Rabbit Plasma

CK2002 10 x 3ml
CK2003 6 x 20ml
For the detection of coagulase enzyme activity in staphylococci.

Introduction

Some species of staphylococci, notably *Staphylococcus aureus* in humans and *Staphylococcus intermedius* and *Staphylococcus hyicus* in animals, are associated with acute infections[1]. The organisms can all clot plasma and this ability, due to the presence of the enzyme coagulase, is widely used as a criterion for identification of these pathogens[2]. Coagulase production is very closely linked with the capability of *S. aureus* to form enterotoxins[3].

The coagulase test can be used to detect both free coagulase and bound coagulase (clumping factor)

The tube coagulase test may be used to identify coagulase positive staphylococci including methicillin resistant strains, provided that only a firm clot (one that does not move when the tube is tipped) is considered a strong positive result[5]. BioConnections Rabbit Plasma contains EDTA as an anti-coagulant in preference to citrate because citrate-utilising organisms such as *Pseudomonas* species, *Serratia marcesens*, *Enterococcus faecalis* and strains of *Streptococcus* are able to clot citrated plasma in 18 hours[6], and can produce false positive results.

The slide agglutination test has an approximate 96% agreement with the tube coagulase test[7]. However, the slide agglutination technique can occasionally generate false-positive results. This is due to the fact that some strains, such as *S. lugdunensis* and *S. schleiferi* subsp. *Schleiferi*, produce clumping factor resulting in a positive slide test only. The tube coagulase method can be used to differentiate these species from *S. aureus*[7]. In addition, spontaneous agglutination may occur on the slide agglutination test, when rough cultures are used. When the slide test is employed, all negative slide reactions must be confirmed by the tube test.

Description

CK2002
10 x 3ml vials, freeze dried rabbit plasma, each sufficient for 6 tests

CK2003
6 x 20ml vials, freeze dried rabbit plasma, each sufficient for 40 tests

Quality Control

Use of the following control organisms is recommended:

Organism	Expected Result
Staphylococcus aureus NCTC 6571	Weak positive
Staphylococcus aureus ATCC 25923	Strong positive
Staph. epidermidis ATCC 14990	Negative

Directions

Tube Coagulase Test:

1. Rehydrate the Coagulase Plasma using sterile deionised water, 3ml CK2002 or 20 ml CK2003. The best method is to aseptically add the diluent using a sterile needle and syringe. Draw the diluent into the syringe and after removing the plastic cap of the vial; inject through the rubber stopper of the vial. The coagulase plasma will rapidly dissolve and may be withdrawn into the syringe. DO NOT TRY TO RESHEATH AN EXPOSED NEEDLE. Discard the used needle into an approved container.
2. Aseptically transfer 0.5ml of rehydrated plasma to a suitable sterile tube for each test.
3. If taking organisms from agar plates, make a suspension of each isolate to be tested to a density equivalent to a McFarland 1.0 opacity standard. Add 100µl of isolate suspension to a labelled coagulase tube.
4. Incubate at 37°C for 4 hours and examine for clot formation at hourly intervals.
5. Examine tubes for clot formation by gently tipping to one side. Do not shake or agitate the tube as this may cause breakdown of the clot and consequent false negative test results.
6. If negative after 4 hours (i.e. no clot observed), tubes should be left at room temperature overnight and examined aga for clot formation[3,4,7].
7. Record formation of a clot as a positive re and note its consistency according to the scale below.

Interpretation of Results

Negative result: No evidence of fibrin clot formation after 24 hours.

Positive result:
1+ Small unorganised clots.
2+ Small organised clots.
3+ Large organised clots.
4+ Entire tube content coagulates and is not displaced when tube is inverted.
A positive result is indicative of *S. aureus*

Slide Agglutination Test:

1. Rehydrate the Coagulase Plasma using sterile deionised water.
2. Place a drop of Coagulase Plasma onto a clean, dry glass slide.
3. Place a drop of sterile water onto the slide act as a control.
4. With a sterile loop or wooden stick, emulsi an amount of the isolated colony being tes into both the Coagulase Plasma and the water. Inoculate the water first.
5. Observe for clumping in the Coagulase Plasma and a homogenous suspension in control.

Interpretation of Results

Negative result

- Colonies mix smoothly into suspension in both Coagulase Plasma and the water control, or
- Clumping in both the Coagulase Plasma ar water control. This indicates that the organism autoagglutinates. When autoagglutination is observed, the tube coagulase test should be employed as an alternative to the slide agglutination test.

Positive result: Colonies form clumps that will no mix uniformly into Coagulase Plasma. A positive result is indicative of
S. aureus.