

Blood Agar (Base)

356-4524

DEFINITION

This blood-enriched medium is used for the isolation of fastidious bacteria without affecting their haemolytic response.

STANDARDS

FOOD MICROBIOLOGY

- **NF EN ISO 11290-2/A1 (February 2005):** Food microbiology - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Enumeration method.
- **NF EN ISO 11290-1/A1 (February 2005):** Food microbiology - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Detection method.
- **FIL 143A (1995):** Milk and dairy products - Detection of *Listeria monocytogenes*.
- Enumeration method for *Listeria monocytogenes* in vegetable products or products of vegetable origin (BOCCRF dated 30 May 1992, modified 5 September 1992).

PRINCIPLE

The nutrient substances provided by the peptone and the yeast extract favor the growth of most bacteria.

PRESENTATION

Dehydrated

500 g

code 356-4524

STORAGE

- Dehydrated: +15°C to 25°C, in carefully-sealed bottles in a cool, dry place.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

Peptone	15 g
Liver extract	2.5 g
Yeast extract	5 g
Sodium chloride	5 g
Agar	12 g
Distilled water	1,000 ml
Final pH (25°C) = 7.2 ± 0.2	

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

- Distilled water
- Horse blood/Additive (code 355-6641)
See corresponding Technical Sheet(s)

EQUIPMENT REQUIRED (NOT SUPPLIED)

(non-exhaustive)

- Hotplate
- Mixer-homogenizer
- Sterile Petri dishes (Ø = 90 mm)
- Sterile Pasteur pipettes (code 355-0751) or inoculating loop
- Sterile spreaders
- Water-bath at 80°C for the "chocolate" agar
- Water-bath precise to ± 1°C
- Thermostatically-controlled incubator or incubation room, precise to ± 1°C
- Autoclave
- All usual laboratory equipment

PREPARATION OF DEHYDRATED MEDIUM

Always shake before use

Dissolve 40 g of powder in 1 liter of distilled water. Bring to boiling point until completely dissolved. Dispense 100 ml per bottle, then sterilize in autoclave at 121°C ± 1°C for 15 minutes.

Reconstitution ratio: 40 g/l.

500 g of powder makes 12.5 liters of medium.

PROTOCOL

• Inoculation and incubation

In general this medium is used for the preparation of a fresh blood agar:

To 100 ml of sterile base agar, melted then cooled to 44°C - 47°C, add 5 - 10% of sterile horse blood (code 355-6641) or sheep blood (code 355-6652).

After shaking carefully, avoiding the creation of air bubbles, pour the mixture into Petri dishes. Inoculate by spreading on the surface or by isolation, and incubate at 37°C ± 1°C for 24 h ± 2 hours.

For primary isolation of strains of *Haemophilus* from samples containing an associated flora, the use of horse blood is recommended. For best results, 2 drops of 10% saponine can be spread on the surface of the agar to be inoculated (Waterworth, 1955).

This base agar medium can also be used for the preparation of a "chocolate" agar (cooked blood agar): 10% sterile horse blood is added to the base.

The bottles are maintained at 80°C in a waterbath for 10-15 minutes, while being agitated, until a chocolate shade is obtained.

Dispense in Petri dishes.

After inoculation by spreading over the surface or by isolation, the plates are incubated at 37°C ± 1°C for 18 to 24 hours.

READING AND INTERPRETATION

Colonies of the principal bacteria have typical size, color and appearance on this agar.

The type of haemolysis varies according to the strains.

PRECAUTIONS

- Do not add the blood to a base medium at a temperature exceeding 47°C.
- Comply with Good Laboratory Practice.

PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains:

STRAINS	Results after 24h culture at 37°C
<i>Streptococcus bovis</i> CIP 5623	Good growth No hemolysis
<i>Staphylococcus aureus</i> ATCC 25923	Good growth Weak β hemolysis
<i>Staphylococcus epidermidis</i> ATCC 12228	Good growth No hemolysis
<i>Streptococcus pyogenes</i> ATCC 19615	Good growth β hemolysis
<i>Streptococcus</i> du groupe C CIP A7	Good growth β hemolysis
<i>Enterococcus faecalis</i> var <i>zymogenes</i> ATCC 29212	Good growth β hemolysis
<i>Streptococcus pneumoniae</i> ATCC 6303	Good growth α-hemolysis turning green
<i>Neisseria meningitidis</i> (+CO ₂) ATCC 13090	Good growth No hemolysis

QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

KEY WORDS

Base for blood agar / Fastidious bacteria / Blood / Isolation / Medium.