

HiCombi™ Blood -Chocolate Agar Plate

HB008

Intended Use

Recommended for isolation of *Neisseria* and other fastidious microorganisms.

Composition**

Blood Agar Base

Ingredients	g / L
HM peptone B#	10.000
Tryptose	10.000
Sodium chloride	5.000
Agar	15.000
Blood	50ml
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef Heart peptone

Chocolate Agar

Ingredients	g / L
Proteose peptone	20.000
Dextrose (Glucose)	0.500
Sodium chloride	5.000
Disodium hydrogen phosphate	5.000
Agar	15.000
FO Growth Supplement (FD022)	495 ml
Yeast Autolysate Supplement (FD027)/	1 vial
Vitamins Growth Supplement (FD025)	
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Streak the test inoculum (50-100 CFU) aseptically.

Principle And Interpretation

Blood Agar Base

Blood Agar Base is a highly nutritious medium generally used as a basal medium for preparing blood agar by supplementation with blood. It can also be used as general-purpose media without the addition of blood. Blood Agar Base media can be used with added phenolphthalein phosphate (1) for the detection of phosphate producing Staphylococci, with added salt and agar for assessment of surface contamination on equipment and pig carcass (2) and to determine salinity range of marine *Flavobacteria* (3). It can also be used for preparation of *Salmonella* Typhi antigens (4). Blood Agar Base is recommended by APHA (5) and Standard Methods (6,7) for testing of food samples.

HM peptone B and tryptose provides carbon, nitrogen, amino acids and vitamins. Sodium chloride helps in maintaining the osmotic equilibrium of the medium. Addition of blood makes the medium more nutritious by providing additional growth factors required by fastidious organisms. It also helps in visualizing the haemolytic reactions. However, haemolytic reactions depend on the animal blood used. Sheep blood gives best results for Group A Streptococci (8). But sheep blood fails to support growth of *Haemophilus haemolyticus* since sheep blood is deficient in pyridine nucleotides. However when horse blood is used *H. haemolyticus* colonies produce haemolysis and mimic *Streptococcus pyogenes* (9).

Chocolate Agar Base

Neisseria gonorrhoeae is a gram-negative bacteria and the causative agent of gonorrhoea, however it is also occasionally found in the throat. The cultivation medium for gonococci should ideally be a rich nutrients base with blood, either partially lysed or completely lysed. The diagnosis and control of gonorrhoea have been greatly facilitated by improved laboratory methods for detecting, isolating and studying *N. gonorrhoeae*.

Chocolate Agar Base, with the addition of supplements, gives excellent growth of the gonococcus without overgrowth by contaminating organisms. G.C. Agar (M434) can also be used in place of Chocolate Agar Base, which gives slightly better results than Chocolate Agar (10). The diagnosis and control of gonorrhoea have been greatly facilitated by improved laboratory methods for detecting, isolating and studying *N. gonorrhoea*. Interest in the cultural procedure for the diagnosis of gonococcal infection was stimulated by Ruys and Jens, McLeod and co-workers (11), Thompson (12), Leahy and Carpenter (13), Carpenter, Leahy and Wilson (14) and Carpenter (15), who clearly demonstrated the superiority of this method over the microscopic technique. 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 (16) reported that this medium and Haemoglobin (FD022) is useful for cultural detection of the *Gonococcus*.

Type of specimen

Clinical samples - Vaginal samples and other pathological material; food samples.

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (17,18). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

Blood Agar Base

1. Sheep blood is recommended to detect haemolysis. This medium does not support the growth of *H.haemolyticus*
2. Haemolytic pattern varies with the source of blood used.

Chocolate Agar Base

1. Further biochemical tests must be carried out for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Sterile Blood and Chocolate Agar in 90 mm disposable biplates with smooth surface and absence of black particles/cracks/bubbles

Colour of Blood Agar Base

Red coloured medium

Colour of Chocolate Agar Base

Chocolate Brown medium

Quantity of medium

10ml of each medium in biplate

pH of Blood Agar Base

7.10- 7.50

pH of Chocolate Agar Base

7.10- 7.50

Sterility Check

Passes release criteria

Cultural response

Cultural characteristics observed after incubation at 35-37°C for 18-48 hours.

