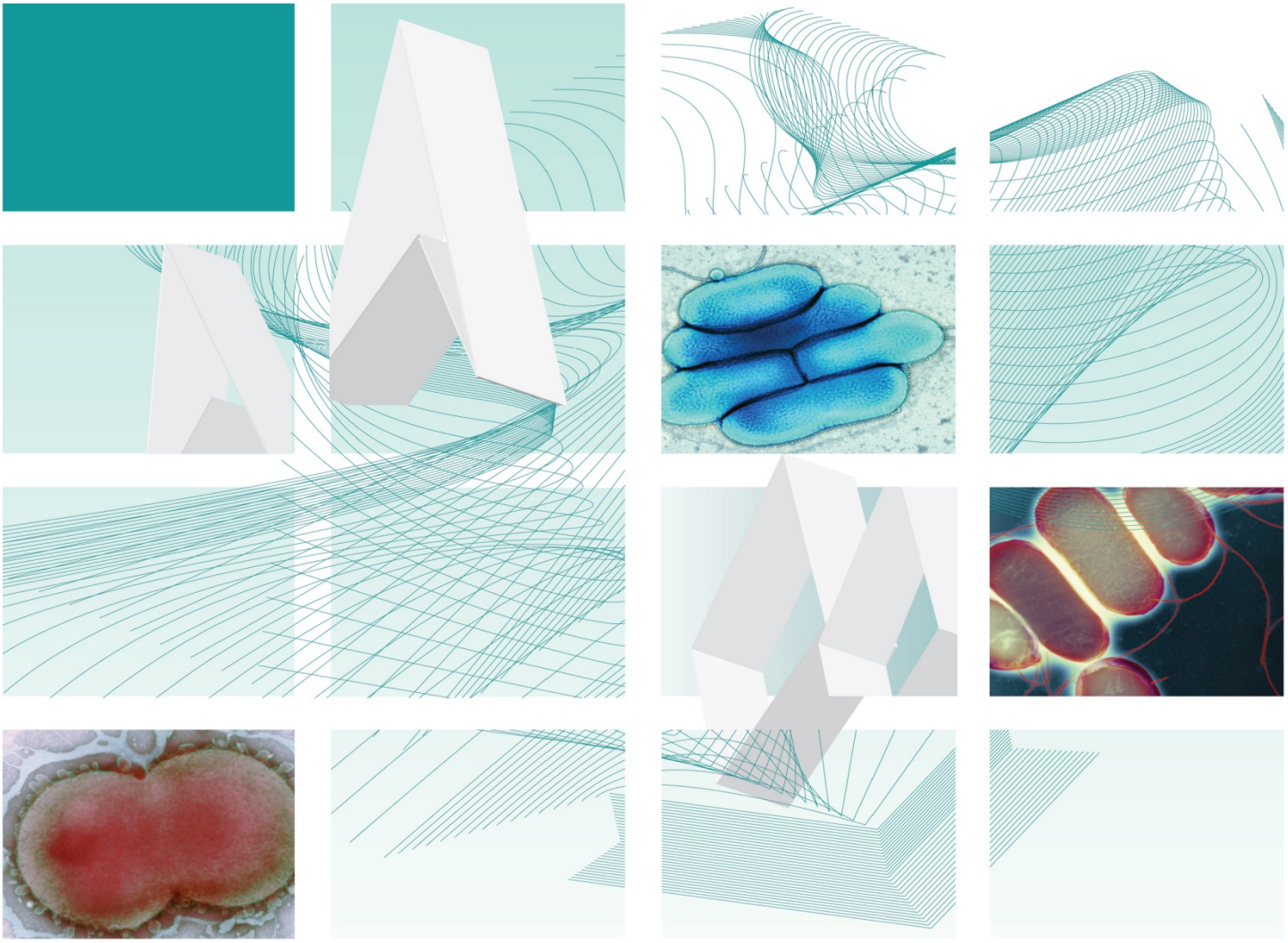
Public Health England

NHS

UK Standards for Microbiology Investigations

Thermonuclease test



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"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011.**"

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Bacteriology – Test Procedures | TP 34 | Issue no: 4 | Issue date: 08.05.19 | Page: 1 of 15

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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Logos correct at time of publishing.

Contents

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	6/08.05.19
Issue number discarded	3
Insert issue number	4
Anticipated next review date*	08.05.22
Section(s) involved	**Amendment**
Whole document.	Document updated. Technical limitations updated with subheadings. Information on use of NCTC 6571 added to the technical limitations/information. References updated with grades.
Quality control organisms.	Alternative bacterial positive NCTC strain listed for this test and EUCAST susceptibility test. The recommended NCTC strains have not been validated in this review.

*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

[#] 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.

accredited and represent neither minimum standards of practice nor the highest level 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.

UK SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested citation for this document

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Scope of document

The thermonuclease test is also known as the 'heat stable nuclease' test. It is a 4hr test based on the production of a heat stable DNase (thermonuclease) by *Staphylococcus aureus.*

It is also used for determining and confirming the presence of *S. aureus* subsp *aureus* DNase from that produced by *S. epidermidis*, enterococci or other micrococci[1].

This is of particular use in determining the presence of *S. aureus* in positive blood culture bottles[2].

This UK SMI should be used in conjunction with other UK SMIs.

Introduction

Unlike other staphylococci, most strains of *S. aureus* and *Staphylococcus intermedius* produce thermonuclease, a heat stable DNase.

Subspecies of *Staphylococcus schleiferi* are DNase positive and produce heat stable nucleases. The thermonuclease test detects the presence of this DNase.

The organism is heated to destroy heat labile thermonucleases. It is then inoculated on medium containing DNA and toluidine blue. The DNA is broken down by heat stable nucleases resulting in the toluidine blue changing to red or pink.

Technical information/limitations

Quality control

Toluidine blue DNA agar is subject to variation and each batch must be controlled and tested prior to use.

Other thermonuclease positive *Staphylococcus* strains

Subspecies of *Staphylococcus schleiferi,* some strains of *Staphylococcus hyicus* and *S. pseudintermedius* are thermonuclease positive.

Interpretation

Results should be interpreted within and up to 4hrs as the metachromatic colour change associated with the production of thermonuclease is stable for 2-4 hours, after which the dye slowly diffuses into the agar and loses its well-demarcated borders[1].

NCTC 6571

NCTC 6571 strain is widely used in diagnostic microbiology laboratories 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 leucocyte destruction and tissue necrosis. Therefore it is recommended that good practice should be adhered to when this organism is handled[3].

Further identification

Identification of thermonuclease positive *S. aureus* can be confirmed by coagulase and deoxyribonuclease tests and the other organisms by appropriate conventional methods[4].

1 Safety considerations[5-22]

Refer to current guidance on the safe handling of all organisms and reagents documented in this UK 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 or positive blood culture with typical staphylococcal morphology on the Gram stain[2].

DNase agar prepared according to the method described by Lachica et al 1971[23].

Bacteriological straight wire/loop (preferably nichrome) or disposable alternative.

Sterile disposable Pasteur pipette.

Sterile capped 13x100mm tubes.

Brain Heart Infusion broth.

Boiling water bath.

3 Quality control organisms

Positive control

Staphylococcus aureus NCTC 6571 or NCTC 12973

Negative control

Staphylococcus haemolyticus NCTC 11042

Note: The recommended NCTC strains have not been validated in this review by NCTC to give this result.

4 Procedure and results

4.1 Blood culture test method[2,4,24-26]

- dispense 2 - 3mL of blood broth from positive blood culture (showing Gram positive cocci in clusters on direct Gram stain) into a sterile capped 13x100mm tube
- heat tube at 100°C for 15min and cool to room temperature
- centrifuge at 1000 x g for 10min and collect the supernatant fluid
- cut 6mm diameter wells in plates of the toluidine blue DNA agar (maximum 12 wells per plate) using blunt end of a sterile pipette and fill each well with 2-3

drops of the supernatant from a different blood culture or controls. Alternatively, boiled blood cultures (not supernatant) may be put in the wells

- negative and positive control wells must be run simultaneously with test specimens on each plate. It should be noted that the controls used must be from blood culture bottles containing blood and the recommended positive and negative control strains used
- incubate the plate at 35-37°C in the upright position (agar side down)
- examine the plate at 1 hour, 2 hours, and 4 hours and again after overnight incubation if negative at 4 hours

4.2 Colony test method[1]

- inoculate several colonies of the isolate to be tested into 1mL of the Brain Heart Infusion broth
- incubate at 35-37°C for 2hr
- heat suspension at 100°C for 15min
- allow to cool to room temperature
- cut 6mm diameter wells (using the blunt end of a sterile pipette) in the plate of the toluidine blue DNA agar (maximum 12 wells per plate)
- fill each well with the cooled broth suspension
- incubate at 35-37°C and examine hourly for up to 4hr. Do not invert the plate

Interpretation

Positive result

Pink zone of clearing at the edge of the well with a darker blue ring at the outer periphery of the zone; indicates thermonuclease activity and that the organism is *Staphylococcus aureus*.

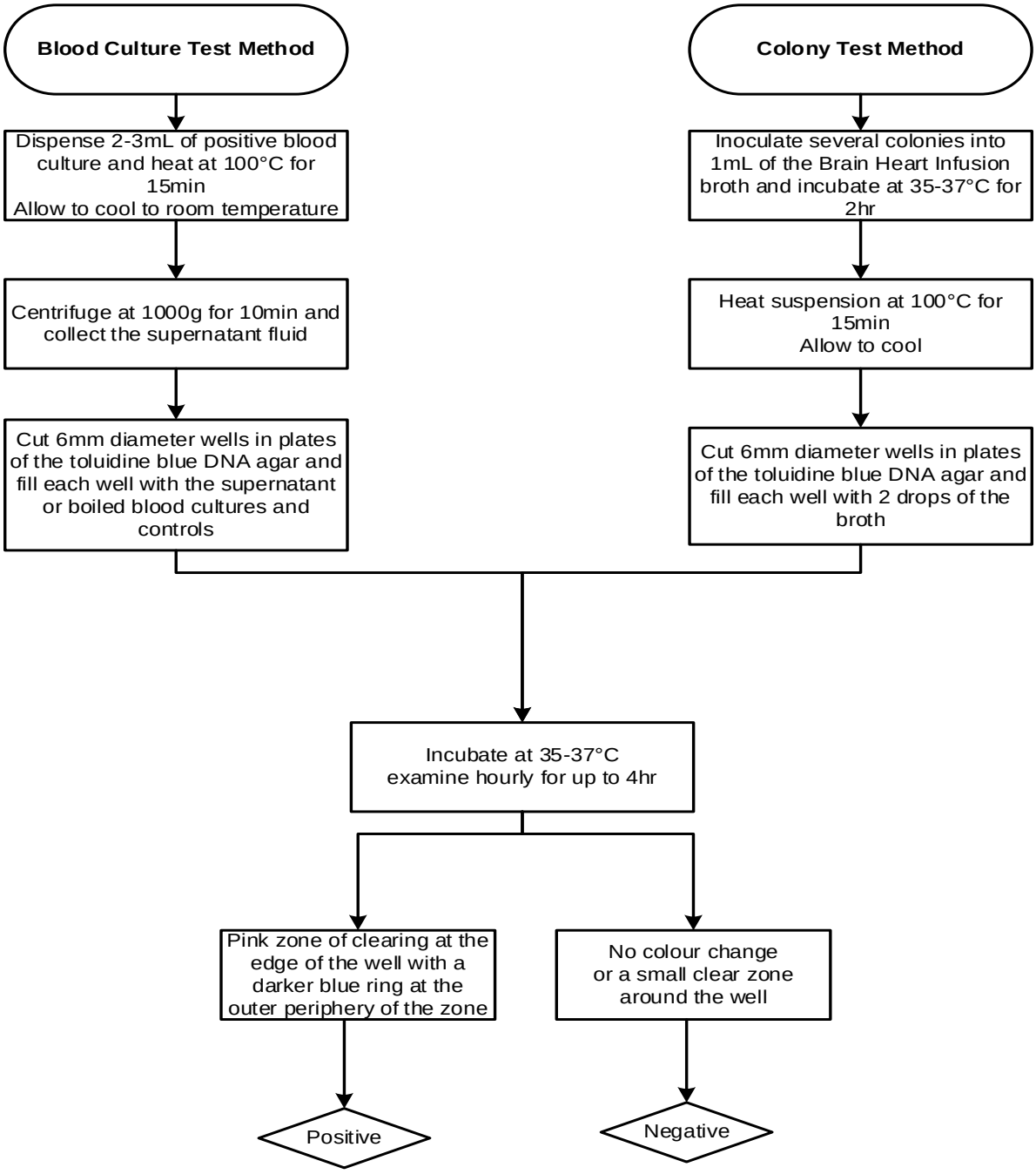
Negative result

No zone or a small clear zone of clearing around the well.

OR

No colour change.

Appendix: Thermonuclease test



Note:

Positive control	*Staphylococcus aureus* NCTC 6571 or NCTC 12973
Negative control	*Staphylococcus haemolyticus* NCTC 11042

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-VIII). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Quality/certainty of evidence	Types 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
	III Evidence from documents describing techniques, methods or protocols
C* Weakly recommended: seek alternatives	IV Non-analytical studies, eg case reports, reviews, case series
D Never recommended	V Expert opinion and wide acceptance as good practice but with no study evidence
	VI Required by legislation, code of practice or national standard/ guideline
	VII Letter /short communication /editorials /conference communication
	VIII Electronic citation

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14. European Parliament. UK Standards for Microbiology Investigations (UK SMIs) use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes". 1998. **A, VI**

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