NATIONAL STANDARD METHOD

COAGULASE TEST

BSOP TP 10

Issued by Standards Unit, Department for Evaluations, Standards and Training
Centre for Infections
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The reader is informed that all taxonomy in this document was correct at time of issue.

Suggested citation for this document:

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AMENDMENT PROCEDURE

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<thead>
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<th>Controlled document reference</th>
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Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

<table>
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SCOPE OF DOCUMENT

Members of the genus Staphylococcus are differentiated by the ability to clot plasma by the action of the enzyme coagulase. The mechanism of coagulase action is not known.

INTRODUCTION

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 free coagulase is detected by the tube coagulase test.

Bound coagulase adsorbs fibrinogen from the plasma and alters it so it precipitates on the staphylococci, causing them to clump resulting in cell agglutination. The tube coagulase test detects both bound and free coagulase. Free coagulase reacts with a substance in plasma to form a fibrin clot.

TECHNICAL INFORMATION/LIMITATIONS

Slide coagulase test

Autoagglutination may occur.

Use water instead of saline as some staphylococci are salt sensitive, particularly if they have been cultured in salt media, and lysis or clumping of cells may occur.

Over mixing may cause the clots to break down.

Tube coagulase test

Citrated plasma may be clotted by any organism that can utilise citrate. Therefore use EDTA, oxalate or heparin plasma.

Longer incubation at 37°C may result in disappearance of the clot. This is due to the production of staphylokinase which can lyse the clot.

Commercial kits

Some strains of Meticillin Resistant Staphylococcus aureus may exhibit a negative or weak positive reaction.

Latex kits can also detect Protein A making them more sensitive than the coagulase test.
1 SAFETY CONSIDERATIONS

Refer to current guidance on the safe handling of all organisms and reagents documented in this NSM.

All work likely to generate aerosols must be performed in a microbiological safety cabinet.

The above guidance should be supplemented with local COSHH and risk assessments.

Compliance with postal and transport regulations is essential.

2 REAGENTS AND EQUIPMENT

Discrete bacterial colonies growing on solid medium.

Test solution

Slide coagulase test: Commercially available plasma (Ethylene diamine-tetraacetic acid, EDTA added)

Tube coagulase test: Commercially available plasma (EDTA added) suitable for tube coagulase. Use the plasma according to manufacturer’s instructions unless an alternative method has been validated. A commercial kit may be used, follow manufacturer’s instructions.

Bacteriological loop (preferably nichrome) or disposable alternative or disposable Pasteur pipette.

3 QUALITY CONTROL ORGANISMS

Positive control: Staphylococcus aureus NCTC 6571

Negative control: Staphylococcus epidermidis NCTC 4276
4 PROCEDURE AND RESULTS \textsuperscript{2,3,12,13}

4.1 SLIDE COAGULASE TEST

- Place a drop of distilled water on a slide
- Emulsify the test strain to obtain a homogenous thick suspension. False negative reactions will occur if the bacterial suspension is not heavy enough
- Observe for auto-agglutination
- Dip a straight wire or loop in the plasma
- Mix gently with the homogenous suspension

NB: Strains which auto-agglutinate must be tested by an alternative procedure.

Positive result: visible clumping within 10 seconds

Negative result: no visible clumping within 10 seconds

NB Should include 2 drops of suspension on the slide. Add plasma to one only and the other serves as an autoagglutination control.

4.2 TUBE COAGULASE TEST\textsuperscript{3,13}

- Place approximately 1 mL of commercially available plasma suitable for tube coagulase in a tube. This should be diluted according to manufacturer's instructions unless an alternative method has been validated
- Emulsify representative colony/colonies of the test organism in the plasma. Incubate at 35-37°C and examine hourly up to 4 h
- Examine for a clot which gels the whole contents of the tube or forms a loose web of fibrin.
- If negative, incubate overnight at 22-25°C and re-examine at 24 hours.

Positive result: formation of a clot up to 4 hours at 37°C or following overnight incubation at 22-25°C

Negative result: no clot, plasma moves freely at 4 hours and 24 hours incubation
<table>
<thead>
<tr>
<th>Species</th>
<th>Tube coagulase test</th>
<th>Slide coagulase test</th>
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<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>+</td>
<td>+</td>
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<tr>
<td>Subspecies aureus</td>
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<tr>
<td><em>Staphylococcus aureus</em></td>
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<td>Subspecies anaerobius</td>
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<td><em>Staphylococcus schleiferi</em></td>
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<td>Subspecies coagulans</td>
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<tr>
<td><em>Staphylococcus intermedius</em></td>
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<td>d</td>
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<tr>
<td><em>Staphylococcus hyicus</em></td>
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</tbody>
</table>

Table taken from reference 2
*rare clinical isolates

d = 11-89% of strains positive
(+)= delayed reaction
5 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the National Standard Methods Working Group for Clinical Bacteriology (http://www.hpa-standardmethods.org.uk/wg_bacteriology.asp). The contributions of many individuals in clinical bacteriology laboratories and specialist organisations who have provided information and comment during the development of this document, and final editing by the Medical Editor are acknowledged.

The National Standard Methods are issued by Standards Unit, Department for Evaluations, Standards and Training, Centre for Infections, Health Protection Agency, London.

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APPENDIX: COAGULASE TEST FLOWCHART

Isolate from pure culture

Slide coagulase test
- Place a drop of distilled water on two sides.
- Emulsifying the inocula to obtain a suspension

Tube coagulase test
- Test of plasma
  - Emulsify one colony

Mix with plasma
- Do not mix with plasma (preferably side)

Positive
- Visible clumping within 10 seconds

Negative
- No visible clumping

Positive
- Positive agglutination

Negative
- No visible clumping

Positive
- Clot formation following 1-4 hours incubation

Negative
- No clot formation following incubation

Note:
Positive control: Staphylococcus aureus NCTC6571
Negative control: Staphylococcus epidermidis NCTC4276
REFERENCES


