Page's Saline

M1988

Intended Use:

Used as a rinsing solution of membranes in water filtration for *Legionella* detection.

Composition**

Ingredients	Gms / Litre
Sodium chloride (NaCl)	0.120
Magnesium sulphate ($MgSO_4.7H_2O$)	0.004
Calcium chloride ($CaCl_2.2H_2O$)	0.004
Disodium hydrogen phosphate (Na_2HPO_4)	0.142
Potassium dihydrogen phosphate (KH_2PO_4)	0.136

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 0.403 grams(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 and sterilize by autoclaving at 15 lbs pressure (121 ± 3°C) for 15 ± 1 minute.

Principle And Interpretation

Page's saline is recommended for concentration of bacteria, including *Legionella* organisms by membrane filtration for detection and enumeration of *Legionella* (5).This medium is also recommended by ATCC (2). When 1.5% Agar is added to Page's saline it is used for the isolation of *Naegleria* and Acanthamoeba from tissues and soil samples (4).
For sample processing add the membrane used for filtration of water in a sterile flask containing 5-25ml of Page's saline then shake vigorously for atleast 2 minutes. The concentrated solution of organisms is plated directly on Buffered Charcoal Yeast Extract Agar (M813). Alternatively the sample is subjected to heat treatment or acid treatment to reduce non *Legionella* bacteria. As the growth of *Legionella* may be inhibited by overgrowth of other bacterial colonies on the membrane, the method is only suitable for waters containing low bacterial counts.

Type of specimen

Natural and artificial aquatic enviorments, soils and composts.

Specimen Collection and Handling

For natural and artificial aquatic enviorments, soils and composts samples follow appropriate techniques for handling specimens as per established guidelines (5).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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

Limitations :

1. As the growth of *Legionella* may be inhibited by overgrowth of other bacterial colonies on the membrane, the method is only suitable for waters containing low bacterial counts.
2. Further biochemical test 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

White to cream homogeneous free flowing powder

Colour and Clarity of prepared medium

colourless clear solution without any precipitate

Cultural Response

Satisfactory results are obtained when used as a diluent during bacteriological examination of water.

Storage and Shelf Life

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

Disposal

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

Reference

1. ATCC Medium 1323 Page's Amoeba Saline,Page, f.c.1988.A new key to freshwater and soil gymnamoebae. Freshwater Biological Association, Ambleside.
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. Practicals & Viva in Medical Parasiotology by Sehgal-2003.
5. Water quality-Detection and enumeration of *Legionella* - Part 2: Direct membrane filtration method for waters with low bacterial counts. ISO 11731-2:2004(E).

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