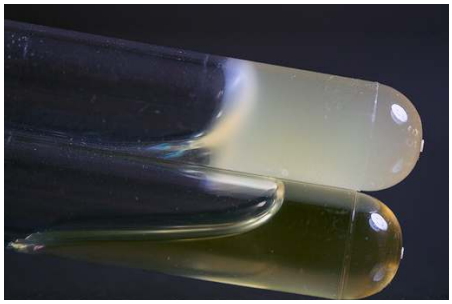
INSTRUCTIONS FOR USE

COAGULASE PLASMA EDTA

Biochemical identification reagent



Coagulase Plasma. From the top to bottom: *S.aureus* (+) and *S.epidermidis* (-)

1 - INTENDED USE

In vitro diagnostic. For the qualitative detection of coagulase enzyme in staphylococci.

2 – TYPICAL COMPOSITION – VIAL CONTENT *

REF 429936: Rabbit Plasma EDTA, 5.0 mL (lyophilized)
REF 429937: Rabbit Plasma EDTA, 2.5 mL (lyophilized)
REF 429938: Rabbit Plasma EDTA, 1.0 mL (lyophilized)

*The vial content may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The coagulase test was developed from observations, in the early 1900s, that certain staphylococci clotted plasma from the goose,[1] man, horse, and sheep[2]. Today, the widely used and generally accepted method of differentiating staphylococci associated with acute infection or food poisoning is the identification of extracellular coagulase production using a tube coagulase test.[3,4] Based on their ability to clot plasma, staphylococci may be divided into coagulase-positive or coagulase-negative ones.
The enzyme coagulase acts on a “coagulase reacting factor” present in rabbit plasma, producing a thrombin-like substance which activates fibrinogen to form fibrin, causing the plasma to clot. Coagulase exists in two forms: “bound coagulase” (or clumping factor) which is bound to the cell wall and “free coagulase” which is liberated by the cell wall. Bound coagulase is detected by the slide coagulase test, whereas both free and bound coagulase are detected by the tube coagulase test.[5]
Coagulase Plasma EDTA, in the different proposed formats, is recommended for performing the tube test. Typically, rabbit plasma is inoculated with growth from isolated colonies and examined for the presence of a gel or clot at 4 hours and, if negative, examined again at 24 hours. In addition to *Staphylococcus aureus* also other species, including *Staphylococcus schleiferi* and *Staphylococcus intermedius* may give positive results in the tube coagulase test but are not common isolates from human infections.[3,5]
For the tube coagulase test, EDTA plasma is superior to citrated plasma because citrate-utilizing organisms such as *Pseudomonas* species, *Serratia marcescens, Enterococcus faecalis* and strains of *Streptococcus* will clot citrated plasma.[5]

4 - METHOD OF PREPARATION

REF 429936: reconstitute the content of one vial with 15 mL of sterile distilled water under aseptic conditions.
REF 429937: reconstitute the content of one vial with 7.5 mL of sterile distilled water under aseptic conditions.
REF 429938: reconstitute the content of one vial with 3 mL of sterile distilled water under aseptic conditions.
Stir for 30 seconds by means of a vortex to dissolve completely. The rabbit plasma results to be diluted 1:3.

5 - PHYSICAL CHARACTERISTICS

Appearance of lyophilized pellet	dense pastille
Appearance of coagulase plasma after reconstitution	yellowish to pink opalescent solution

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Coagulase Plasma EDTA	Identification reagent	429936	4 vials with 5 ml of rabbit plasma ⟶ (4 x 15 mL: 120 tests)
Coagulase Plasma EDTA	Identification reagent	429937	4 vials with 2,5 ml of rabbit plasma ⟶ (4 x 7,5 mL: 60 tests)
Coagulase Plasma EDTA	Identification reagent	429938	10 vials with 1 ml of rabbit plasma ⟶ (10 x 3 mL: 60 tests)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Small sterile culture tubes, water bath or incubator, sterile loops, sterile pipettes, sterile purified water, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

In clinical and non-clinical microbiology, the specimens consist of suspected staphylococcal colonies grown on plating media. Coagulase Test EDTA cannot be used for the direct testing of clinical specimens.

9 - TEST PROCEDURE

From the surface of each selected colony, remove an inoculum with a sterile loop and transfer it to a tube of Brain Heart Infusion Broth. Incubate the broth at 35-37°C for 18-24 hours.
Aseptically add 0.5 mL of each culture to 0.5 mL of Coagulase Plasma EDTA in small sterile tubes, and incubate at 35-37°C.

10- READING AND INTERPRETATION

Observe every 60 minutes in the first 4 hours of incubation for clotting by gently slanting the tube. Do not shake.
If no clot is observed by 4 hours, the tube should be read again after 18-24 h of incubation at 35-37°C.

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Any degree of clotting represents a positive test. A flocculent or fibrous precipitate is not a true clot and should be recorded as negative.

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed the test strains useful for the quality control.
Positive control: *S.aureus* ATCC 25923
Negative control: *S.epidermidis* ATCC 12228

ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of Coagulase Plasma EDTA is tested with positive and negative strains.
Coagulase positive strains (reading after 2, 4, 24 hours of incubation at 35-37°C): *S.aureus* ATCC 25923, *S.aureus* ATCC 6538, *S.aureus* ATCC 33862, *S.aureus* MR ATCC 33592.
Coagulase negative strains (reading after 24 hours of incubation at 35-37°C): *S.epidermidis* ATCC 12228, *S.xylosus* ATCC 35033 , *S. saprophyticus* ATCC 15305, *S.sciuri* CB-BX 16.1, *E.faecalis* ATCC 19433
All the tested strains give a coagulase reaction according to the specifications.

13 - LIMITATIONS OF THE METHOD

- Some species of staphylococci other than *S. aureus* (*S.intermedius, S.hycus, S.schleiferi* subsp. *coagulans, S.delphini, S.lutrae, S.pseudointermedius, S.argenteus*) may give positive reaction to coagulase test.[3-6]
- The colony inoculum used for testing must be pure because a contaminant may produce false results after prolonged incubation.[5]
- The tube coagulase test should not be unduly agitated as this can cause the clot to shrink also giving a false negative result.[5]
- Care should be taken when using tube coagulase test directly on presumptive positive coagulase blood culture broth while recent reports have indicated no loss of sensitivity when the tube coagulase test is performed directly on uncentrifuged blood culture broths.[7,8]
- Observation for clotting should be made within the first 4 h since some staphylococci produce fibrinolysin, which may lyse clots early in the incubation period.[9,10] If no clotting is observed, however, the tube should be incubated overnight and observed again for delayed clotting.
- False-negative coagulase reactions may occur if the test isolate is older than 18-24 hours or if there is scant growth.
- Slide coagulase test has a sensitivity lower than tube test since it detects only bound coagulase enzyme.
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed for complete identification of the isolates. If relevant, perform antimicrobial susceptibility testing.
- This reagent is intended as an aid in the diagnostic procedures of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

14 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation
- This product contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the product be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as the reagent, the culture media or the microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused reagent and the tubes inoculated with microbial strains in accordance with current local legislation.
- Do not use this product as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases, existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store the product in the original pack at 2-8°C away from direct light. If properly stored, the product may be used up to the expiration date. Do not use the product beyond this date. Opened and reconstituted vial under aseptic conditions can be stored at 2-8°C for up to 5 days or aliquot into 0.5 ml amounts and stored at -20ºC for 14 days. Allow plasma to equilibrate to room temperature before use. Do not use the plasma if is clotted upon rehydration, if it is contaminated or if there are other signs of deterioration (precipitate, atypical colour).

16 – REFERENCES

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5. Public Health England. (2018). UK Standards for Microbiology Investigations: Coagulase test test Procedures TP 10, Issue no: 6, Issue date: 01.08.18
6. Staphylococcus argenteus: Another Coagulase Positive Staphylococcus. May 28, 202, Podcast. American Society for Microbiology.

7. Davis TE, Fuller DD, Aeschleman EC. Rapid, direct identification of Staphylococcus aureus and Streptococcus pneumoniae from blood cultures using commercial immunologic kits and modified conventional tests. Diagn Microbiol Infect Dis 1992; 15:295-300. B, II
8. McDonald CL, Chapin K. Rapid identification of Staphylococcus aureus from blood culture bottles by a classic 2-hour tube coagulase test. J Clin Microbiol 1995; 33:50-2. B, II
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TABLE OF APPLICABLE SYMBOLS

REF or **REF** Catalogue number	**LOT** Batch code	**IVD** *In vitro* Diagnostic Medical Device	Manufacturer	This side up	
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content	2021/10
Revision 2	Removal of obsolete classification	2023/04

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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