



Dehydrated Culture Media
Bases / Media Supplements

Technical Information

Diphtheria Virulence Agar Base

Product Code: DM 1882

Application: - Diphtheria Virulence Agar Base with supplements is recommended for testing 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

Principle & Interpretation

Diphtheria Virulence Agar Base 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 supplies the carbon and nitrogen sources required for good growth of a wide variety of organisms and also for toxin production. Sodium chloride helps to maintain the osmotic balance of the medium. Agar is incorporated as the solidifying agent. Potassium tellurite enhanced 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).

Methodology

Suspend 37.5 grams of dehydrated powder media in 1000 ml distilled water. Mix thoroughly & heat to boil 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 (MS2072) and 0.5 ml sterile 1% Potassium Tellurite (MS2052) 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.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder



Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity

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 Range

7.60-8.00

Cultural Response

DM1882: Cultural characteristics observed with added KL Virulence Enrichment (MS2072) and 0.5 ml of 1% Potassium tellurite solution (MS2052) after an incubation at 35-37°C for 24-72 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Line of precipitin
<i>Bacillus subtilis</i> ATCC 6633	≥10 ³	inhibited	0%	-
<i>Corynebacterium diphtheriae</i> type <i>gravis</i>	50-100	luxuriant	≥50%	positive
<i>Corynebacterium diphtheriae</i> type <i>intermedius</i>	50-100	luxuriant	≥50%	positive
<i>Corynebacterium diphtheriae</i> type <i>mitis</i>	50-100	luxuriant	≥50%	positive
<i>Staphylococcus epidermidis</i> ATCC 12228	50-100	none-poor	≤10%	-

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

1. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone
2. Elek S. D., 1948, Br. Med. J., 1:493.
3. King E. O., Frobisher M. and Parsons E. I., 1949, Am. J. Public Health, 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.
6. Murray P. R., Baron E. J., Jorgensen J. H., Tenover F. C., Tenover F. C., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
7. Branson, 1972, Methods in Clinical Bacteriology, Charles C. Thomas, Springfield, Ill

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