PRODUCT SPECIFICATION SHEET

Diphtheria Virulence Agar Base (DM1067)

Intended Use
Diphtheria Virulence Agar Base (DM1067) is recommended for toxigenicity determination of *Corynebacterium diphtheriae*.

Product Summary and Explanation
Corynebacterium diptheriae is a rod-shaped, non-motile, non capsulated, non-sporing and gram positive bacteria. It is also known as the Klebs-Löffler bacillus, because it was discovered in 1884 by German bacteriologists Edwin Klebs (1834–1912) and Friedrich Löffler (1852 – 1915). *C. diphtheriae* 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] Using a standardized medium, Eleks technique was further improved by King, Frobisher and Parsons.[3] 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.

Principles of the Procedure
Diphtheria Virulence Agar Base contains 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. Potassium tellurite inhibits most gram-negative bacteria except *Corynebacterium* species, *Streptococcus mitis, Streptococcus salivarius* and *Enterococci*. *Staphylococcus epidermidis may exhibit growth*.

Formula / Liter

Ingredients	Gms / Liter
Proteose peptone	20.00
Sodium chloride	2.50
Agar	15.00
Final pH: 7.8 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 37.5 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely, with frequent agitation to avoid charring.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 55-60°C.
5. Aseptically add 2 ml sterile KL Virulence Enrichment (MS145) and 0.5 ml sterile 1% Potassium Tellurite (MS024) to a 100 mm Petri plate and quickly add 10 ml of sterile Diphtheria Virulence Agar Base.
6. 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.
7. Inoculate the plate with heavy inoculum across the strip.

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Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Medium amber coloured, slightly opalescent gel forms in Petri plates
Reaction of 3.75% Solution	pH : 7.8 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed with added KL Virulence Enrichment (MS145) and 0.5 ml of 1% Potassium tellurite solution (MS024) after an incubation at 35-37°C for 24-72 hours.

Sr. No.	Organisms		Results to be achieved		
		Inoculum (CFU)	Growth	Recovery	Line of Precipitin
1.	*Bacillus subtilis ATCC 6633*	>=10^3	inhibited	0%	--
2.	*Corynebacterium diphtheriae type gravis*	50 - 100	good- luxuriant	>=50%	positive
3.	*Corynebacterium diphtheriae type intermedius*	50 - 100	good- luxuriant	>=50%	positive
4.	*Corynebactrium diphtheria type mitis*	50 - 100	good- luxuriant	>=50%	positive
5.	*Staphylococcus epidermidis ATCC 12228*	50 - 100	none-poor	<=10%	--

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure
Refer to appropriate references for standard test procedures.

Results
Refer appropriate references and procedures for interpretation of results.

Storage
Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration
Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure
1. A positive control has to always be run in parallel as false positive results may also be encountered.
2. *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis* may also produce line of precipitation.
3. Consult appropriate texts for detailed information and recommended procedures.

Packaging
Product Name : Diphtheria Virulence Agar Base
Product Code : DM1067
Available Pack sizes : 500gm

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References

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 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.

Further Information

For further information please contact your local MICROMASTER Representative.

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