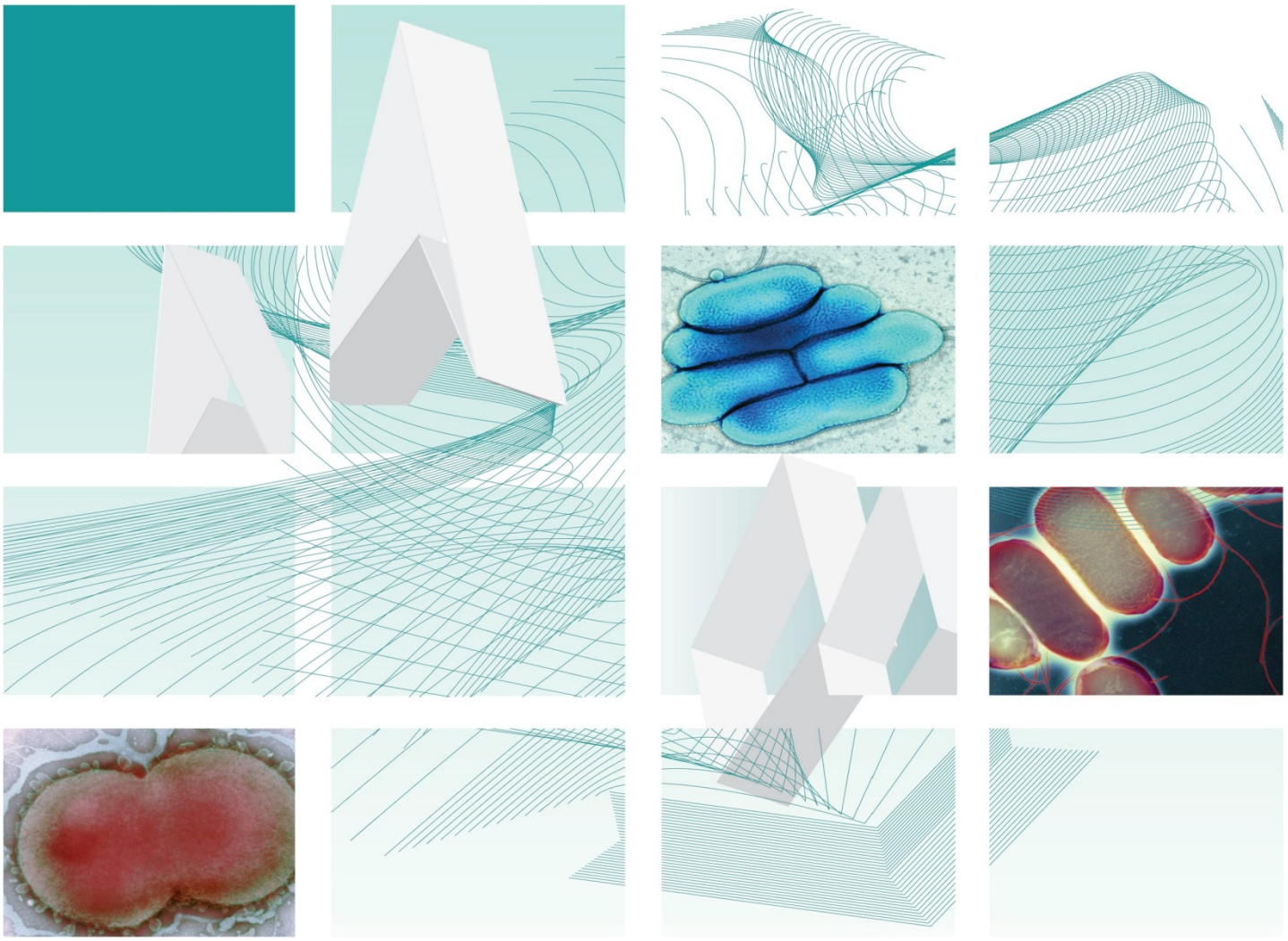
Public Health England

NHS

UK Standards for Microbiology Investigations

Coagulase Test



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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 https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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UK Standards for Microbiology Investigations are produced in association with:

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Contents

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.	8/18.11.14
Issue no. discarded.	4.3
Insert Issue no.	5
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Technical information/Limitations.	This section has been updated and references added. Additional information on rapid screening coagulase test and commercial kits are mentioned.
Reagents/Equipment.	This section has been updated with references.
Quality control Organisms.	The negative control strain for the coagulase test has been updated from NCTC 4276 to NCTC 11042.
Procedures and Results.	This has been updated with references.
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 https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. 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 https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity. 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

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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.

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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[1].

This SMI should be used in conjunction with other SMIs.

Introduction

Coagulase is a protein enzyme produced by several microorganisms that enables the conversion of fibrinogen to fibrin. Coagulase binds plasma fibrinogen, causing the organisms to agglutinate or 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 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

The colony inoculum used for testing must be pure because a contaminant may produce false results after prolonged incubation.

Slide coagulase test

This test is unsuitable for isolates that are not easily emulsified[2].

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[3].

S. schleiferi and *S. lugdunensis* may give positive results in the slide coagulase test[2,4].

Tube coagulase test

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].

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[5].

Some other species of staphylococci, including *Staphylococcus schleiferi* and *Staphylococcus intermedius* may give positive results in the tube coagulase test but are not common isolates from human infections[2,4].

The tube coagulase test should not be unduly agitated as this can cause the clot to shrink also giving a false negative result[6].

Rapid screening coagulase test

The main advantage of the direct tube coagulase test is its rapidity in identifying *S. aureus* in blood culture broths and reducing turnaround times and it is also helpful in initiating appropriate antimicrobial treatment[7,8]. It is a valuable adjunct in the routine microbiology laboratory because of its good performance, technical simplicity and low cost.

Care should be taken when using this test directly on presumptive positive coagulase blood culture broths. Several investigators have evaluated the accuracy of the two agglutination tests in rapid identification of *S. aureus* from positive blood culture broths[7,9,10]. Although overall specificity has been excellent, a wide range of sensitivities have been reported for a variety of latex tests. Studies have confirmed that slide coagulase kits should not be used for the rapid identification of *S. aureus* from blood culture broths as they have been principally designed for isolates grown on solid culture media and lack sensitivity. Manufacturer's instruction should be followed.

However, the tube coagulase test is the preferred method for the rapid screening test for *S. aureus* in blood culture broths and negative blood culture results should be re-tested using standard laboratory techniques (Gram stain, subculturing of broths and retesting from the culture plates). There have been reports of negative blood culture results when tested directly, but when repeated from culture plates are then positive[7]. Another limitation that may deter laboratories from its use is the labour intensive, double centrifugation step. Moreover, two recent reports have indicated no loss of sensitivity when the tube coagulase test is performed directly on uncentrifuged blood culture broths[10,11].

If a rapid screening coagulase test is done on blood culture broths, confirmation should be done using conventional methods.

Some strains of Meticillin Resistant *Staphylococcus aureus* may exhibit a negative or weak positive reaction. In addition, rare strains of *S. aureus* are negative in coagulase tests[2].

Commercial kits

Commercial kits are available using latex technology. These kits can detect Protein A and or clumping factor but can also detect various surface antigens, making them more sensitive than the coagulase test but at some expense to specificity due to cross-reaction with Coagulase Negative Staphylococci. In addition, any test including clumping factor may give false positive results with *Staphylococcus lugdunensis* and *Staphylococcus schleiferi*[2].

1 Safety Considerations[12-28]

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

Aseptic technique and established precautions against microbiological hazards throughout all procedures must be observed.

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

Fresh discrete bacterial colonies growing on solid medium including the positive and negative control organisms:

Distilled water

Microscope slide

Bacteriological loop (preferably nichrome) or disposable alternative

Disposable Pasteur pipette

Marking pen or wax pencil

Sterile test tubes

Test tube rack

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.

Commercially lyophilized products (or kits) are also available and manufacturer's instructions should be followed.

3 Quality Control Organisms

Refer to TP 1- Example Reference Strains for UK SMI Test Procedures

Positive Control

Staphylococcus aureus NCTC 6571

Negative Control

Staphylococcus haemolyticus NCTC 11042

4 Procedure and Results

4.1 Slide Coagulase Test[1,29]

- Place two drops of distilled water on a clean glass slide. Identify where the test strain (T) and the control(C) will be placed by labelling the slide. An additional slide will be required for the control strains and this should be clearly labelled.
- Set up the positive and negative control organisms on the same slide to be tested simultaneously.
- 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 autoagglutination. Strains which autoagglutinate must be tested by an alternative procedure.
- Dip a straight wire or loop in the plasma and stir gently with the homogenous suspension. If using a reusable loop, sterilize the loop before proceeding.

Note: Plasma is added only to the test strain and the control organisms but not the control (C) as it serves as an autoagglutination control.

- Observe for immediate formation of white clumps.

Positive Result

Visible clumping within 10s.

Negative Result

No visible clumping within 10s.

Note: The positive control species should show clumping only when emulsified in the plasma and the negative control species should not show clumping in either water (saline) or plasma[4].

4.2 Tube Coagulase Test[1-3,6,29-31]

4.2.1 Tube coagulase Test direct from colonies

- Use commercially available plasma and this should be diluted according to manufacturer's instructions unless an alternative method has been validated
- Label the test tubes with the organism to be tested as well as the control organisms
- 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 by the end of 4hr, incubate overnight at room temperature (22°C) and re-examine at 24hr.This is because a small proportion of strains require longer than 4hr for clot formation[2]

4.2.2 Tube coagulase Test direct from blood culture broth

This can be used for a rapid presumptive identification of *S. aureus* in Blood culture broths.

- Add 1 – 2 drops of the positive flagged blood culture broth to 2mL of the diluted plasma in a tube or bijou
- 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 by the end of 4hr, incubate overnight at room temperature (22°C) and re-examine at 24hr.This is because a small proportion of strains require longer than 4hr for clot formation[2]

Note: Always check identification the following day from the culture plate using a slide coagulase or latex test

Positive Result

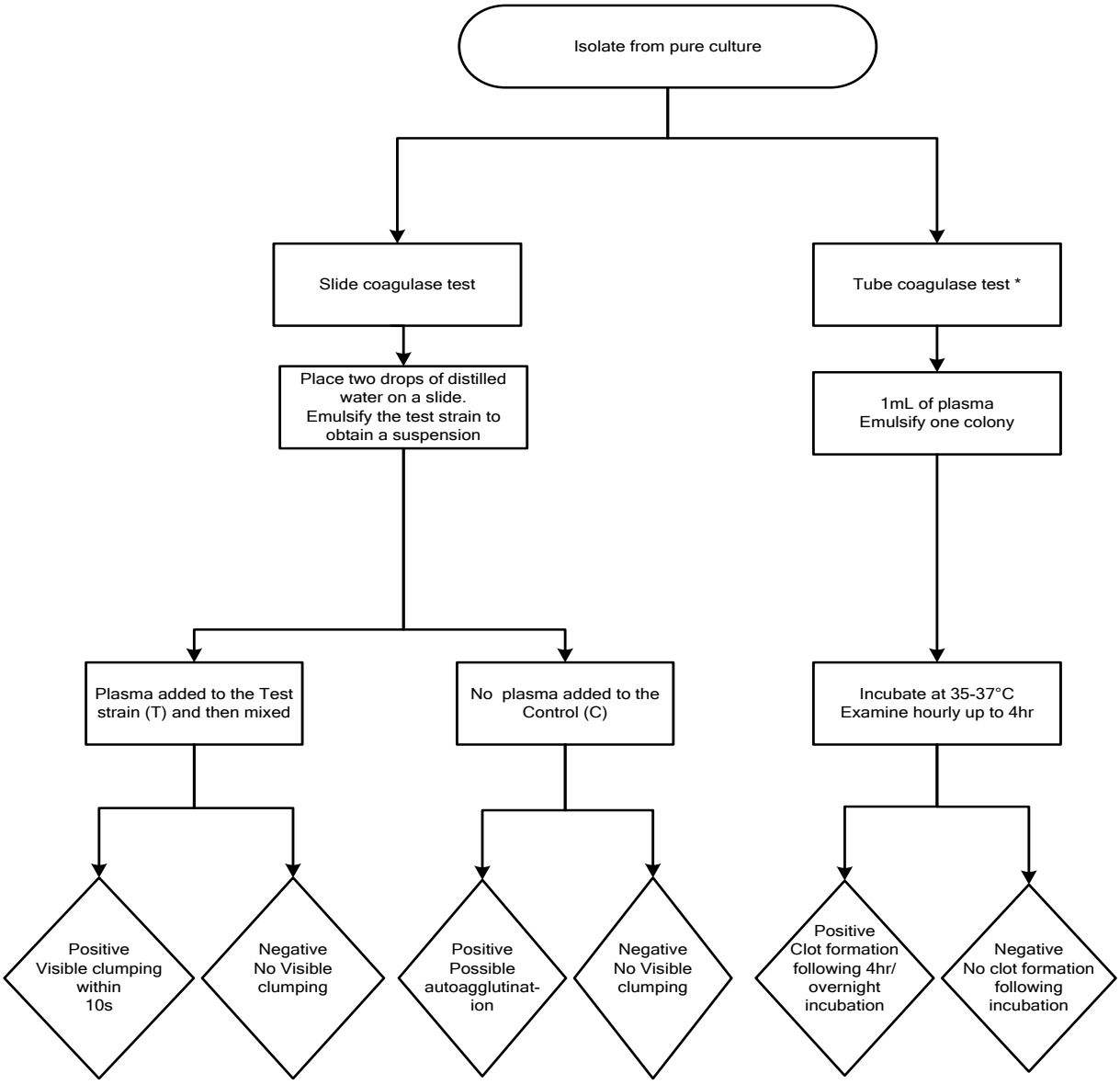
Formation of a clot up to 4hr at 37°C or following overnight incubation at room temperature (22°C).

Negative Result

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

Species	**Tube coagulase test**	**Slide coagulase test**
Staphylococcus aureus subspecies *aureus*	+	+
Staphylococcus aureus subspecies *anaerobius*	+	-
Staphylococcus schleiferi subspecies *coagulans*	-	+
Staphylococcus lugdunensis	-	+
Staphylococcus schleiferi subspecies *schleiferi*	-/+	+
*Staphylococcus delphini**	+	-
*Staphylococcus intermedius**	+	v
*Staphylococcus hyicus**	v	-
Table taken from reference[1,2] V= variable reaction - = negative reaction *rare clinical isolates +=positive reaction		

Appendix: Coagulase Test



Note:

Positive control: *Staphylococcus aureus* NCTC 6571

Negative control: *Staphylococcus haemolyticus* NCTC 11042

*** If blood cultures are tested directly by tube coagulase test and is found to be negative, follow the procedure for culture above for re-testing.**

The flowchart is for guidance only.

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