

## **Technical Data Sheet**

# C Blood Agar Base

Ordering number: 1.10886.0500

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.

The medium complies with the recommendations of APHA (1992). This culture medium is a nonselective general purpose medium which may be enriched with blood or serum but can also be used without blood e.g. for setting up blood cultures (UPDYKE 1970) and as a base for preparing special culture media. Blood agar plates are routinely used in the clinic to test for pathenogenic bacteria in throat swabs.

IVD in vitro diagnosticum - For professional use only

#### **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**

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**

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

pH:  $6.8 \pm 0.2$  at 25 °C.

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

#### **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.

### **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.

Poured blood plates can be stored for a maximum of 3 months in there frigerator.

Preparation of boiled blood agar: after adding the blood, heat the culture mediumfor about 10 minutes at approx. 80°C with frequent swirling until it turns brownish (chocolate colour).

### **Quality Control**

Control Strains	ATCC#	Inoculum CFU	Incubation	Expected Results
Staphylococcus	25923	10 <sup>3</sup> -10 <sup>5</sup>	24 h at 35 °C	Recovery ≥ 70 %
aureus		10 10	2111 41 00 0	110001019 = 70 70
Streptococcus	12344	10 <sup>3</sup> -10 <sup>5</sup>	24 h at 35 °C	Recovery ≥ 70 %
pyogenes	12544	10-10-	24 11 at 33 C	Recovery ≥ 70 %
Streptococcus	13813	10 <sup>3</sup> -10 <sup>5</sup>	24 h at 35 °C	Bacayony > 70 %
agalactiae	13013	10°-10°	24 11 at 35 C	Recovery ≥ 70 %
Streptococcus	6301	10 <sup>3</sup> -10 <sup>5</sup>	24 h at 35 °C	Decovery > 70 %
pneumoniae	0301	10°-10°	24 11 at 35 C	Recovery ≥ 70 %
Listeria	19118	10 <sup>3</sup> -10 <sup>5</sup>	24 h at 35 °C	Decovery > 70 %
monocytogenes	19110	10-10-	24 11 at 33 C	Recovery ≥ 70 %
Bacillus cereus	11778	10 <sup>3</sup> -10 <sup>5</sup>	24 h at 35 °C	Recovery ≥ 70 %
Clostridium	13124	10 <sup>3</sup> -10 <sup>5</sup>	24 h at 35 °C	Recovery ≥ 70 %
perfringens	13124			(anaerobic incubation)

Please refer to the actual batch related Certificate of Analysis.



#### 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.

## **Ordering Information**

Product	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

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