

# TMP 020 – CHOCOLATE AGAR PLATE

#### **INTENDED USE**

For isolation and cultivation of fastidious microorganisms like Neisseria gonorrhoeae.

## **PRODUCT SUMMARY AND EXPLANATION**

Chocolate agar or chocolate blood agar (CBA), is a nonselective, enriched growth medium used for isolation of pathogenic bacteria. It is a variant of the blood agar plate, containing red blood cells that have been lysed by slowly heating to 80°C. Chocolate agar is used for growing fastidious respiratory bacteria, such as *Haemophilus influenzae* and *Neisseria meningitidis*. In addition, some of these bacteria, most notably *H. influenzae*, need growth factors such as nicotinamide adenine dinucleotide (factor V or NAD) and hemin (factor X), which are inside red blood cells; thus, a prerequisite to growth for these bacteria is the presence of red blood cell lysates. The heat also inactivates enzymes which could otherwise degrade NAD.

Chocolate Agar Base, with the addition of supplements, gives excellent growth of the gonococcus without overgrowth by contaminating organisms. Chocolate Agar Base with addition of supplement not only supports the growth of the gonococcus in pure culture but also permits its development from the mixed flora encountered in chronic gonococcal infections. Carpenter reported that this medium and Haemoglobin Powder (TS 021) is useful for cultural detection of the gonococcus.

## COMPOSITION

Ingredients	Gms / Ltr		
Proteose peptone	20.000		
Disodium phosphate	5.000		
Haemoglobin	2.000		
Dextrose	0.500		
Agar	15.000		
Sodium chloride	5.000		

#### PRINCIPLE

Medium contains proteose peptone as nitrogen source, required for the growth of wide variety of organisms. Dextrose acts a carbon energy source. Disodium phosphate buffers the medium whereas sodium chloride maintains the osmotic equilibrium. Agar is the solidifying agent. This medium is supplemented with cofactor, which provides NAD to facilitate the growth of *Haemophilus influenzae*, *Neisseria gonorrhoeae* and *Neisseria meningitidis*. Heated sheep blood is added to give the medium its "chocolate" appearance. This medium is prepared, stored and dispensed under oxygen-free conditions to prevent the formation of oxidized products prior to use.

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#### **INSTRUCTION FOR USE**

Either streak, inoculate or surface spread the test inoculum aseptically on the plate.

#### **QUALITY CONTROL SPECIFICATIONS**

Appearance	:	Chocolate brown coloured medium
Quantity of Medium	:	25ml of medium in 90mm plates.
pH (at 25°C)	:	7.3±0.2
Sterility Check	:	Passes release criteria







### **INTERPRETATION**

Cultural characteristics observed after an incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Incubation Temperature	Incubation Period
Neisseria gonorrhoeae	19424	50-100	luxuriant	>=70%	35-37°C	24-48 Hours
Neisseria meningitidis	13090	50-100	luxuriant	>=70%	35-37°C	24-48 Hours
Streptococcus pneumoniae	6303	50-100	luxuriant	>=70%	35-37°C	24-48 Hours
Streptococcus pyogenes	19615	50-100	luxuriant	>=70%	35-37°C	24-48 Hours
Haemophilus influenzae	19418	50-100	luxuriant	>=70%	35-37°C	24-48 Hours

#### PACKAGING:

Doubled layered packing containing 5 No. of plates with one silica gel desiccant bag packed inside it.

### **STORAGE**

On receipt, store the plates at 2-8 °C. Avoid freezing and overheating. Do not open until ready to use. Prepared plates stored in their original sleeve wrapping until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

#### DISPOSAL

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

#### REFERENCES

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\*For Lab Use Only

Revision: 21<sup>st</sup> March. 2022

