TM 072 – COLUMBIA BLOOD AGAR BASE W/ HEMIN

INTENDED USE

An efficient and enriched base for fastidious microorganisms.

PRODUCT SUMMARY AND EXPLANATION

Columbia Agar Base is used as the base for media containing blood and for selective media formulations, which incorporates various combinations of antimicrobial agents as additives. Sheep blood allows detection of hemolytic reactions and supplies the X-factor (hemin) necessary for the growth of many bacterial species but lacks V-factor (Nicotinamide Adenine Dinucleotide), since it contains NADase, which destroys the NAD. Therefore, *Haemophilus influenzae*, which requires both the X and Vfactors, will not grow on this medium. 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*. 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 Blood Agar Base w/ 1 % Agar is used as a base for preparing media containing blood and for selective media formulations in which different combinations of antimicrobial agents are used as additives.

Sheep blood permits the detection of haemolysis and also provides heme (X-factor), which is required for the growth of many bacteria. As these media have a relatively high carbohydrate content, beta-haemolytic Streptococci may exhibit a greenish haemolytic reaction, which may be mistaken for alpha haemolysis. Confirmatory tests of all the presumptive colonies are needed.

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 microaerophillic atmosphere. Plates with *Gardenerella* supplements plates should be incubated at 35°C for 48 hours containing 7% CO_2. 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, X and V factors will not grow on this medium. Hemin stimulates growth of various fastidious organisms. As this medium has a relatively high carbohydrate content, beta-haemolytic Streptococci may exhibit a greenish haemolytic reaction which may be mistaken for the alpha haemolysis. Carry out confirmatory tests of all the colonies.

COMPOSITION

Ingredients	Gms / Ltr
Peptone, special	23.000
Corn starch	1.000
Sodium chloride	5.000
Hemin	0.010
Agar	15.000

PRINCIPLE

Columbia Agar Base supplemented with sheep, rabbit or horse blood derives its superior growth-supporting properties from the combination of peptones prepared from pancreatic digest of casein, peptic digest of animal tissue and beef extract. Cornstarch serves as an energy source and also neutralizes toxic metabolites.

INSTRUCTION FOR USE

- Dissolve 44.01 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. On standing the molten medium shows haziness. 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.

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	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
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

PACKAGING:

In pack size of 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.

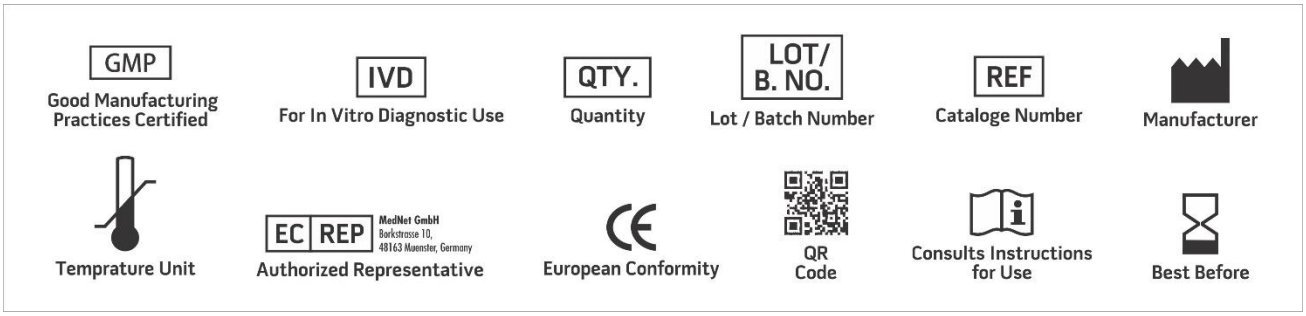
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

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., 17:11.
2.Ellner P. P., Stoessel C. J., DrE.
3. Fildes P., 1920, Br. J. Exp. Pathol., 1:129.
4. Fildes P., 1921, Br. J. Exp. Pathol., 2.



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

***For Lab Use Only**

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