmonella</i> Enteritidis ATCC50-100 13076 (00030*)		good-luxuriant	Positive reaction, grey-white turbid zone
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50-100	good-luxuriant	Positive reaction, grey-white turbid zone
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	none-poor	Negative reaction, no turbid zone
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	$\geq 10^4$	inhibited	-

Key : (*) Corresponding WDCM numbers

Storage and Shelf Life

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

Disposal

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

Reference

1. De Smedt J.M., Balderdijk R., Rappold H. and Lautenschlaeger D., 1986, J. Food Prot., 49:510.
2. International Organization for Standardization 6579-1:2017(E), Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella*.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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