Columbia agar base (Ph.Eur., USP, JP) Code 84621.0500

Also known as
Columbia blood agar base

Intended use
Base for the preparation of culture media for the cultivation of a variety of fastidious and non-fastidious microorganisms.

Formula * - Composition in g/L
Special peptones…… 23.0
Maize starch…… 1.0
Sodium chloride…… 5.0
Agar…… 12.0

* Adjusted and/or supplemented as required to meet performance criteria

Final pH 7.3 ± 0.2 at 25 °C.

Instructions for preparation
Dissolve 41 g in 1 litre of purified water by bringing to the boil with frequent shaking. Sterilise in the autoclave at 121 °C for 15 minutes. If necessary, cool to 45-50 °C. and aseptically add enrichments. Blood is generally added at a concentration of 5-10%.

Principle of the method and general information
Ellmer et al. of the Columbia University, found that the combination of meat and casein peptones used in Columbia agar gave better results than those obtained with current blood agar bases: it allows a more rapid and abundant growth of Streptococci, Staphylococci, *Neisseria* and *Haemophilus,* with better defined haemolytic reactions.
Columbia agar base can be prepared as blood agar or chocolate agar, with the addition of 5-10 % sheep, rabbit or horse blood, for isolating, cultivating and determining the haemolytic reactions of fastidious pathogenic microorganisms. Without enrichment, Columbia agar base can be used as a general purpose medium.
The formulation complies with the requirements of European Pharmacopoeia and the medium can be used for the sub-culturing of the growth obtained in reinforced medium for clostridia.

Instruction for use
For laboratory use only.
Columbia agar base supplemented with blood can be used as following.

- Firmly roll the swab, charged with the specimen to be examined, over one sixth of the blood agar plate.
- Use a sterile wire loop to cross hatch the remainder of the plate.
- Stab the agar several times with the wire loop to obtain subsurface growth and to permit the detection of both streptolysine O and S.
- Incubate for 18-24 hours in an aerobic atmosphere, or with 5-10% CO_2.

The procedure described by European Pharmacopoeia for the detection of Clostridia is the following:

- Prepare a sample using a 1 in 10 dilution (with a minimum total volume of 20 ml) of not less than 2 g or 2 ml of the product to be examined. Divide the sample into 2 portions of at least 10 ml. Heat 1 portion at 80°C for 10 minutes and cool rapidly. Do not heat the other portion.
- Use 10 ml or the quantity corresponding to 1 g or 1 ml of the product to be examined of both portions to inoculate suitable amounts of Reinforced clostridial medium (Art.N° 84699.0500).
- Incubate under anaerobic conditions at 30-35 °C for 48 hours.

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- After incubation, make subcultures form each container on Columbia agar base and incubate under anaerobic conditions at 30-35 °C for 48-72 hours.
- The occurrence of anaerobic growth of rods (with or without endospores) giving a negative catalase reaction indicates the presence of clostridia: This is confirmed by identification tests.

Limitations

- It is recommended that biochemical and/or serological tests be performed on colonies from pure culture for complete identification.
- Columbia agar base has a relatively high carbohydrate content and therefore beta-haemolytic streptococci may produce a greenish haemolytic reaction (on media containing blood) that may be mistaken for alpha-haemolysis.

Quality Control

Physical characteristics:

Appearance of powder	Pale yellow, fine, homogeneous hygroscopic powder
Appearance of prepared medium	Pale yellow, slightly opalescent
pH (25°C)	7.3 ± 0.2

Microbiological characteristics:

Test strains	Incubation T° / t / At.	Inoculation method	Growth characteristics	Productivity Ratio
Columbia agar base without defibrinated sheep blood				
E. faecalis ATCC 29212	35-37 °C / 24 h / AE	EC	Good growth	
S. epidermidis ATCC 12228	35-37 °C / 24 h / AE	EC	Good growth	
C. albicans ATCC 18804	35-37 °C / 24 h / AE	EC	Good growth	
S. lutea ATCC 9341	35-37 °C / 24 h / AE	EC	Good growth	
Columbia agar base with 5% defibrinated sheep blood				
S. pyogenes ATCC 19615	35-37 °C / 24 h / AE	EC	Good growth, beta haemolysis	
Group B *Strept.* ATCC 12386	35-37 °C / 24 h / AE	EC	Good growth small beta haemolysis	
Group C *Strept.* ATCC 12388	35-37 °C / 24 h / AE	EC	Good growth large beta haemolysis	
S. pneumoniae ATCC 6305	35-37 °C / 24 h / AE	EC	Good growth alpha haemolysis	
Columbia agar base without defibrinated sheep blood, according to Ph.Eur.				
C. sporogenes ATCC 19404	30-35° C / 48 h / AN	QT / 80-120 CFU	Good growth	PR ≥ 0.7

Notes

PR (Productivity Ratio): CFU obtained on the culture medium under test / CFU obtained on the Reference Batch
Inoculation method QT : quantitative surface plating method; EC: semi-quantitative, ecometric technique
Incubation atmosphere AE: aerobic incubation, AN: anaerobic incubation
ATCC is a registered trade mark of American Type Culture Collection

References

- Balows, A., Hausler, W.J., Herrmann, K.L., Isenberg H.D. and Shadomy, H.J. (ed) (1991) Manual of Clinical Microbiology, 5 th edition, ASM, Washington D.C.
- Ellner, P.D. Stoessel, C.J. Drakeford, E. & Vasi, F. (1966. Am. J. Clin. Path. **45**, 502-504.
- European Pharmacopoeia: 2.6.13 Microbiological examination of non-sterile products: tests for specified micro-organisms

Storage conditions

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+10 ºC to 30 ºC and <60% RH).

Ordering information

84621.0500 Columbia agar base (Ph.Eur., USP, JP) Bottle of 500 g

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