Colonisation Medium w/ Dulbecco's Phosphate Buffer

M1239

Intended Use:

Recommended for the preparation of suspension of enterotoxigenic *Escherichia coli* used for HeLa cell lines.

Composition**

Ingredients	Gms / Litre
BHI Powder	0.700
Bile salts mixture	0.140
Mannose	1.400
Potassium dihydrogen phosphate	0.200
Potassium chloride	0.200
Sodium chloride	8.000
Dipotassium hydrogen phosphate	1.150
Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 11.80 grams in 1000 ml purified / distilled water. Cool to 45-50°C. Mix well and sterilize by filtration through a 0.45 mm membrane. DO NOT AUTOCLAVE OR HEAT the medium.

Principle And Interpretation

This medium is formulated as recommended by APHA (3) for preparing suspension of enterotoxigenic *Escherichia coli* used for colonization test in HeLa cell lines.

Virulence prerequisites for enterotoxigenic strains of *E. coli* include the ability to attach to the jejunal lining to proliferate in-situ, and to elaborate one or more toxins. Host specificity is manifested by possession of unique colonization factors like antigens and lectins. Because of commercial non-availability of these factors several types of mammalian cells have been proposed to show colonization, and HeLa is one of them. It was found that many *E.coli* strains attach to HeLa cells with two different attachment patterns i.e. diffused adherence and localized adherence. By adding mannose to the culture medium it was possible to distinguish between mannose-sensitive and mannose-resistant adherence. Mannose-resistant adherence was not related to colonization factor antigens.

Colonization Medium w/ Dulbeccos Phosphate Buffer is used in the HeLa adherence assay.

BHI Powder in the medium provides necessary nutrients like amino acids, growth factors and trace ingredients for the growth of enterotoxigenic *E. coli*. Mannose acts as an energy sources as well as differentiates mannose-sensitive and mannose-resistant adherence. Phosphates buffer the medium. Bile salts mixture inhibits the contaminating flora while sodium chloride maintains the osmotic equilibrium.

Type of specimen

Isolated Microorganism

Specimen Collection and Handling:

For isolated Microorganism samples follow appropriate techniques for handling specimens as per established guidelines (3). 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 :

NA

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light to medium amber coloured, clear solution without any precipitate

Reaction

Reaction of 1.18% w/v aqueous solution at 25°C. pH : 7.5±0.2

pH

7.30-7.70

Cultural Response

Satisfactory results are obtained when used for preparation of suspension of enterotoxigenic *Escherichia coli* used for HeLa cells test for colonisation .

Storage and Shelf Life

Store dehydrated 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. 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.

3. Vanderzant C. and Splittstoesser D. F., (Eds.), 1992, Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed., APHA, Washington, D.C.

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