CM 20601 – DIPHTHERIA VIRULENCE AGAR BASE

INTENDED USE

For determination of toxigenicity of Corynebacterium diphtheriae.

PRODUCT SUMMARYANDEXPLANATION

Corynebacteriumdiphtheriae is a principle human pathogen and owes its pathogenicity to the production of a potent exotoxin activeonavariety of tissue including heart muscles and peripheral nerves. 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 diffusingfroma strip of filter paper embedded in the agar. In vitro toxigenicity (virulence) of C. diphtheriae was first describedbyElek.Eleks technique was further improved by King, Frobisher and Parsons by the use of a standardized medium. Thismediumgave results comparable with animal inoculation test. Also it was found that proteose peptone supported toxinproduction in addition to maintaining the consistency of results. Hermann et al developed a non-serum based enrichmenttoovercome the irregularities encountered during the usage of horse, sheep or rabbit serum based enrichments.Thesenon-sera based enrichments consist of Acicase, tween 80 and glycerol. Upon incubation of the inoculated plate,alineof precipitin is observed for toxigenic strains.

COMPOSITION

Ingredients	Gms / Ltr
Proteose peptone	20.000
Sodium chloride	2.500
Agar	15.000

PRINCIPLE

The medium consists of proteose peptone which 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. Corynebacterium ulcerans and Corynebacterium pseudotuberculosis may also produce line of precipitation.

INSTRUCTION FOR USE

Dissolve 37.5 gramsin 1000 ml purified/distilled water.
Heat to boiling to dissolve the medium completely.
Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes. Cool to 55-60°C.
Aseptically add 2 ml sterile KL Virulence Enrichment and 0.5 ml sterile 1% Potassium Tellurite 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 SPECIFICATIONS

Appearance of Powder	: Cream to yellow homogeneous free flowing powder.
Appearance of prepared medium	: Medium amber coloured, slightly opalescent gel forms in Petri plates.
pH (at 25°C)	: 7.8 ± 0.2

INTERPRETATION

Cultural characteristics observed with added KL Virulence Enrichment and 0.5 ml of 1% Potassium tellurite solution after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Line of precipitin	Incubation Temperature	Incubation Period
Bacillus subtilis subsp. spizizenii	6633	>=10^4	Inhibited	0%	-	35-37°C	24-72 Hours
Corynebacterium diphtheria	8028	50-100	Luxuriant	>=70%	Positive	35-37°C	24-72 Hours
Corynebacterium diphtheriae	8032	50-100	Luxuriant	>=70%	Positive	35-37°C	24-72 Hours
Staphylococcus aureus	25923	>=10^4	Inhibited	0%	-	35-37°C	24-72 Hours
Staphylococcus epidermidis	12228	50-100	None-poor	0-10%	-	35-37°C	24-72 Hours

PACKAGING:

Inpacksizeof500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

Afteruse,prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

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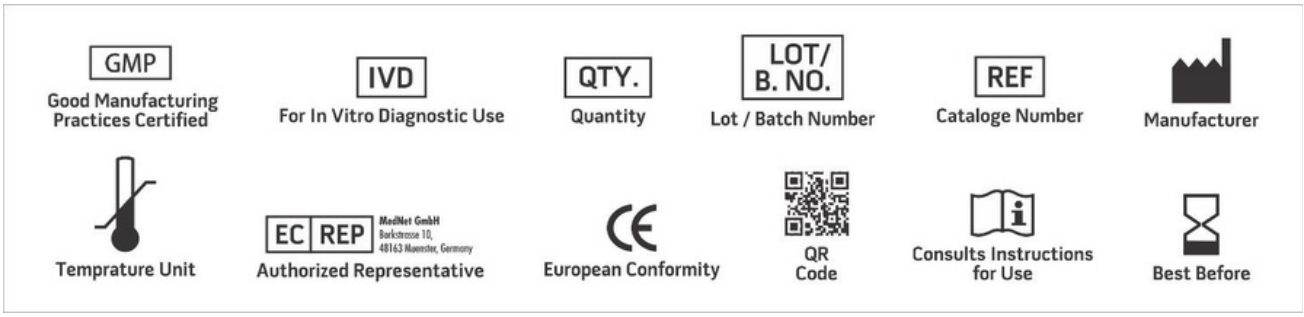

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

*For LabUse Only