



UK Standards for Microbiology Investigations

Coagulase Test



Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website <http://www.hpa.org.uk/SMI/Partnerships>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <http://www.hpa.org.uk/SMI/WorkingGroups>).

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Contents

ACKNOWLEDGMENTS	2
CONTENTS	3
AMENDMENT TABLE	4
UK STANDARDS FOR MICROBIOLOGY INVESTIGATIONS: SCOPE AND PURPOSE.....	5
SCOPE OF DOCUMENT	8
INTRODUCTION	8
TECHNICAL INFORMATION/LIMITATIONS.....	8
1 SAFETY CONSIDERATIONS	9
2 REAGENTS AND EQUIPMENT	9
3 QUALITY CONTROL ORGANISMS	9
4 PROCEDURE AND RESULTS.....	9
APPENDIX: COAGULASE TEST.....	11
REFERENCES	12



NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment Table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	7/13.03.14
Issue no. discarded.	4.2
Insert Issue no.	4.3
Section(s) involved	Amendment
Whole document.	<p>Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England.</p> <p>Front page has been redesigned.</p> <p>Status page has been renamed as Scope and Purpose and updated as appropriate.</p> <p>Professional body logos have been reviewed and updated.</p> <p>Standard safety and notification references have been reviewed and updated.</p> <p>Scientific content remains unchanged.</p>

Amendment No/Date.	6/21.10.11
Issue no. discarded.	4.1
Insert Issue no.	4.2
Section(s) involved	Amendment
Whole document.	Document presented in a new format.
References.	Some references updated.

UK Standards for Microbiology Investigations[#]: Scope and Purpose

Users of SMIs

- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK.
- SMIs provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests.
- SMIs provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post-analytical (result interpretation and reporting) stages.

Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies.

The list of participating societies may be found at <http://www.hpa.org.uk/SMI/Partnerships>. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process.

SMIs are developed, reviewed and updated through a wide consultation process.

[#]Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development.

The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information Governance and Equality

PHE is a 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 conditions.

The development of SMIs are subject to PHE Equality objectives http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1317133470313. The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

SMIs are Crown copyright which should be acknowledged where appropriate.

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UNDER REVIEW

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 known¹.

This SMI should be used in conjunction with other SMIs.

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 down².

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.

Comments/kits

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

Later kits can also detect Protein A making them more sensitive than the coagulase test.

1 Safety Considerations³⁻¹⁹

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

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 nitrome) or disposable alternative or disposable Pasteur pipette.

3 Quality Control Organisms²⁰

Positive Control

Staphylococcus aureus NCTC 6571

Negative Control

Staphylococcus haemolyticus NCTC 4276

4 Procedure and Results^{1,2,21,22}

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

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

Positive Result

Visible clumping within 10s.

Negative Result

No visible clumping within 10s.

Note: Two drops of suspension should be included on the slide. Add plasma to one only and the other serves as an autoagglutination control.

4.2 Tube Coagulase Test^{2,22}

- Place approximately 1ml 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 4hr
- 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 24hr

Positive Result

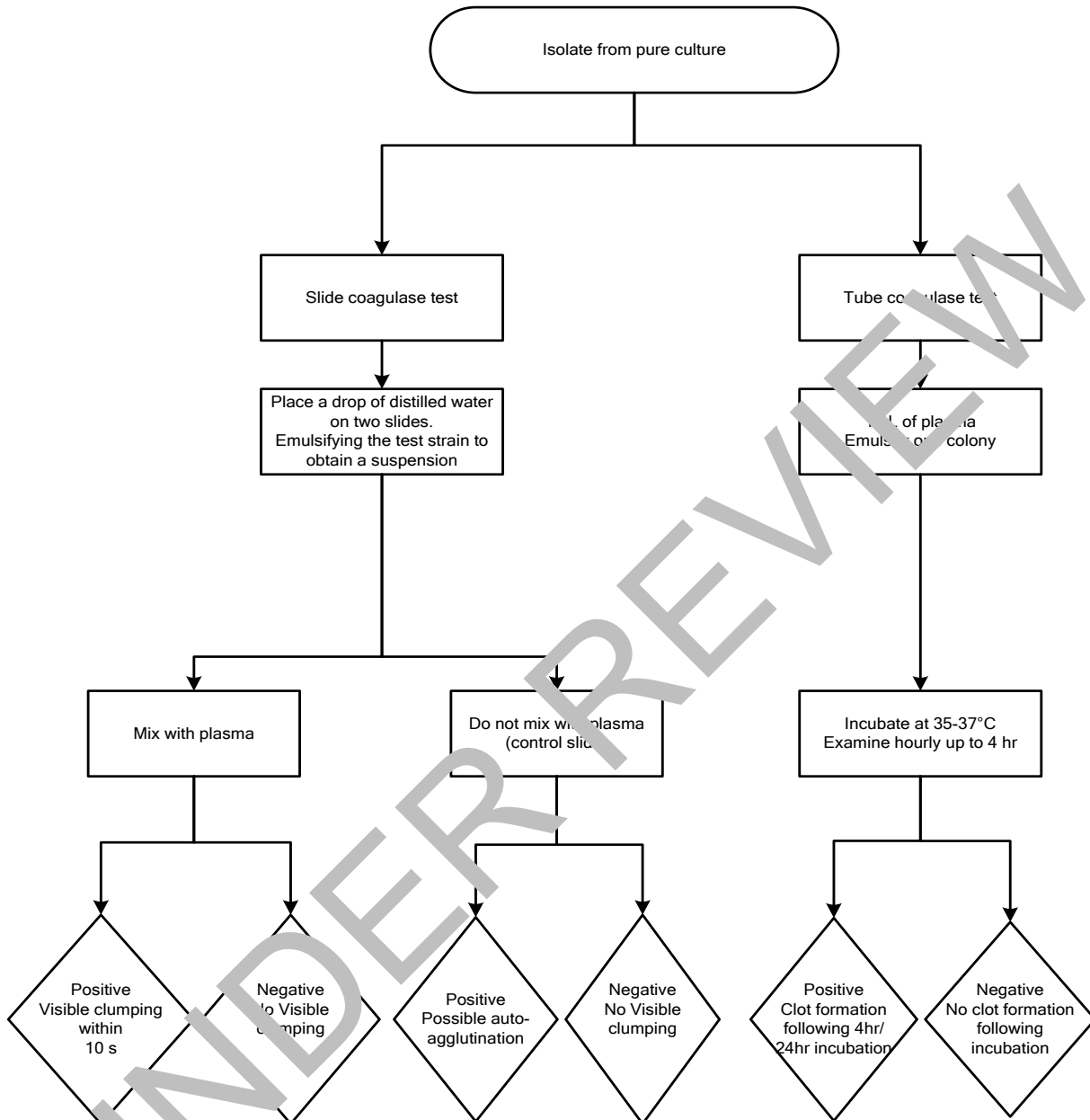
Formation of a clot up to 4hr at 37°C or following overnight incubation at 22-25°C or following overnight incubation at 22-25°C

Negative Result

No clot, plasma moves freely at 4hr and 24hr incubation.

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>regularis</i>	+	-
<i>Staphylococcus lugdunensis</i>	-	(+)
<i>Staphylococcus schleiferi</i> Subspecies <i>schleiferi</i>	-	+
<i>Staphylococcus delphini</i> *	+	-
<i>Staphylococcus intermedius</i> *	+	d
<i>Staphylococcus hyicus</i> *	d	-
Table taken from reference ¹ D=11-89% of strains positive *rare clinical isolates (+)=delayed reaction		

Appendix: Coagulase Test



Note:

Positive control: *Staphylococcus aureus* NCTC 6571

Negative control: *Staphylococcus haemolyticus* NCTC 4276

The flowchart is for guidance only.

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UNDER REVIEW