Diphtheria Virulence Agar Base

M882

Diphtheria Virulence Agar Base with supplements is used 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

Directions

Suspend 37.5 grams in 1000 ml 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).

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

M882: 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 ATCC 6633	>=10^3	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	50-100	none-poor	<=10%	

Storage and Shelf Life

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

Reference

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5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. I, Williams and Wilkins, Baltimore.
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