

COAGULASE RABBIT PLASMA

CONFIRMATION OF COAGULASE POSITIVE *STAPHYLOCOCCUS*

1 INTENDED USE

Rabbit Plasma recovered over EDTA and freeze-dried is used for the detection of staphylocoagulase.

Its use is recommended in the standards NF EN ISO 6888-1 & NF EN ISO 6888-3 ; NF V08-057-1 ; and NF EN ISO 22718.

2 HISTORY

Coagulase is an enzyme that can coagulate blood plasma and was demonstrated for the first time by Loeb in 1903 in some staphylococci. Since that time, a number of authors have tried to link coagulase production in *Staphylococcus aureus* to its capacity to produce an enterotoxin and thus to its pathogenic power. Today, the presence of a coagulase is considered to be the main factor in the determination of the pathogenicity of staphylococci. Nevertheless, it is not rare to isolate coagulase negative strains with proven pathogenicity.

3 PRINCIPLES

Staphylococcus aureus produces two types of coagulase :

- free extracellular coagulase that reacts with plasma prothrombin;
- bound coagulase, localized in the bacterial wall, that reacts with plasma fibrinogen to produce a clot.

In tests done in tubes, free coagulase reacts primarily by forming a clot in plasma, indicating a positive reaction. In the case of coagulase-negative staphylococci, it is recommended to screen for the presence of other enzymes such as phosphatase or deoxyribonuclease, that are also indicators of pathogenicity.

4 TYPICAL COMPOSITION

Rabbit Plasma, EDTA, lyophilized.

5 PREPARATION

- Using aseptic techniques, fill the vial of lyophilisate with 6 mL of sterile distilled water.
- Turn end-over-end to dissolve. Avoid frothing the solution.
- Dispense into hemolysis tubes at 0,3 mL per tube.

✓ **Rehydration :**
6 mL sterile distilled water

6 INSTRUCTIONS FOR USE

- Inoculate a colony to confirm into Brain Heart broth (BK015) and incubate 24 h at 37°C.
- Inoculate 0,1 mL of the Brain Heart culture into a tube of rabbit plasma.
- Mix well.
- Incubate in a thermostated water bath at 37 ± 1 °C for 4 to 24 hours.

✓ **Inoculation :**
0,1 mL per tube

✓ **Incubation :**
4 to 24 h at 37 °C

Note : As a control, add 0,1 mL of sterile Brain Heart broth (BK015) to 0,3 mL of reconstituted Rabbit Plasma. This negative control should not show any signs of coagulation after the 24 hours of incubation.

7 RESULTS

The reaction is considered positive when the clot occupies 3/4 of the initial volume. Coagulation in principle occurs within less than 3 hours and most often the clot adheres to the tube walls. Coagulation sometimes occurs more slowly and in this case the reaction can be considered positive if a clot appears in less than 24 hours.

8 QUALITY CONTROL

Appearance : light beige pellet giving an amber yellow solution after reconstitution, opalescent.

Coagulation test after 24 hours of incubation at 37 °C :

| Microorganisms | | Coagulase |
|-----------------------------------|------------|-----------|
| <i>Staphylococcus aureus</i> | WDCM 00033 | Positive |
| <i>Staphylococcus aureus</i> | WDCM 00034 | Positive |
| <i>Staphylococcus epidermidis</i> | WDCM 00036 | Negative |
| <i>Escherichia coli</i> | WDCM 00013 | Negative |

9 STORAGE / SHELF LIFE

Freeze-dried supplement : 2-8 °C.

The expiration date is indicated on the label.

Rehydrated supplement (*) : 30 days at 2-8 °C.

(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Freeze-dried supplement :

10 vials (20 reactions per vial) BR00208

11 BIBLIOGRAPHY

NF EN ISO 6888-1. Octobre 1999. Microbiologie des aliments. Méthode horizontale pour le dénombrement des staphylocoques à coagulase positive (*Staphylococcus aureus* et autres espèces). Partie 1 : Technique utilisant le milieu gélosé de Baird-Parker.

NF EN ISO 6888-3. Juin 2003. Microbiologie des aliments. Méthode horizontale pour le dénombrement des staphylocoques à coagulase positive (*Staphylococcus aureus* et autres espèces). Partie 3 : Recherche et méthode NPP pour les faibles nombres.

NF V 08-057-1. Janvier 2004. Microbiologie des aliments. Méthode de routine pour le dénombrement des staphylocoques à coagulase positive par comptage des colonies à 37 °C. Partie 1 : Technique avec confirmation des colonies.

NF EN ISO 22718. Septembre 2009. Cosmétiques. Microbiologie. Détection de *Staphylococcus aureus*.

9 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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