

TM 074 – COLUMBIA C.N.A. AGAR BASE

INTENDED USE

For selective isolation of gram positive cocci from clinical and non-clinical samples.

PRODUCT SUMMARY AND EXPLANATION

Columbia Agar Base is a nutritionally rich formula containing 5% defibrinated blood, which provides more nutrients and capability of displaying haemolytic reactions. Columbia Blood Agar Base is utilized as a base for preparation of media containing blood and in selective media preparations where various combinations of antimicrobial agents are used as additives. Ellner et al formulated the medium and found that the combination of peptones used gave more rapid and abundant growth of Streptococci, Staphylococci, *Neisseria* and *Haemophilus* with better-defined haemolytic reactions. Columbia C.N.A. Agar Base is prepared with the same formula as Columbia Agar Base with the addition of 10 mg/litre of colistin and 15 mg/litre of nalidixic acid to inhibit the growth of gram-negative bacteria and to support the growth of Staphylococci, haemolytic Streptococci and Enterococci when supplemented with 5% blood.

Sheep blood allows detection of haemolytic reactions and supplies X-factor necessary for the growth of many bacterial species. Horse blood supplies X-factor and V-factor, therefore is mostly preferred in most laboratories. Yeast extract and cornstarch serve as energy source and neutralizer respectively.

It should be noted that this medium has relatively high carbohydrate content and, therefore, beta-hemolytic streptococci may produce a greenish hemolytic reaction that may be mistaken for alpha haemolysis. The addition of the antimicrobial agents, colistin (or polymyxin B) and nalidixic acid, renders the medium selective for gram-positive microorganisms. Colistin and nalidixic acid disrupt the cell membrane of gram-negative organisms, whereas nalidixic acid blocks DNA replication in susceptible gram-negative bacteria

Columbia C.N.A. Agar Base with addition of blood gives selective isolation of gram-positive cocci, Staphylococci and Streptococci, particularly when gram-negative bacilli are present and tend to overgrow on conventional blood agar plates. Also used for selective isolation of *Gardnerella vaginalis*. This medium supports growth of *Brucella abortus*, *Yersinia pestis*, *Clostridium perfringens* and all commonly occurring Enterobacteriaceae without addition of blood.

COMPOSITION

Ingredients	Gms / Ltr
Biopeptone	20.000
Tryptose B	3.000
Corn starch	1.000
Sodium chloride	5.000
Colistin sulphate	0.010
Nalidixic acid	0.015
Agar	15.000

PRINCIPLE

Biopeptone and tryptose B supports luxuriant growth of microorganisms and visualization of good haemolytic reactions. Yeast extract and cornstarch serve as energy source and neutralizer respectively.

INSTRUCTION FOR USE

- Dissolve 44.02 grams 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 and aseptically add 5% v/v sterile, defibrinated blood.



- Mix well and pour into sterile Petri plates.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder	: Cream to yellow homogeneous free flowing powder.
Appearance of prepared medium	: Basal medium: Yellow coloured clear to slightly opalescent gel. After addition of 5% v/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% v/v sterile, defibrinated blood.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Haemolysis	Incubation Temperature	Incubation Period
<i>Escherichia coli</i>	25922	50-100	Inhibited	0%	-	35-37°C	40-48 Hours
<i>Neisseria meningitidis</i>	13090	50-100	Inhibited	0%	-	35-37°C	40-48 Hours
<i>Staphylococcus aureus subsp. aureus</i>	25923	50-100	Luxuriant	≥70%	Beta/gamma	35-37°C	40-48 Hours
<i>Staphylococcus epidermidis</i>	12228	50-100	Luxuriant	≥70%	Gamma	35-37°C	40-48 Hours
<i>Streptococcus pneumoniae</i>	6303	50-100	Luxuriant	≥70%	Alpha	35-37°C	40-48 Hours
<i>Streptococcus pyogenes</i>	19615	50-100	Luxuriant	≥70%	Beta	35-37°C	40-48 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 2-8°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. Ellner et al, 1966, Am. J. Clin. Path., 45:502.
2. Estevez, 1984, Lab. Med., 15:258.
3. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Tenover F. C., (Ed.), 2003, Manual of Clinical Microbiology, 9th Edition, ASM Press

 GMP Good Manufacturing Practices Certified	 IVD For In Vitro Diagnostic Use	 QTY. Quantity	 LOT/ B. NO. Lot / Batch Number	 REF Catalogue Number	 Manufacturer
 Temperature Unit	 EC REP Authorized Representative <small>MedNet GmbH Barkstrasse 10, 49163 Moenster, Germany</small>	 European Conformity	 QR Code	 Consults Instructions for Use	 Best Before

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

***For Lab Use Only**
Revision: 08 Nov., 2019