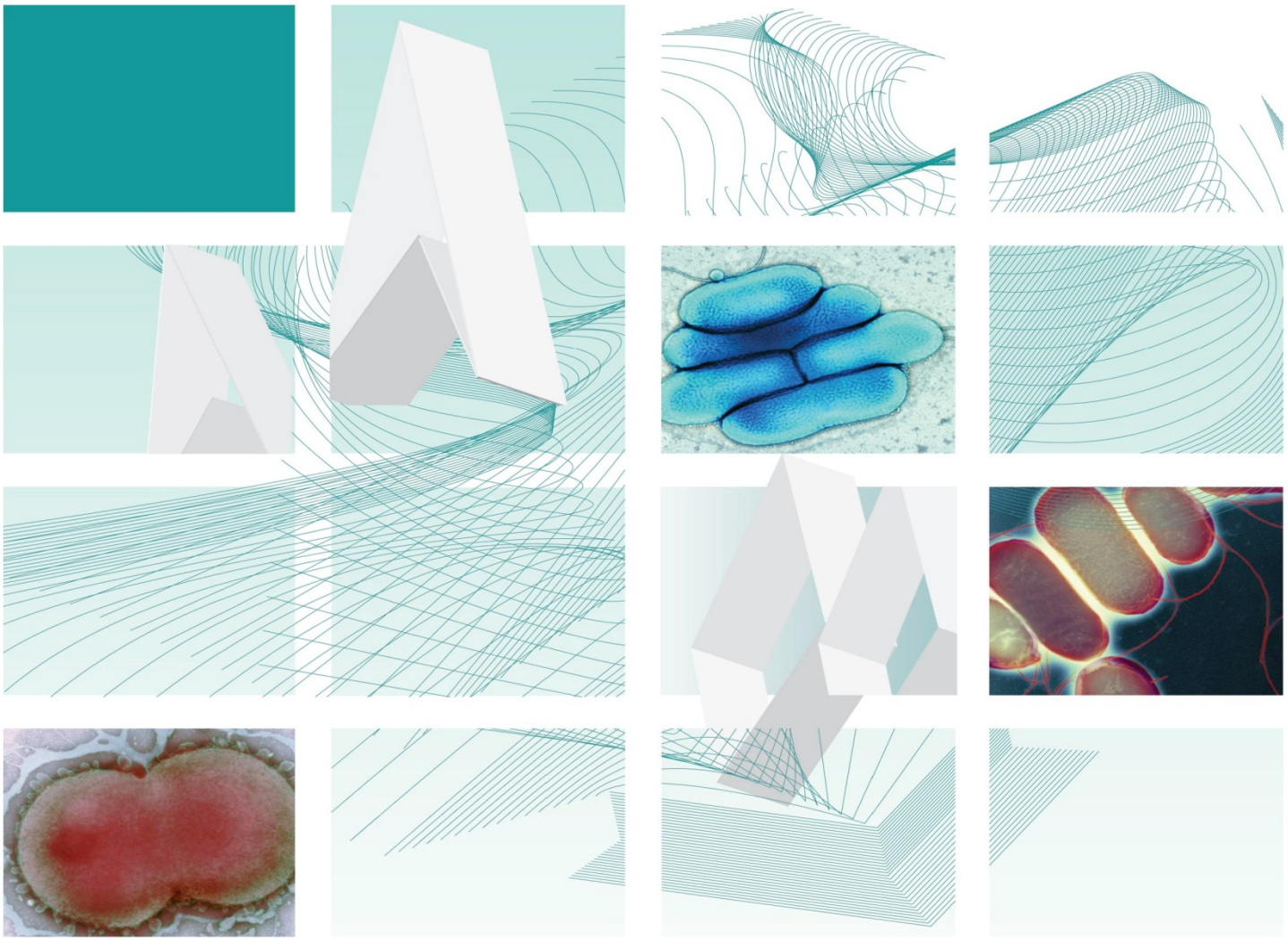
Public Health England

NHS

UK Standards for Microbiology Investigations

Thermonuclease Test



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Bacteriology – Test Procedures | TP 34 | Issue no: 3 | Issue date: 08.01.15 | Page: 1 of 13

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:

Logos correct at time of publishing.

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.	5/08.01.15
Issue no. discarded.	2.2
Insert Issue no.	3
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Scope of the Document.	The scope has been updated and references added.
Safety Considerations.	References updated.
Quality Control Organisms.	Negative control for the thermonuclease test has been amended; *Staphylococcus haemolyticus* NCTC 4276 has been replaced by *Staphylococcus haemolyticus* NCTC 11042. The quality control organisms have been validated by NCTC.
Procedures and Results.	Information and references updated.
Flowchart.	This flowchart has been modified for easy guidance.
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

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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Scope of Document

This 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* or other micrococci[1].

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

This SMI should be used in conjunction with other 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

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

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

1 Safety Considerations[3-19]

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

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[20]

Bacteriological straight wire/loop or disposable alternative

Sterile Pasteur pipette

Brain Heart Infusion broth

Boiling Water bath

3 Quality Control Organisms

Positive Control

Staphylococcus aureus NCTC 6571

Negative Control

Staphylococcus haemolyticus NCTC 11042

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

4 Procedure and Results

4.1 Blood Culture Test Method[2,21,22]

- Dispense 2 - 3mL of blood broth from positive blood culture 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
- 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 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 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

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*.

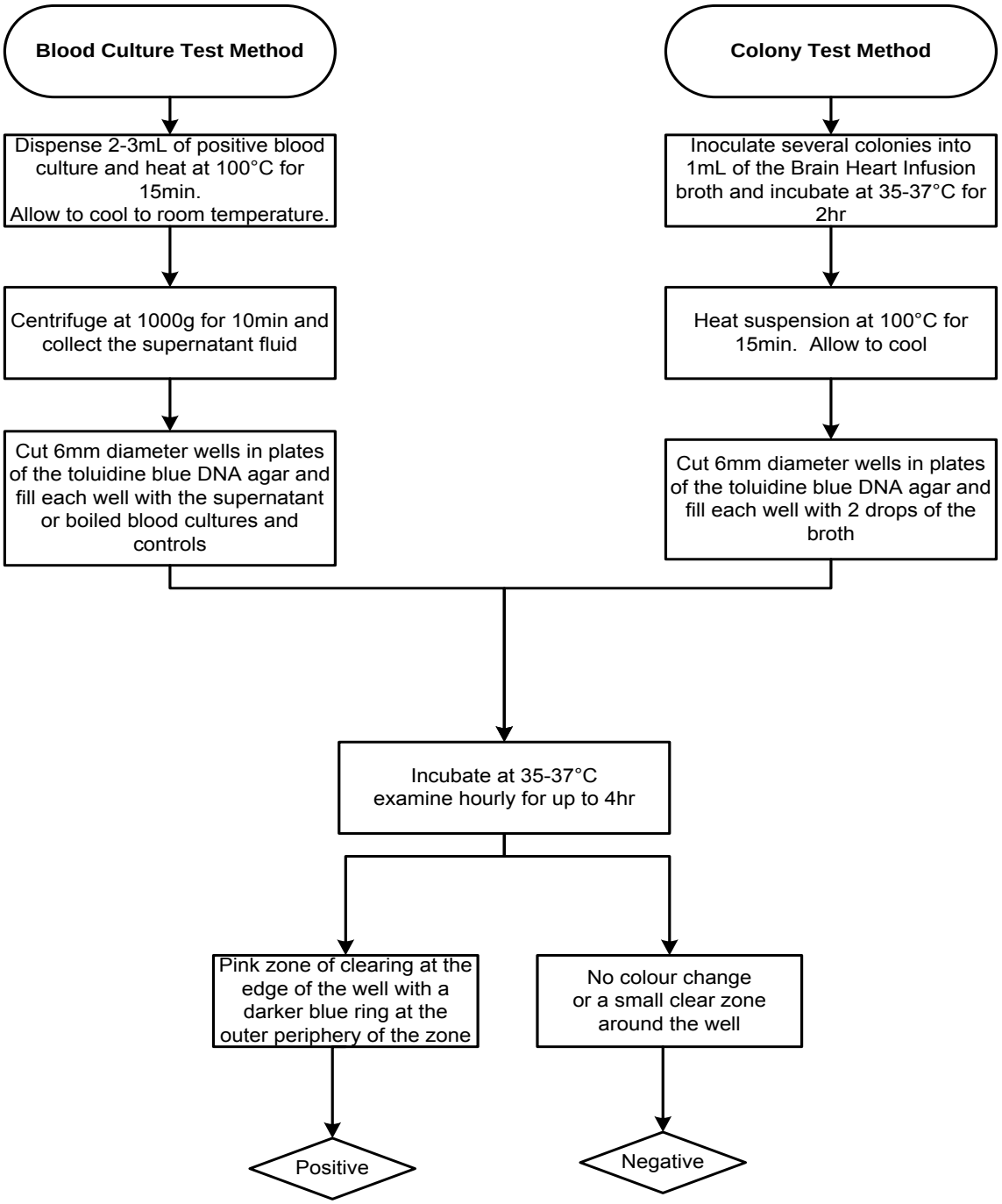
Negative result

No zone or a small clear zone around the well.

OR

No colour change.

Appendix: Thermonuclease Test



Note:

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

The flowchart is for guidance only.

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