Columbia Blood Agar Base

Intended Use
Columbia Blood Agar Base is used as a highly nutritious, general-purpose medium for the isolation and cultivation of non-fastidious and fastidious microorganisms, with or without addition of blood, from a variety of clinical and non-clinical specimens.

Summary
Columbia Blood Agar Base was developed by Ellner *et. al.,* and is used for isolating, cultivating and determining the haemolytic reactions of fastidious pathogenic microorganisms. Without enrichment, it can be used as a general-purpose medium. It is also used for the selective cultivation of *Brucella* and *Campylobacter* species by the addition of the respective selective supplement. Columbia Blood Agar Base is recommended by APHA for the examination of foods.

Principle
Pancreatic Digest of casein, Proteose peptone No.3, Yeast extract and Beef Heart Infusion provides essential nutrients. Corn starch serves as the energy source and also neutralizes toxic metabolites. Columbia Blood Agar Base is used as a base for media containing blood and for selective media formulations in which different combinations of antimicrobial agents are added as additives. It also promotes typical colonial morphology; better pigment production and more sharply defined hemolytic reaction. Sheep blood permits the detection of hemolysis 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.

Formula*

Ingredients	**g/L**
Pancreatic Digest of casein	10.0
Proteose peptone No.3	5.0
Yeast Extract	5.0
Beef Heart Infusion from 500 g	3.0
Corn Starch	1.0
Sodium Chloride	5.0
Agar	15.0
Final pH (at 25°C)	7.3 ± 0.2

*Adjusted to suit performance parameters.

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2ºC-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of Specimen
Clinical samples – Blood, Respiratory exudates

Specimen Collection and Handling
Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.
The samples must be stored and tested within the permissible time duration.
After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 44.00 g of powder in 1000 mL purified / distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation and boil for 1 minute to dissolve the powder completely.
4. Autoclave at 121°C (15 psi) for 15 minutes as per validated cycle.
5. For preparation of blood agar, Cool the base to 45°C - 50°C and add 5% sterile defibrinated blood.

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 this 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 (204020680005) to 500 mL sterile molten base.
For Cocci: Add rehydrated contents of 1 vial of Staph-Strepto Supplement (204191340005) or Strepto Supplement (204191350010) to 500 mL sterile molten base.

Quality Control

Dehydrated Appearance: Light yellow coloured, homogenous and free flowing powder.
Prepared Appearance: Basal medium: Light amber coloured slightly opalescent gel.
With addition of 5% defibrinated blood: Cherry red coloured opaque gel forms in petridishes.
Cultural Response: Cultural characteristics observed after an incubation of 24-48 hours at 35°C - 37°C under anaerobic condition.

Organism (ATCC)	**Growth**	**Haemolysis**
Streptococcus pyogenes Strain Bruno (19615)	Good	Beta
Staphylococcus aureus subsp. *aureus* (25923)	Good	Beta
Staphylococcus aureus subsp. *aureus* (6538)	Good	Beta
Streptococcus pneumoniae (6303)	Good	Alpha

Interpretation of Results

1. After incubation most plates will show an area of confluent growth.
2. Because the streaking procedure is, in effect, a “dilution” technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen.
3. Further, growth of each organism may be semi-quantitatively scored on the basis of growth in each of the streaked areas.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions/Limitations

1. *Brucella* cultures are highly infective and must be handled under properly protected conditions.
2. *Campylobacter* species are best grown at 42ºC (except *C. fetus* subspecies *fetus*) in a microaerophilic
1. atmosphere.
2. Staph/Strepto supplemented plates should be incubated aerobically at 35ºC for 18 hours. Incubation in carbon
3. dioxide-enriched air will cause inhibition of Staphylococcal growth.
4. Strepto supplemented plates should be incubated aerobically or anaerobically at 35ºC for 18 hours.
5. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
6. Columbia Blood Agar Base has a relatively high carbohydrate content and therefore beta-hemolytic Streptococci may produce a greenish hemolytic reaction that may be mistaken for alpha-hemolysis.
7. Hemolytic reactions of some strains of group D Streptococci are affected by differences in animal blood. Such strains are beta-hemolytic on horse, human and rabbit Blood Agar and alpha-hemolytic on sheep Blood Agar.

8. Prepared plates of supplemented media should be used within 18 hours of preparation for maximum selectivity.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Ellner P. P., Stoessel C. J., Drakeford E. and Vasi F., 1966, Am. J. Clin. Pathol., 45:502.
2. Fildes P., 1920, Br. J. Exp. Pathol., 1:129.
3. Fildes P., 1921, Br. J. Exp. Pathol., 2:16.
4. Chapin K. C. and Doern G. V., 1983, J. Clin. Microbiol., 17:1163.
5. Bailey R. K., Voss J. L. and Smith R. F., 1979, J. Clin. Microbiol., 9; 65-71.
6. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201030140100	Dehydrated Culture Media	100 g
201030140500	Dehydrated Culture Media	500 g