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Blood Agar Base

For preparing blood plates and boiled blood (chocolate) plates used for the isolation and cultivation of various fastidious microorganisms, especially of pathogenic species, and for establishing their forms of haemolysis.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

This culture medium can be used without blood e.g. for setting up blood cultures (UPDYKE 1970) and as a base for preparing special culture media

The medium complies with the recommendations of APHA (1992) for the examination of foodstuffs.

Principle

Microbiological method

Mode of Action

This culture medium represents a rich nutrient base, which provides optimal growth conditions for all relevant microorganisms. The pH value of 6.8 stabilizes the red blood corpuscles and favours the formation of clear haemolysis zones (NORTON 1932). Fresh, defibrinated sheep blood is most suitable for determining haemolysis forms. Boiled blood agar ("chocolate agar") is an extremely rich culture medium and can be prepared by heating after the blood has been added.

If the culture medium base is to be used without blood, the pH should, however, be adjusted to 7.2 to 7.4 since most bacterial colonies appear somewhat earlier and grow better in a slightly alkaline medium.

TARSHIS and FRISH (1951) recommended addition of 1% glycerol and 25 % human blood when isolating tubercle bacilli from sputum, since recognizable mycobacteria colonies grow from even minimal amounts of sample material.

HOSTY et al. (1953) reported, however, that 0.1 % glycerol and 2.5 % human blood together with 100 IU/mol of penicillin as a selective agent are sufficient. According to SONDAG et al. (1977) and BLACK a. VAN BUSKIRK (1973), addition of 5 mg/l gentamicin (e.g. 0.1 ml gentamicin solution) to blood agar permits selective cultivation of Streptococcus pneumoniae and other Streptococci as well as bacterioides, Clostridium and yeasts. For the selective cultivation of Aeromonas MISHRA et al. (1987) recommend an ampicillin sheep blood agar (ASBA 30).

Typical Composition (g/litre)

Nutrient substrate (heart extract and peptones) 20.0; sodium chloride 5.0: agar-agar 15.0.

Also to be added:

Blood 50-80 ml.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25 °C. Protect from light.After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25 °C.

Suspend 40 g/litre, autoclave (15 min at 121 °C), cool to 45-50°C, add 5-8 % defibrinated blood, mix.

pH: 6.8 ± 0.2 at 25 °C.

Before adding blood, the prepared medium is clear and yellowish-brown, then blood coloured and not haemolytic.

- **Poured blood plates can be stored for a maximum of 3months in the refrigerator.**
  Preparation of boiled blood agar: after adding the blood, heat the culture medium for about 10 minutes at approx. 80 °C with frequent swirling until it turns brownish (chocolate colour).

Specimen

e.g. Secretions of respiratory tract, sputum.Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Inoculate the surface of the plates.

Incubation: under optimal conditions usually 24 hours at 35 °C aerobically (Cl. perfringens anaerobically).

Check the plates for kind of hemolysis.

Literature

American Public Health Association: Compendium of Methods for the Microbiological Examination of Foods. 3rd ed., 1992.

BLACK, W.A. a. VAN BUSKIRK, F.: Gentamicin blood agar used as a general-purpose selective medium. – **Appl. Microbiol., 25** ; 905-907 (1973).HOSTY, FREEMAN a. IRWIN: **Publ. Hlth. Lab., 11**; 143 (1953).

MISHRA, S., NAIR, G.B., BHADRA, R.K., SIKDER, S.N., a. PAL, S.C.: Comparison of selective media for primary isolation of Aeromonas species from human and animal faeces. – **J. Clin. Microbiol., 25**; 2040-2043 (1987).

NORTON, J.F.: Bacteriology of pus. – **J. Lab. Clin. Med., 17**; 558-565 (1932).

SONDAG, J.E., MORGENS, R.K., HOPPE, J.E., a. MARR, J.J.: Detection of pneumococci in respiratory secretions: clinical evaluation of gentamicin blood agar. – **J. Clin. Microbiol. 5**; 397-400 (1977).

TARSHIS, M.S., a. FRISCH, A.W.: Blood media for the cultivation of Mycobacterium tuberculosis. – **Amer. J. Clin. Pathol. 21**; 101-113 (1951).
UPDYKE, E.L.: Pneumococcal Infections – in **Diagnostic Procedures for Bacterial, Mycotic and Parasitic Infections, 5th Edition**, APHA New York 1970.

Blood Agar Base

Ordering Information

Product	Merck Cat. No.	Pack size
Blood Agar Base	1.10886.0500	500 g
Anaerobic jar	1.16387.0001	1 ea
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® A	1.13829.0001	1 x 10
Anaerocult® A mini	1.01611.0001	1 x 25
Anaerocult® P	1.13807.0001	1 x 25
Anaerotest®	1.15112.0001	1 x 50
Gentamicin solution	1.11977.0001	10 ml
Glycerol (about 87 %)	1.04094.0500	500 ml
Plate basket	1.07040.0001	1ea
Blood		
Ampicillin mono-sodium salt	CN Biosciences	
Penicillin G potassium salt	CN Biosciences	

Quality control

Test strains	Inoculum(cfu/ml)	Recovery rate (%)	Hemolysis	Bacitracin test
Staphylococcus aureus ATCC 25923	10^3-10^5	≥ 70	β	
Streptococcus pyogenes ATCC 12344	10^3-10^5	≥ 70	β	+
Streptococcus agalactiae ATCC 13813	10^3-10^5	≥ 70	-	
Streptococcus pneumoniae ATCC 6301	10^3-10^5	≥ 70	α	-
Listeria monocytogenes ATCC 19118	10^3-10^5	≥ 70	-	
Bacillus cereus ATCC 11778	10^3-10^5	≥ 70	β	
Clostridium perfringens ATCC 13124	10^3-10^5	≥ 70 (anaerobic incubation)	β	