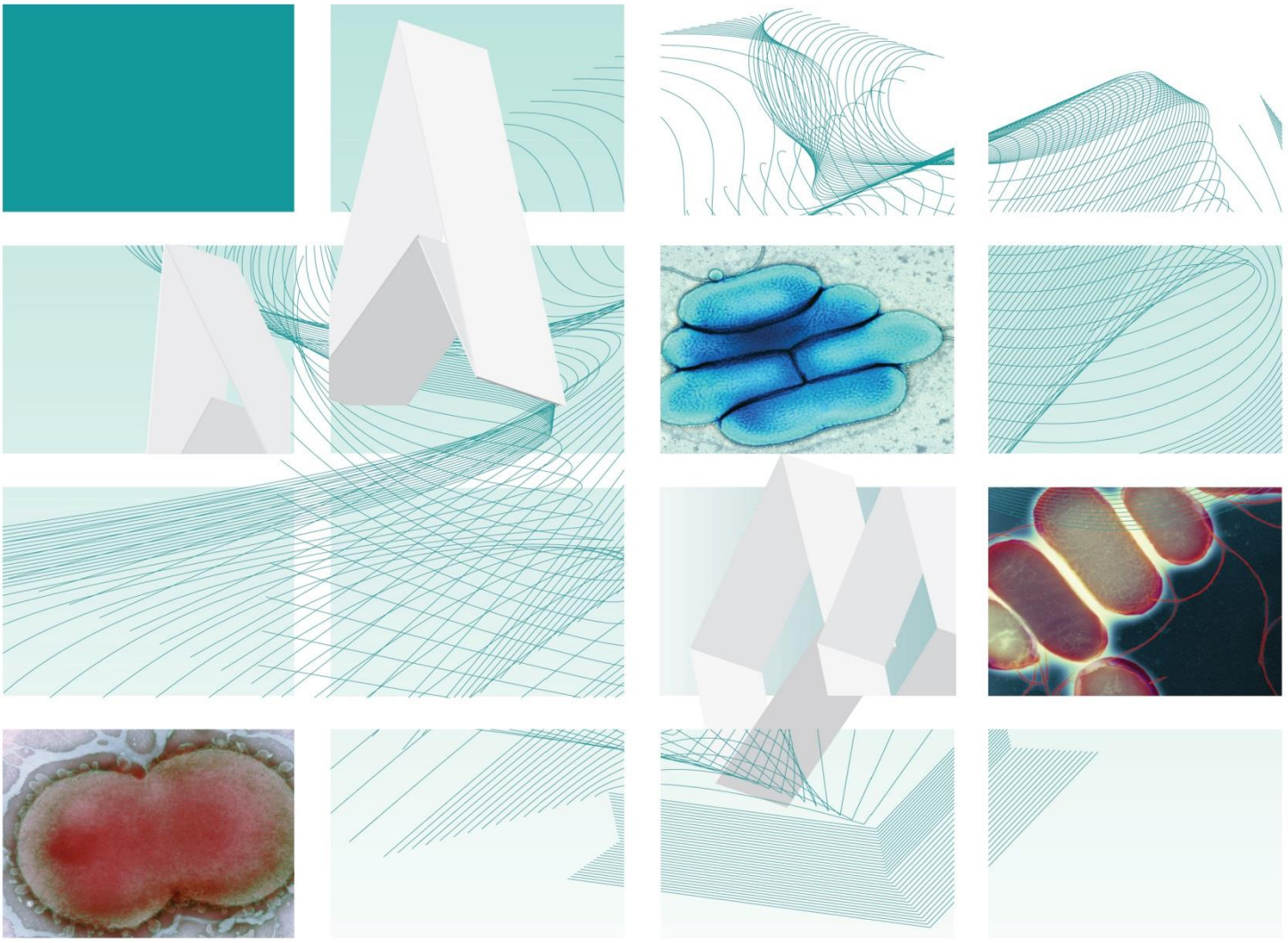
Public Health England

NHS

UK Standards for Microbiology Investigations

Coagulase test



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Issued by the Standards Unit, National Infection Service, PHE

Acknowledgments

UK Standards for Microbiology Investigations (UK 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. UK 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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Amendment table

Each UK 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 number/date	9/01.08.18
Issue number discarded	5
Insert issue number	6
Anticipated next review date*	01.08.21
Section(s) involved	**Amendment**
Whole document.	Document updated. Technical limitations updated with subheadings. Information on plasma use and NCTC 6571 added to the technical limitations/information. Link to UK SMI TP 34: Thermonuclease test document added to this document. References updated with grades.

*Reviews can be extended up to five years subject to resources available.

UK SMI[#]: scope and purpose

Users of UK SMIs

Primarily, UK SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. UK SMIs also 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. The documents also 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 UK SMIs

UK 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. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of UK 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

UK 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 UK SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing UK SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop UK 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. UK SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the UK SMI working groups to produce UK SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of UK SMIs is certified to ISO 9001:2008. UK SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. UK SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level

[#] 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.

of complex laboratory investigation possible. In using UK SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. UK SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. UK SMIs also provide a reference point for method development. The performance of UK 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 UK SMI working groups are committed to patient and public involvement in the development of UK SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting UK 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 UK SMIs is subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The UK 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

While every care has been taken in the preparation of UK SMIs, PHE and the partner organisations, 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 UK SMI or any information contained therein. If alterations are made by an end user to an UK SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as UK SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

The evidence base and microbial taxonomy for the UK SMI is as complete as possible at the date 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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Suggested citation for this document

Public Health England. (2018). Coagulase test. UK Standards for Microbiology Investigations. TP 10 Issue 6. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

Scope of document

This test detects the ability of bacteria to clot plasma by the action of the enzyme, coagulase. This test is used specifically to differentiate the species within the genus *Staphylococcus*. There are many hypotheses but the precise mechanism of coagulase action is not known[1].

This UK SMI should be used in conjunction with other UK 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 both free and bound coagulase are 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. Free coagulase reacts with a substance in plasma to form a fibrin clot.

Technical information/limitations

Purity of inoculum used

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 on salt containing media (such as Mannitol Salt Agar), as lysis or clumping of cells may occur. Do not perform slide coagulase tests from colonies grown from salt containing media.

Over mixing may cause the clots to break down[3].

S. schleiferi and *S. lugdunensis* may give positive results on 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].

Plasma use

Commercially available lyophilized products (or kits) are available and manufacturers' instructions should be followed when using these products.

Tube coagulase test direct from blood culture broth

The main advantage of the direct tube coagulase test is its rapidity in presumptive identification of *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 two agglutination tests in rapid identification of *S. aureus* from positive blood culture broths and although overall specificity has been excellent, a wide range of sensitivities have been reported with a variety of latex tests[7,9,10]. 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.

The tube coagulase test is the preferred method for the rapid screening test for *S. aureus* in blood culture broths and when reported negative, blood culture isolates 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 coagulase test results when tested directly on blood cultures, but when repeated from culture isolates, are reported 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].

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].

Refer to UK SMI TP 34: Thermonuclease test procedure for information on how to use this test to determine the presence of *S. aureus* in positive blood culture bottles.

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].

NCTC 6571

The NCTC strain known as the "*Oxford Staphylococcus*" is widely used in clinical diagnostic microbiology laboratories throughout the UK as a reference control strain for antimicrobial susceptibility testing and other phenotypic tests such as coagulase test (regarded as a weak positive control), determination of DNase activity, etc.

Recent studies have shown that this strain produces Panton-Valentine Leukocidin (PVL), a pore-forming cytotoxin produced by fewer than 5% of *Staphylococcus aureus* strains that causes leukocyte destruction and tissue necrosis. Therefore it is recommended that good practice should be adhered to when this organism is handled[12].

1 Safety considerations[13-30]

Refer to current guidance on the safe handling of all organisms and reagents documented in this UK 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 (non-salt containing media) 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 treated with ethylene diamine-tetraacetic acid (EDTA).

Tube coagulase test:

Commercially available plasma treated with EDTA added suitable for tube coagulase. Use the plasma according to manufacturer's instructions unless an alternative method has been validated.

Note: 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 or NCTC 12973

Negative control

Staphylococcus haemolyticus NCTC 11042

Note: These strains have been validated by NCTC to give this result.

4 Procedure and results

4.1 Slide coagulase test[1,31]

- 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[1-3,6,31-33]

4.2.1 Tube coagulase test direct from colonies

- use commercially available plasma and dilute 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. Do not shake or agitate the tube
- gently slant and 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 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

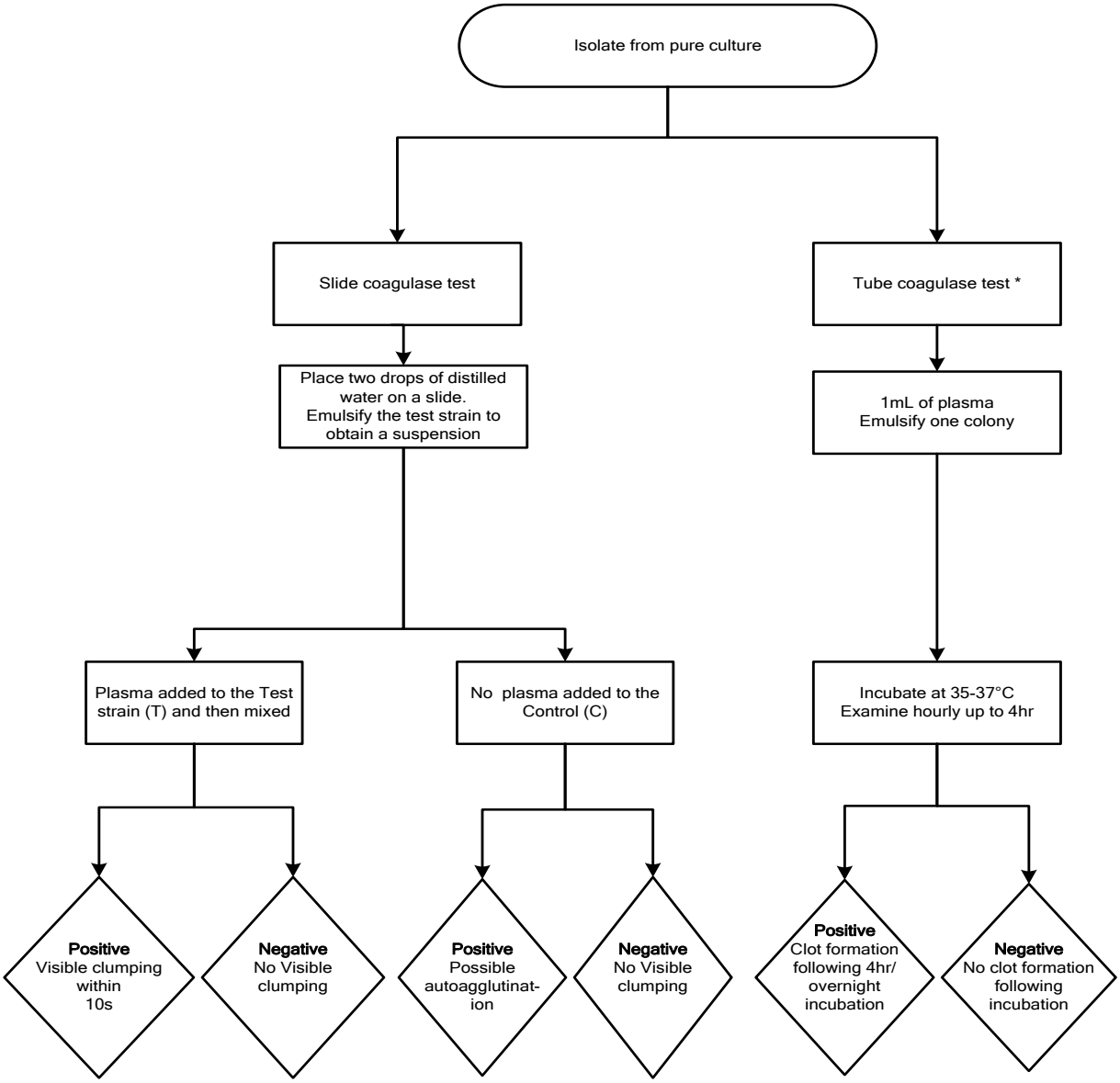
No clot, plasma moves freely at 4hr and 24hr incubation.

Table 1: Summary of coagulase test of some *Staphylococcus* species that may cause infections in humans[1, 2].

Species	Tube coagulase test	Slide coagulase test
Staphylococcus aureus subspecies *aureus*	+	+
Staphylococcus aureus subspecies *anaerobius*	+	-
Staphylococcus epidermidis	-	-
Staphylococcus haemolyticus	-	-
Staphylococcus saprophyticus subspecies saprophyticus	-	-
Staphylococcus schleiferi subspecies *coagulans*	-	+
Staphylococcus lugdunensis	-	+
Staphylococcus schleiferi subspecies *schleiferi*	-/+	+
*Staphylococcus delphini**	+	-

*Staphylococcus intermedius**	+	v
*Staphylococcus hyicus**	V	-
V= variable reaction *rare clinical isolates	- = negative reaction +=positive reaction	

Appendix: Coagulase test



Note:

Positive control: *Staphylococcus aureus* NCTC 6571 or NCTC 12973

Negative control: *Staphylococcus haemolyticus* NCTC 11042

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

The flowchart is for guidance only.

References

Modified GRADE table used by UK SMIs when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMIs for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VI). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Strength of recommendation	**Quality of evidence**
A Strongly recommended	I Evidence from randomised controlled trials, meta-analysis and systematic reviews
B Recommended but other alternatives may be acceptable	II Evidence from non-randomised studies
C Weakly recommended: seek alternatives	III Non-analytical studies, for example, case reports, reviews, case series
D Never recommended	IV Expert opinion and wide acceptance as good practice but with no study evidence
	V Required by legislation, code of practice or national standard
	VI Letter or other

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