Technical Data

Diphtheria Virulence Agar Base

M882

Intended Use:

Recommended for determining toxigenicity of *Corynebacterium diphtheriae.*

Composition**

Ingredients	Gms / Litre
Proteose peptone	20.000
Sodium chloride	2.500
Agar	15.000
Final pH (at 25°C)	7.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37.5 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 55-60°C. Aseptically add 2 ml sterile KL Virulence Enrichment (FD072) and 0.5 ml sterile 1% Potassium Tellurite (FD052) to a 100 mm Petri plate and quickly add 10 ml of sterile Diphtheria Virulence Agar Base. Before the medium solidifies, place a filter paper strip saturated with potent Diphtheria antitoxin across the diameter of the plate. Allow the strip to sink to the bottom of the plate. Inoculate the plate with heavy inoculum across the strip.

Principle And Interpretation

Corynebacterium diptheriae is a principle human pathogen and owes its pathogenicity to the production of a potent exotoxin active on a variety of tissue including heart muscles and peripheral nerves (1). Toxin diffusing from a streak culture of suspected *C. diphtheriae* is demonstrated by the formation of a white line of precipitate where it meets with diphtheria antitoxin diffusing from a strip of filter paper embedded in the agar. In vitro toxigenicity (virulence) of *C. diphtheriae* was first described by Elek (2). Eleks technique was further improved by King, Frobisher and Parsons (3) by the use of a standardized medium. This medium gave results comparable with animal inoculation test. Also it was found that proteose peptone supported toxin production in addition to maintaining the consistency of results. Hermann et al (4) developed a non-serum based enrichment to overcome the irregularities encountered during the usage of horse, sheep or rabbit serum based enrichments. These non-serum based enrichments consist of casein acid hydrolysate, tween 80 and glycerol (5).

Upon incubation of the inoculated plate, a line of precipitin is observed for toxigenic strains.

Proteose peptone provides the carbon and nitrogen sources required for good growth of a wide variety of organisms and also for toxin production. Sodium chloride maintains the osmotic balance of the medium. Agar is incorporated as the solidifying agent. Potassium tellurite inhibits most gram-negative bacteria except *Corynebacterium* species, *Streptococcus mitis, Streptococcus salivarius* and Enterococci. *Staphylococcus epidermidis may exhibit growth*. False positive results may also be encountered. Therefore, a positive control has to always be run in parallel (6). *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis* may also produce line of precipitation (7).

Type of specimen

Clinical samples - Throat swab

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

False positive results may also be encountered. Hence, a positive control has to always be run in parallel (6)

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

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Medium amber coloured, slightly opalescent gel forms in Petri plates

Reaction
Reaction of 3.75% w/v aqueous solution at 25°C. pH : 7.8±0.2

pH
7.60-8.00

Cultural Response
Cultural characteristics observed with added KL Virulence Enrichment (FD072) and 0.5 ml of 1% Potassium tellurite solution (FD052) after an incubation at 35-37°C for 24-72 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Line of precipitin
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)	>=10^4	inhibited	0%	
Corynebacterium diphtheriae type gravis	50-100	luxuriant	>=50%	positive
Corynebacterium diphtheriae type intermedius	50-100	luxuriant	>=50%	positive
Corynebactrium diphtheriae type mitis	50-100	luxuriant	>=50%	positive
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	none-poor	<=10%	

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container 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. Use before expiry date on the label.
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 (4,5).

Reference

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3. King E. O., Frobisher M. and Parsons E. I., 1949, Am. J. Public Heal th, 39:1314.
4. Hermann G. J., Moore M. S., and Parsons E. I., 1958, Am. J. Clin. Pathol., 29:181.
5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. I, Williams and Wilkins, Baltimore.
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7. Branson, 1972, Methods in Clinical Bacteriology, Charles C. Thomas,Springfield, III

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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.

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