

Staphylocoagulase/Broth (Rabbit plasma)

355-3544
355-6352

DEFINITION

Confirmation test for *Staphylococcus aureus* through detection of staphylocoagulase by means of oxalated rabbit plasma.

STANDARDS

- **NF EN ISO 6888-1 (October 1999):** Food microbiology - Horizontal method for the detection of coagulase-positive *staphylococci* (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird Parker agar.
- **FIL 60 B (1990):** Dried dairy products - Enumeration of *Staphylococcus aureus* - Most Probable Number technique.
- **FIL 138 (1986):** Dried milk - Enumeration of *Staphylococcus aureus* - Technique of colony count at 37°C.
- **FIL 145 (1990):** Milk and milk-based products - Enumeration of *Staphylococcus aureus* - Technique of colony count at 37°C.

PRINCIPLE

The principle of the coagulation reaction relies on the ability of *Staphylococcus aureus* to coagulate oxalated rabbit plasma within 24 hours, unlike strains of *Staphylococcus epidermidis* and of *Micrococcus*.

PRESENTATION

- **Special broth**
Ready-to-use
9 ml x 25 tubes **code 355-3544**
- **Freeze-dried rabbit plasma**
Pack for 20 reactions **code 355-6352**

STORAGE

- Rabbit plasma
 - freeze-dried: + 4°C until expiration date.
 - reconstituted: + 4°C for 48 hours.
- Special broth, ready-to-use: + 2°C to 25°C.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

Broth

Meat peptone	4 g
Gelatine peptone	1 g
Meat extract	2 g
Sodium chloride	5 g
Distilled water	1,000 ml
Final pH (25°C)	= 7.0 ± 0.2

PREPARATION OF FREEZE-DRIED RABBIT PLASMA

Re-hydrate the rabbit plasma using the equivalent volume of solvent (10 ml). This volume must be measured accurately, since it ensures optimal dilution of the plasma in addition to rehydration.

N.B.: Reconstitution of a suspension from the lyophilized substance is facilitated by slight stirring, while avoiding a build-up of froth.

PROTOCOL

• Inoculation and incubation

Inoculate the special broth from a pure culture of the test strain for testing coagulase (*). Incubate the culture at 37°C for 18 hours.

In a sterile haemolysis tube, mix 0.5 ml of rehydrated plasma and 0.5 ml of broth culture of the test strain. Incubate the mixture at 37°C for 24 hours.

(*) Some non-inoculated, ordinary broth preparations mixed with oxalated plasma may cause coagulation of the latter. A control broth plasma must therefore be prepared in advance if the special broth is not used.

READING AND INTERPRETATION

S. aureus strains usually trigger coagulation of the plasma during the first 3 hours (> 1/2 h and < 24 h). The plasma generally forms a complete mass to the extent that it is possible to turn the tube over. In some cases, coagulation is lighter and occurs at a later stage, but the reaction must be considered positive if the phenomenon occurs before the 24th hour.

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 into contact with the culture medium must not exceed 15 minutes.

- For an optimal result, the volumes for rehydration of the plasma must be measured very precisely.
- Comply with Good Laboratory Practice.

PERFORMANCES / QUALITY CONTROL OF THE TEST

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

STRAINS	Detection of coagulase after 24h at 37°C
<i>Staphylococcus aureus</i> ATCC 25923	Good growth Positive coagulase
<i>Staphylococcus aureus</i> ATCC 6538P	Good growth Positive coagulase
<i>Staphylococcus saprophyticus</i> ATCC 15305	Good growth Negative coagulase
<i>Staphylococcus epidermidis</i> ATCC 12228	Good growth Negative coagulase

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

Staphylocoagulase / *Staphylococcus aureus* / Food products / Sterility test / Confirmation / Medium.