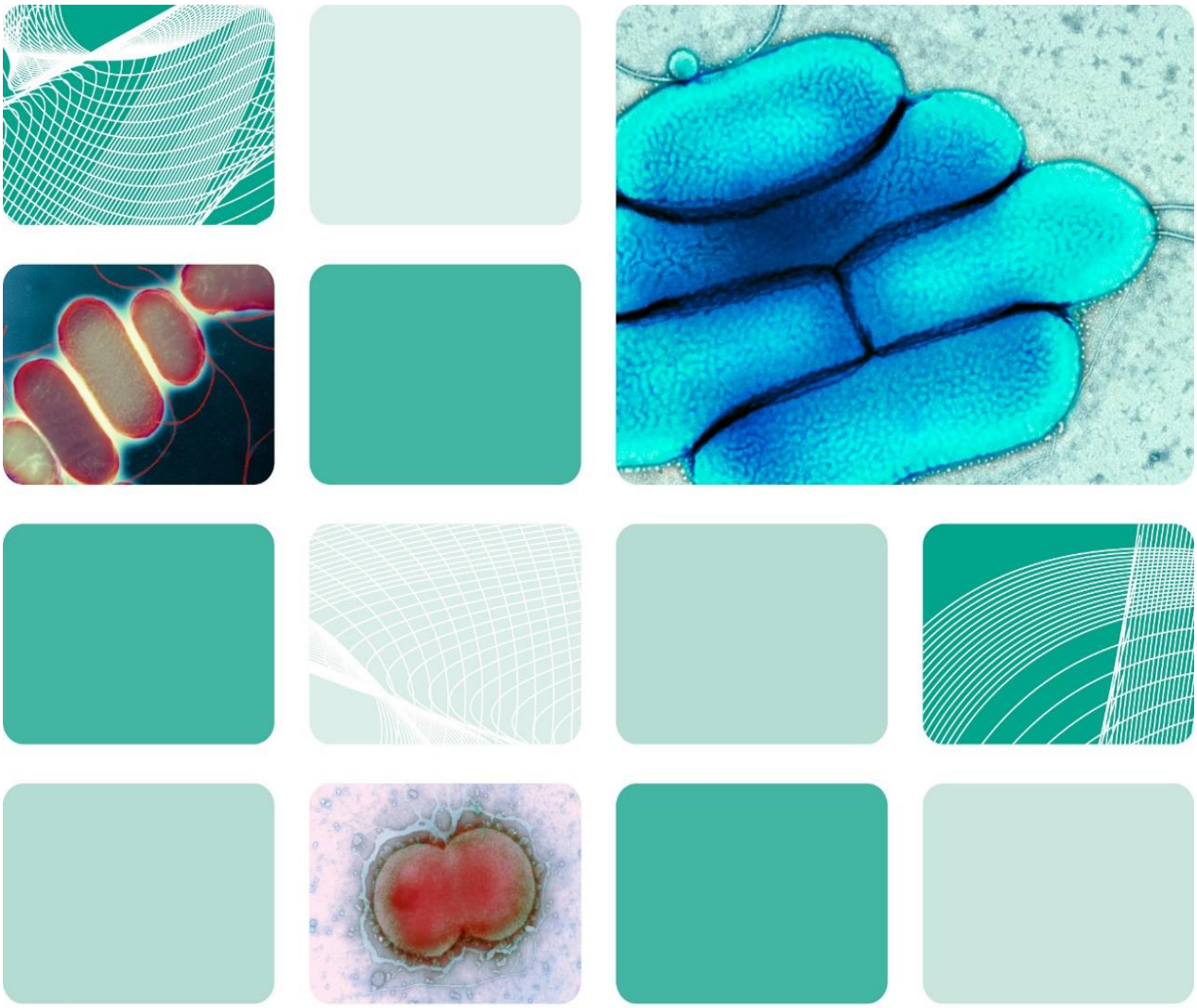
UK Health Security Agency

UK Standards for Microbiology Investigations

Thermonuclease test



Issued by the Standards Unit, UK Standards for Microbiology Investigations, UKHSA

Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on the UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a 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.

UK SMIs are produced in association with:

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Contents

Amendment table

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

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	7/12.03.25
Issue number discarded	4
Insert issue number	4.1
Section(s) involved	**Amendment**
Whole document.	**This is an administrative point change.** **The content of this UK SMI document has not changed.** **The last scientific and clinical review was conducted on 08/05/2019.** Hyperlinks throughout document updated to Royal College of Pathologists website. Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms Partner organisation logos updated. Broken links to devolved administrations replaced. References to NICE accreditation removed. Scope and Purpose replaced with General and Scientific information to align with current UK SMI template.

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 5 years where appropriate

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 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.

4 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.

5 Technical information/limitations

5.1 Quality control

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

5.2 Other thermonuclease positive Staphylococcus strains

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

5.3 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].

5.4 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].

5.5 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].

6 Safety considerations[5-22]

The section covers specific safety considerations (1-22) related to this UK SMI, and should be read in conjunction with the general safety considerations.

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.

7 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.

8 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.

9 Procedure and results

9.1 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

9.2 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*.

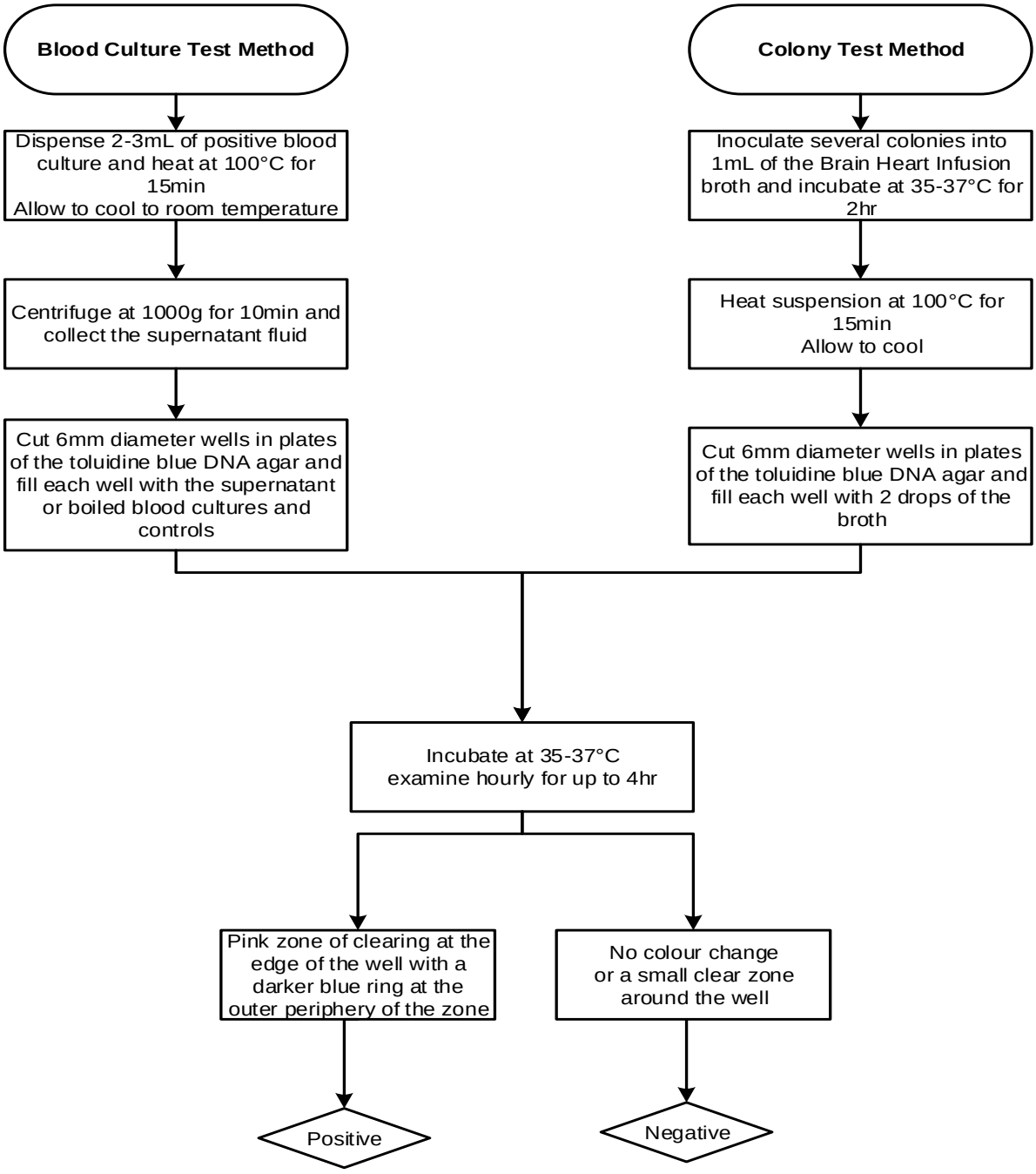
Negative result:

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

OR

No colour change.

Algorithm: Thermonuclease test



Note:

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

References

An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.

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