

NATIONAL STANDARD METHOD

COAGULASE TEST

BSOP TP 10

Issued by Standards Unit, Department for Evaluations, Standards and Training
Centre for Infections



COAGULASE TEST

Issue no: 4.1 Issue date: 09.12.10 Issued by: Standards Unit, Department for Evaluations, Standards and Training Page no: 1 of 11
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STATUS OF NATIONAL STANDARD METHODS

National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

National Standard Methods are developed, reviewed and updated through an open and wide consultation process where the views of all participants are considered and the resulting documents reflect the majority agreement of contributors.

Representatives of several professional organisations, including those whose logos appear on the front cover, are members of the working groups which develop National Standard Methods. Inclusion of an organisation's logo on the front cover implies support for the objectives and process of preparing standard methods. The representatives participate in the development of the National Standard Methods but their views are not necessarily those of the entire organisation of which they are a member. The current list of participating organisations can be obtained by emailing standards@hpa.org.uk.

The performance of standard methods depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

Whereas every care has been taken in the preparation of this publication, the Health Protection Agency or any supporting organisation cannot be responsible for the accuracy of any statement or representation made or the consequences arising from the use of or alteration to any information contained in it. These procedures are intended solely as a general resource for practising professionals in the field, operating in the UK, and specialist advice should be obtained where necessary. If you make any changes to this publication, it must be made clear where changes have been made to the original document. The Health Protection Agency (HPA) should at all times be acknowledged.

The HPA is an independent organisation dedicated to protecting people's health. It brings together the expertise formerly in a number of official organisations. More information about the HPA can be found at www.hpa.org.uk.

The HPA aims to be a fully Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions¹.

More details can be found on the website at www.evaluations-standards.org.uk. Contributions to the development of the documents can be made by contacting standards@hpa.org.uk.

The reader is informed that all taxonomy in this document was correct at time of issue.

Please note the references are now formatted using Reference Manager software. If you alter or delete text without Reference Manager installed on your computer, the references will not be updated automatically.

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AMENDMENT PROCEDURE

Controlled document reference	BSOP TP 10
Controlled document title	Coagulase Test

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.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
4/ 11.03.10	3	4		Whole document	Document reviewed, no updates required
5/ 9.12.10	4	4.1	1	Front page	The Association of Medical Microbiologist logo replaced with new British Infection Association logo.
			6	Quality control organisms	Negative control amended from <i>Staphylococcus epidermidis</i> to <i>Staphylococcus haemolyticus</i>
			10	Appendix	Flowchart amended

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COAGULASE TEST

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 known².

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 down³.

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.

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1 SAFETY CONSIDERATIONS⁴⁻¹⁰

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 ORGANISMS¹¹

Positive control: *Staphylococcus aureus* NCTC 6571

Negative control: *Staphylococcus haemolyticus* NCTC 4276

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4 PROCEDURE AND RESULTS^{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^{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

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.Species	Tube coagulase test	Slide coagulase test
<i>Staphylococcus aureus</i> Subspecies <i>aureus</i>	+	+
<i>Staphylococcus aureus</i> Subspecies <i>anaerobius</i>	+	-
<i>Staphylococcus schleiferi</i> Subspecies <i>coagulans</i>	+	-
<i>Staphylococcus lugdunensis</i>	-	(+)
<i>Staphylococcus schleiferi</i> Subspecies <i>shleiferi</i>	-	+

<i>Staphylococcus delphini</i> *	+	-
<i>Staphylococcus intermedius</i> *	+	d
<i>Staphylococcus hyicus</i> *	d	-

Table taken from reference ²

*rare clinical isolates

d = 11-89% of strains positive

(+) = delayed reaction

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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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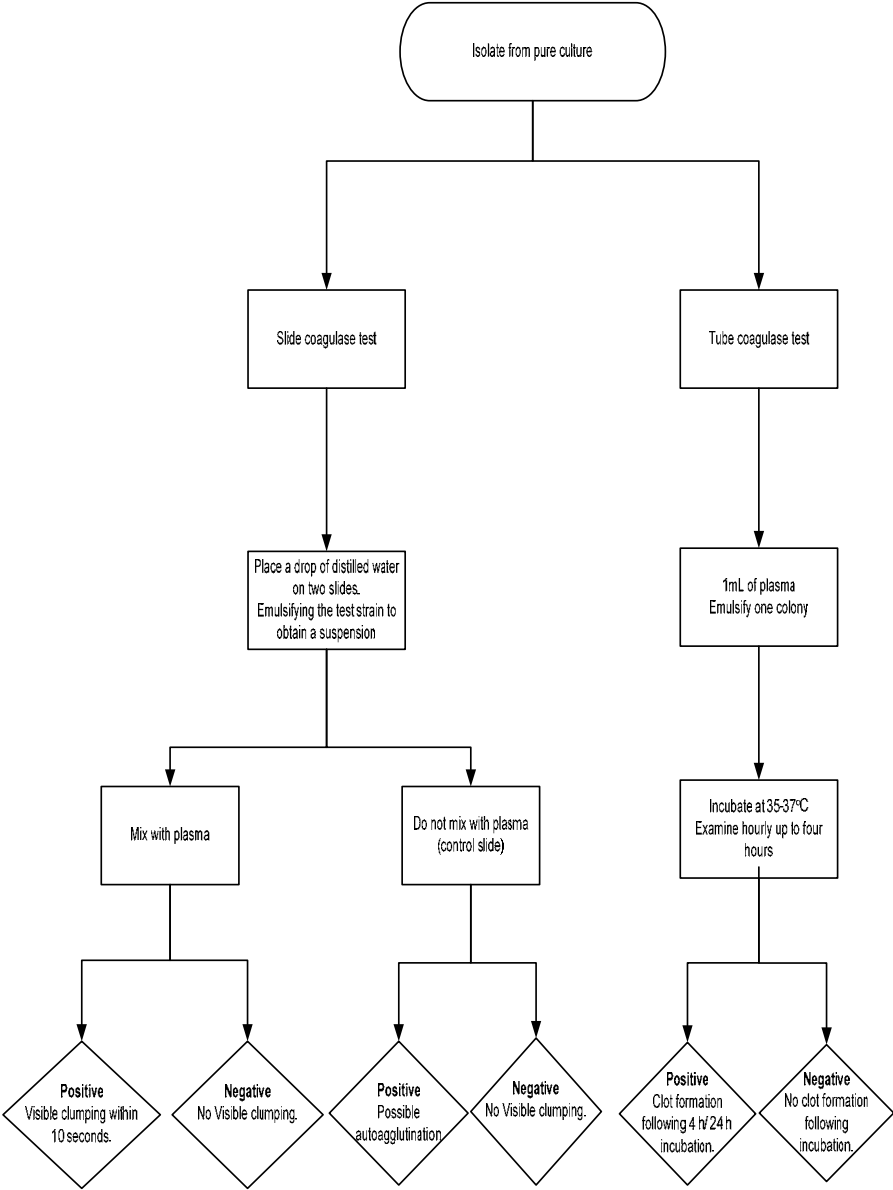
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APPENDIX: COAGULASE TEST FLOWCHART



Note:

Positive control: *Staphylococcus aureus* NCTC 6571

Negative control: *Staphylococcus haemolyticus* NCTC 4276

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