

Mueller-Hinton Blood Agar

(Test of antibiotic susceptibility of *Streptococci*)

356-3825
356-3902

DEFINITION

Medium used in the analysis of food products for investigating the sensitivity of *streptococci* to antibiotics.

STANDARDS

FOOD MICROBIOLOGY

- **NF EN ISO 10272-1 (April 2006):** Food microbiology - Horizontal method for detection and enumeration of *Campylobacter* spp. Part 1: Detection method.

PRINCIPLE

The nutrient substances provided by the beef infusion, the casein hydrolysate and the sheep blood favor the growth of most *streptococci*.

PRESENTATION

Pre-poured

20 dishes x 90 mm
10 dishes x 120 mm

code 356-3825
code 356-3902

STORAGE

- + 2°C to 8°C.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

Meat infusion	6 g
Casein hydrolysate	17.5 g
Corn starch	1.5 g
Agar + 5% sheep blood	12 g
Distilled water	1,000 ml
Final pH (25°C) = 7.3 ± 0.2	

EQUIPMENT REQUIRED (NOT SUPPLIED)

(non-exhaustive)

- Sterile pipettes (0,1 ml, etc)
- Sterile Pasteur pipettes (code 355-0751) or inoculating loops
- Equipment for working in anaerobiosis (Durham bell jar, catalyser, etc)
- Thermostatically-controlled incubator or incubating room, precise to ± 1°C
- All usual laboratory equipment.

PROTOCOL

Inoculation and incubation

Starting with a 6 to 8 hours culture of liquid nutrient medium (buffered glucose broth or heart-brain infusion, with or without the addition of 5% horse serum and human ascites), prepare a dilution appropriate for the group under examination and inoculate the surface of

the agar in a uniform manner.

The recommended dilutions are as follows (for 10 ml of distilled water):

- *Streptococci* in groups A, C, F, G: 15 to 20 drops,
- *Streptococci* in groups B and D (*E. faecalis*, *E. faecium*): 1 to 2 drops,
- *Streptococci* in other ungroupable groups (*S. pneumoniae*, *S. bovis*): 6 to 10 drops.

After deposition of antibiotics on the dishes, incubate at 37°C ± 1°C in an atmosphere enriched with 5 % CO₂ for 18 hours.

High level of resistance to aminoglycosides:

The beta-lactam-aminoglycoside combination cannot be synergic unless the streptococci do not present a high level of resistance to aminoglycosides (more than 1 mg/ml). This level can be examined by incorporating the antibiotic into the agar (1 to 2 mg/ml) or for antibiotic sensitivity by using dishes loaded as follows:

- Streptomycin 500 µg
- Gentamicin 250 µg

READING AND INTERPRETATION

Measure the diameter:

	DIAMETER	
	High level of resistance	Low level of resistance
Streptomycin 500 µg	< 12 mm	≥ 14 mm
Gentamicin 250 µg	< 10 mm	≥ 17 mm

For intermediate diameters, it is advisable to verify the high level of resistance of the strain on agar media containing 1 to 2 mg/ml of Streptomycin or of Gentamicin.

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PRECAUTIONS

- The time lapse between the end of preparation of the stock solution (or the 10^{-1} dilution in the case of a solid product) and the moment when the dilutions come in contact with the culture medium must not exceed 15 minutes.
- 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
<i>Pseudomonas aeruginosa</i> ATCC 27853	Zone diameter complies with N.C.C.L.S. performance standards
<i>Streptococcus pneumoniae</i> ATCC 49619	Zone diameter complies with N.C.C.L.S. performance standards

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

Mueller-Hinton Blood Agar / *Streptococci* / Food Products / Susceptibility / Antibiotics / Sheep blood / Antibiogram / Medium.