TM 071 – COLUMBIA BLOOD AGAR BASE

INTENDED USE
For preparation of various selective & identification media & isolation of organisms from clinical specimens.

PRODUCT SUMMARY AND EXPLANATION
Columbia Blood Agar Base was devised by Ellner et al. This medium promotes typical colonial morphology; better pigment production and more sharply defined haemolytic reactions. Fildes found that Nutrient Agar supplemented with a digest of sheep blood supplied both of these factors and the medium would support the growth of *H. influenzae*. Sheep blood permits the detection of haemolysis and also provides heme (X factor) which is required for the growth of many bacteria. However, it is devoid of V factor (Nicotinamide adenine dinucleotide) and hence *Haemophilus influenzae* which needs both the X and V factors, will not grow on this medium. The inclusion of bacitracin makes the enriched Columbia Agar Medium selective for the isolation of Haemophilus species from clinical specimens, especially from upper respiratory tract. Columbia Agar Base is used as the base for the media containing blood and for selective media formulations in which different combinations of antimicrobial agents are used as additives.
Columbia Agar Base with added sterile serum provides an efficient medium for *Corynebacterium diphtheriae* virulence test medium. After following the established technique for *C. diphtheriae*, lines of toxin-antitoxin precipitation are clearly visible in 48 hours. Many pathogens require carbon dioxide; therefore, plates may be incubated in an atmosphere containing approximately 3-10% CO_2.
Precaution: *Brucella* cultures are highly infective and must be handled carefully; incubate in 5-10% CO_2. Campylobacter species are best grown at 42°C in a micro aerophillic atmosphere. Plates with Gardenerella supplements plates should be incubated at 35°C for 48 hours containing 7% CO_2.

COMPOSITION

Ingredients	Gms / Ltr
Peptone, special	23.000
Corn starch	1.000
Sodium chloride	5.000
Agar	15.000

PRINCIPLE
This medium contains special peptone which supports rapid and luxuriant growth of fastidious and non-fastidious organisms. Corn starch serves as an energy source and also neutralizes toxic metabolites.

INSTRUCTION FOR USE
- Dissolve 44.0 grams of in 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 45-50°C before adding heat sensitive compounds.
- For Blood Agar: Add 5% v/v sterile defibrinated sheep blood to sterile cool base.
- For Chocolate Agar: Add 10% v/v sterile defibrinated sheep blood to sterile cool base. Heat to 80°C for 10 minutes with constant agitation. The medium can be made selective by adding different antimicrobials to sterile base.
- For *Brucella* species: Add rehydrated contents of 1 vial of Brucella Selective Supplement to 500 ml sterile molten base.
- For *Campylobacter* species: Add rehydrated contents of 1 vial of Campylobacter Supplement- I (Blaser-Wang) or Campylobacter Supplement- II, (Butzler) or Campylobacter Supplement- III (Skirrow) or Campylobacter Selective

Supplement or Campylobacter Supplement- VI (Butzler) to 500 ml sterile molten base along with rehydrated contents of 1 vial of Campylobacter Growth Supplement and 5-7% v/v horse or sheep blood.

- For *Gardnerella* species: Add rehydrated contents of 1 vial of G.Vaginalis Selective Supplement to 500 ml sterile molten base.
- For *Cocci*: Add rehydrated contents of 1 vial of Staph-Strepto Supplement or Strepto Supplement or Streptococcus Selective Supplement to 500 ml sterile molten base.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder.

Appearance of prepared medium : Basal medium: Light amber coloured clear to slightly opalescent gel. After addition of 5%w/v sterile defibrinated blood : Cherry red coloured opaque gel forms in Petri plates.

pH (at 25°C) : 7.3±0.2

INTERPRETATION

Cultural characteristics observed after incubation with added 5% w/v sterile defibrinated blood.

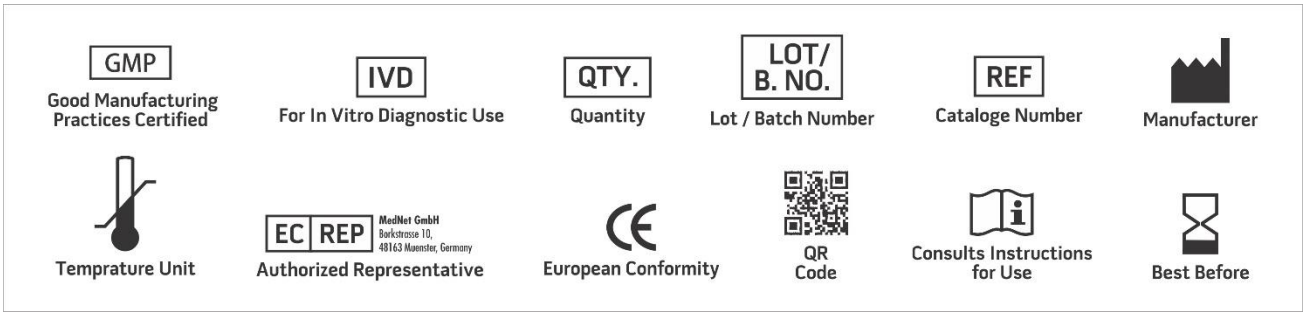
Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Haemolysis	Incubation Temperature	Incubation Period
Neisseria meningitidis	13090	50-100	Luxuriant	>=70%	None	35-37°C	24-48 Hours
Staphylococcus aureus subsp. aureus	25923	50-100	Luxuriant	>=70%	Beta/gamma	35-37°C	24-48 Hours
Staphylococcus epidermidis	12228	50-100	Luxuriant	>=70%	Gamma	35-37°C	24-48 Hours
Staphylococcus aureus subsp. aureus	6538	50-100	Luxuriant	>=70%	Beta/gamma	35-37°C	24-48 Hours
Streptococcus pneumoniae	6303	50-100	Luxuriant	>=70%	Alpha	35-37°C	24-48 Hours
Streptococcus pyogenes	19615	50-100	Luxuriant	>=70%	Beta	35-37°C	24-48 Hours
Clostridium sporogenes	19404	50-100	Luxuriant	>=70%	-	35-37°C	24-48 Hours
Clostridium sporogenes	11437	50-100	Good-luxuriant	>=50%	-	35-37°C	24-48 Hours
Clostridium perfringens	13124	50-100	Luxuriant	>=70%	-	35-37°C	24-48 Hours
Clostridium perfringens	12934	50-100	Luxuriant	>=70%	-	35-37°C	24-48 Hours

PACKAGING:
In pack size of 100 gm and 500 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
After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES
1. Chapin K. C. and Doern G. V., 1983, J. Clin. Microbiol., 17:1163.
2. Ellner P. P., Stoessel C. J., Drakeford E. and Vasi F., 1966, Am. J. Clin. Pathol., 45:502.
3. Fildes P., 1920, Br. J. Exp. Pathol., 1:129.
4. Fildes P., 1921, Br. J. Exp. Pathol., 2:16.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.
***For Lab Use Only**
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