PSB Broth, Modified

M941I

Intended Use:

Recommended for primary enrichment and enumeration of *Yersinia enterocolitica* from food. The composition and performance criteria are in accordance with ISO 1994, ISO/DIS 10273 :2017 and ISO 11133:2014 (E) /Amd. :2020

Composition**

ISO Specification - Peptone, sorbitol and bile salts (PSB) broth

Ingredients	g / L
Enzymatic digest of casein	5.000
Sorbitol	10.000
Sodium chloride	5.000
Disodium hydrogen phosphate	8.230
Sodium dihydrogen phosphate monohydrate	1.200
Bile salts	1.500
Final pH (at 25°C)	7.6±0.2

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**Formula adjusted, standardized to suit performance parameters

Equivalent to Enzymatic digest of casein

Directions

Suspend 30.77 gram (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense in tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Yersinia enterocolitica are ubiquitous, being isolated frequently from soil, water, animals, and a variety of foods. They comprise a biochemically heterogeneous group that can grow at refrigeration temperatures. PSB Broth Base is recommended by APHA (1) for enrichment of *Yersinia* species. PSB Broth, Modified is a modification of PSB Broth (M941I) recommended by ISO Committee (2,3). Supplementation of phosphate buffer with sorbitol and bile salts appears to be better for cold enrichment (4).

Peptone provides essential growth nutrients. Sodium chloride maintains osmotic equilibrium while phosphates buffer the medium well. Sorbitol is the energy source. Bile salts are added to make the medium selective for *Yersinia* species by inhibiting accompanying gram-positive bacteria.

Type of specimen

Food and Animal feeding stuff

Specimen Collection and Handling

Processing (2,3)

Enrichment : For the first initial suspension place the sample (x) in known volume of the PSB broth (M941I), to give a dilution of 1/10 dilution (by mass/volume or volume/volume). Homogenize the suspension using a peristaltic blender for 2 min. Incubate at 22°C to 25°C for 2 to 3 days with or 5 days without agitation.

For the second inition suspension in the same way with the ITC broth (M1220) so as to obtain a test portion/enrichment medium dilution of 1/100 (mass/volume or volume/volume). Incubate at 25°C for 48 hours.

Isolation : 1. Inoculate the culture obtain from PSB culture on the surface of CIN agar plate and incubate at 30°C for 24 to 48 hours.

2.Alkaline treatment : Using sterile pipette transfer 0.5ml of the PSB culture into 4.5 ml of KOH solution and mix for 20 seconds only. Immediately inoculate on CIN agar plate. Incubate at 30°C for 24 to 48 hours.

3.Using ITC culture inoculate the surface of SSDC agar plate (M1703). Incubate at 30°C for 24 to 48 hours.

4.Purification : Streak the selected colonies on the surface of Nutrient Agar (M561A). Incubate at 30°C for 24 hours.

5.Confirmation : Streak the slant of the agar and incubate at 30°C for 24 hours. A black halo around the colonies obtain positive reaction.

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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured clear to very slightly hazy solution

Reaction

Reaction of 3.08% w/v aqueous solution at 25°C. pH : 7.6±0.2

pH

7.40-7.80

Cultural Response

Productivity : Cultural characteristics observed after an incubation at 25 ± 1°C for 3-5 days. Recovery is carried out on SSDC Agar (M1703)

Selectivity : Cultural characteristics observed after an incubation at 25 ± 1°C for 3-5 days Recovery is carried out on SSDC Agar (M1703)

Organism	Inoculum (CFU)	Growth	Characteristic reaction on SSDC Agar (M1703)
Productivity			
Yersinia enterocolitica subsp. *enterocolitica* ATCC 9610 (00038*) +*Escherichia coli* ATCC 8739 (00012*) +*Pseudomonas aeruginosa* ATCC 27853 (00025*)	50-100 >=10^4 >=10^4	>10 colonies	colourless round colonies, approximately 1mm diameter. A finely granulated centre can be seen under 10X magnification
Yersinia enterocolitica subsp. *enterocolitica* ATCC 9610 (00038*) +*Escherichia coli* ATCC 25922 (00013*) +*Pseudomonas aeruginosa* ATCC 27853 (00025*)	50-100 >=10^4 >=10^4	>10 colonies	colourless round colonies, approximately 1mm diameter. A finely granulated centre can be seen under 10X magnification
Yersinia enterocolitica biotype 1 serotype 0:8 ATCC 23715 (00160*) +*Escherichia coli* ATCC 8739 (00012*) +*Pseudomonas aeruginosa* ATCC 27853 (00025*)	50-100 >=10^4 >=10^4	>10 colonies	colourless round colonies, approximately 1mm diameter. A finely granulated centre can be seen under 10X magnification

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+*Escherichia coli* ATCC 25922 (00013*)	>=10^4		
+*Pseudomonas aeruginosa* ATCC 27853 (00025*)	>=10^4		
Selectivity			
Pseudomonas aeruginosa ATCC 27853 (00025*)	>=10^4	inhibited	
Proteus mirabilis ATCC 29906 (00023*)	>=10^4	inhibited	

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. 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.

2. International Organization for Standardization(ISO), 1994, Draft, ISO/DIS 10273:2017

3. Microbiology of food,animal feeding stuffs and water- Preparation, production,storage and performance culture media, EN ISO 11133:2014 (E) /Amd. :2020 .

4. Mehlman I.J., Aulisio C.C.G. and Sander A.C., 1978, J. Assoc. Off. Anal. Chem. 61:761.

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.

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