Diluting Fluid D

M1686

Intended Use:

Recommended as a diluent for testing of pharmaceuticals in accordance with USP.

Composition**

Ingredients	Gms / Litre
Peptone	1.000
Polysorbate 80 (Tween 80)	1.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 2.0 grams in 1000 ml distilled/purified water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes i.e. validated cycle.

Principle And Interpretation

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

Type of specimen

Pharmaceutical sample

Specimen Collection and Handling

For pharmaceutical sample samples follow appropriate techniques for handling specimens as per established guidelines (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. Further serological or biochemical testing is required for complete identification.
2. Use of this method is appropriate only when test samples are clear.

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 coloured homogeneous free flowing powder

Colour and Clarity of prepared medium

Light amber coloured clear solution without any precipitate

Reaction

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

pH

6.90-7.30

Cultural Response

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

Organism	Inoculum (CFU)	Growth
Candida albicans ATCC 10231 (00054*)	50-100	good
Escherichia coli ATCC 25922 (00013*)	50-100	good
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	good
Escherichia coli ATCC 8739 (00012*)	50-100	good
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	50-100	good

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. The United States Pharmacopoeia-National Formulatory (USP-NF), 2022.
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 Clinical Microbiology, 11th Edition. Vol. 1.

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