Diluting Fluid A

M1415

Intended Use:

Recommended for sterility testing of pharmaceuticals in accordance with USP. The composition and performance criteria of this medium is also as per the specifications laid down in ISO 8199:-1998 (E).

Composition**

ISO specification - Peptone Diluent	
Ingredients	**g/ L**
Peptone	1.000
Final pH (after sterilization)	7.1±0.1

Diluting Fluid A - M1415	
Ingredients	**g/ L**
Peptone	1.000
Final pH (after sterilization)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 1.0 gram in 1000 ml purified / distilled 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.

Principle And Interpretation

Diluting Fluid A 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). The composition is also as per ISO Committee (2). 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 and ISO. The resultant growth is compared with positive control to determine presence of fungistasis or bacteriostasis activity in test specimen.

Type of specimen

Pharmaceutical sample, Water samples

Specimen Collection and Handling

For pharmaceutical sample samples follow appropriate techniques for handling specimens as per established guidelines (1).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (2,3).

Preparation of test sample of water - ISO 5667-2, ISO 5667-3: The sample should be mixed thoroughly by vigorous agitation to achieve uniform distribution of microorganisms and, depending on the nature of the water and the bacterial content anticipated, any dilutions necessary made at this stage. Prepare tenfold dilutions of water samples as per ISO 6887. 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. Use of this method is appropriate only when test samples are clear.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
3. Further recovery on solid media is required for identification of 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

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Colourless to light yellow coloured clear solution

Reaction

Reaction of 0.1% 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 20-25°C for 45 mins to 1hour. Recovery is considered on TSA

Organism	Inoculum (CFU)	Growth
Escherichia coli ATCC 25922 (00013)*	50-100	±30% of the original count
Escherichia coli ATCC 8739 (00012*)	50-100	±30% of the original count
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	±30% of the original count

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 (4,5).

Reference

1.The United States Pharmacopoeia / National Formulary, USP 2020, Asian Edition, US Pharmacopeial convention Inc., Rockville, MD.

2.Water quality - General guide to the enumeration of microorganisms by culture, International organization for standardization (ISO): 8199:1988 (E).

3.Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

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.

Revision: 05/2024