

COLUMBIA AGAR BASE

Medium for fastidious microorganisms isolation from clinical samples.

TYPICAL FORMULA	(g/l)
Peptospecial	23.0
Starch	1.0
Sodium Chloride	5.0
Agar	14.0
Final pH = 7.3 ± 0.2 at 25	°C.

DIRECTIONS

Suspend 43.0 g of powder in 1 liter of distilled or deionized water. Heat to boiling until completely dissolved. Sterilize in autoclave at 121 °C for 15 minutes. Cool to 45-50 °C and aseptically add 5% defibrinated sterile sheep blood. Mix well. Dispense in petri dishes.

Columbia Agar Base can be also enriched in various way:

- with 2 vials of CNA (Staf / Strep) supplement (colistin sulphate 5 mg/vial, nalidixic acid 8 mg/vial, code 81048), each one reconstituted with 5 ml of sterile distilled water; final medium will contain colistin sulphate 10 mg/l and nalidixic acid 16 mg/l.
- with 2 vials of *Gardnerella vaginalis* supplement (gentamicin 3 mg/vial, amphotericin B 1mg/vial, nalidixic acid 15 mg/vial, code 81040), each one reconstituted with 5 ml of a 1:1 solution of ethyl alcohol and sterile distilled water; final medium will contain gentamicin 6 mg/l, amphotericin B 2 mg/l and nalidixic acid 30 mg/l.

DESCRIPTION

COLUMBIA AGAR BASE, enriched with sterile sheep blood (5%), is suitable for isolation and growth of fastidious microorganisms such as streptococci, staphylococci, pneumococci and listeriae from clinical samples.

TECHNIQUE

Inoculate the medium with the specimen streaking by a sterile loop and incubate at 36 ± 1 °C for 18-48 hours aerobically, anaerobically or under conditions of increased CO_2 (5-10%), in accordance with established laboratory procedures. Examine plates for growth and hemolytic reactions. Four types of hemolysis on blood agar media can be described:

- 1. a-hemolysis is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony, causing a greenish discolorization of the medium.
- 2. β-hemolysis is the lysis of red blood cells, producing a clear zone surrounding the colony.
- 3. γ -hemolysis indicates no destruction of red blood cells and no change in the color of the medium.
- 4. ά-hemolysis indicates a partial lysis.

QUALITY CONTROL

Dehydrated medium

Appearance: free-flowing, homogeneous.

Color: beige.

<u>Prepared medium</u>

Appearance: opaque.

Color: cherry red.

Incubation conditions: $36 \pm 1^{\circ}$ C for 18-48 hours at 5-10% CO₂.

Microorganism	ATCC	Growth	Characteristics
Streptococcus pyogenes	19615	good	β-hemolysis
Streptococcus pneumoniae	6303	good	a -hemolysis
Staphylococcus aureus	25923	good	β-hemolysis
Gardnerella vaginalis	14018	good	β-hemolysis





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PERFORMANCE AND LIMITATIONS

When this medium is enriched with 10% sterile sheep blood, heated at 80 °C for 10 minutes until a chocolate color is obtained, and an antibiotic mixture is added (vancomycin, colimycin, trimethoprim, amphoterycin B) it is suitable for the selective isolation of the pathogens neisseria. If used without the addition of blood, the medium is suitable for growing of *Brucella abortus*, *Yersinia pestis*, *Clostridium perfrigens and enterobacteria*. Hemolytic reactions of some strains of Group D streptococci have been shown to be affected by differences in animal blood. Such strains are beta –hemolytic on horse and rabbit blood agar and alpha-hemolytic on sheep blood agar.

STORAGE

The powder is very hygroscopic: store the powder at 10-30 °C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident. Store prepared plates at 2-8 °C.

REFERENCES

- 1. Ellner, P.D., C.J. Stoessel., E. Drakeford, and F. Vasi (1966). A new culture medium for medical bacteriology. Am. J.Clin. Path. **45**, 502-504.
- 2. Isenberg, H.D. (ed.) (1992). Clinical microbiology procedures handbook, vol. 1 American Society for Microbiology, Washington, DC.

PRESENTATION					
Product	REF	Σ			
COLUMBIA AGAR BASE (11.6 I)	610013	500 g			
COLUMBIA AGAR BASE (2.3 I)	620013	100 g			
COLUMBIA AGAR BASE (116.2 I)	6100135	5 Kg			
SHEEP BLOOD DEFIBRINATED	83296	50 ml			
CNA (Staf / Strep) supplement	81048	10 vials			
Gardnerella vaginalis supplement	81040	10 vials			

TABLE OF SYMBOLS

LOT Batch code	Caution, consult accompanying documents	Manufacturer	Contains sufficient for <n> tests</n>	IVD In Vitro Diagnostic Medical Device
REF Catalogue number	Fragile, handle with care	Use by	Temperature limitation	Keep away from heat source





