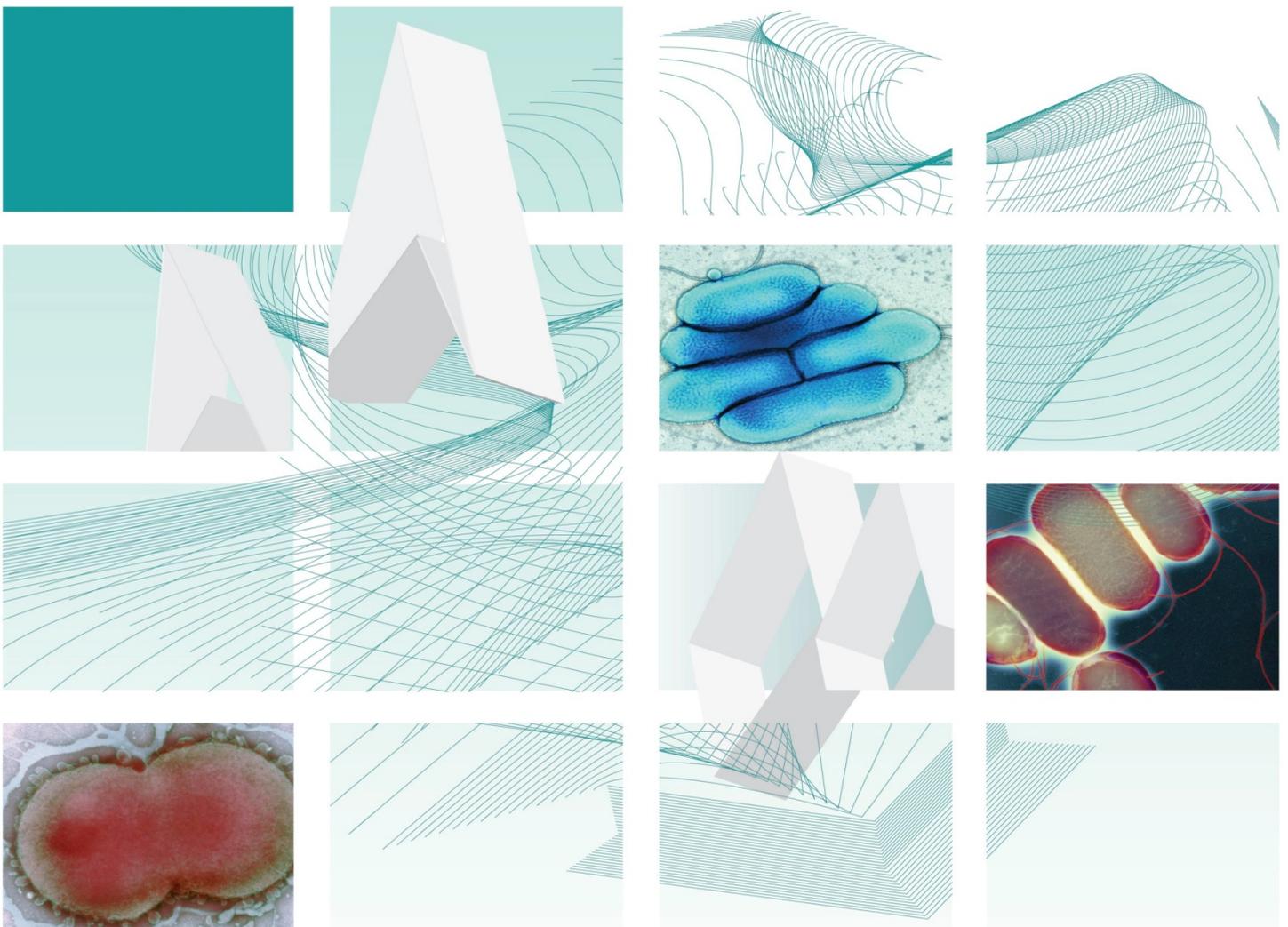




UK Standards for Microbiology Investigations

Deoxyribonuclease Test



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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For full details on our accreditation visit: www.nice.org.uk/accreditation.

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. | 8/20.11.14 |
| Issue no. discarded. | 2.5 |
| 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 amended to add the hyperlink for TP 34 -Thermonuclease Activity Test. |
| Introduction. | Updated to include other organisms with references. |
| Technical information/Limitations. | This section has been updated and references added. |
| Safety Considerations. | Section updated. |
| Reagents/Equipment. | 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 to include the stab technique. |
| Flowchart. | This has been amended 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

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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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Suggested Citation for this Document

Public Health England. (2014). Deoxyribonuclease Test. UK Standards for Microbiology Investigations. TP 12 Issue 3. <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>

Scope of Document

This test is used to determine the ability of an organism to produce deoxyribonuclease (DNase), an enzyme which is capable of degrading deoxyribonucleic acid (DNA)¹.

The thermonuclease test is described in [TP 34 - Thermonuclease Test](#).

Introduction

The test is used primarily as a supplementary presumptive test to distinguish pathogenic staphylococci which produce large quantities of extracellular DNase. The DNase reacts with media containing DNA with the resulting hydrolysis of the DNA. The oligonucleotides liberated by the hydrolysis are soluble in acid and in a positive reaction, the addition of hydrochloric acid results in a clear zone around the inoculum. Due to the precipitation of DNA by hydrochloric acid, in a negative reaction, the solution becomes cloudy. In contrast to hydrochloric acid, toluidine blue produces much more clearly delineated zones of DNase activity².

Most strains of *Staphylococcus aureus* hydrolyse DNA and give positive reactions in this test, but some MRSA strains do not and some strains of the coagulase negative staphylococci may give weak reactions. Some strains of *Staphylococcus intermedius* are DNase positive. Some coagulase negative staphylococci such as *Staphylococcus capitis* give weak reactions. Subspecies of *Staphylococcus schleiferi* are DNase positive and produce heat stable nucleases³.

This test also aids in the differentiation of closely related genera within the *Klebsiella-Enterobacter-Serratia* division of *Enterobacteriaceae* and several other pathogens, including *Pseudomonas aeruginosa*^{3,4}.

Serratia and *Moraxella* species also produce deoxyribonuclease.

Technical Information/Limitations

Spot-inoculate strains, including controls, so as not to overlap.

There are some disadvantages that limit the usefulness of the IM Hydrochloric acid (HCl) procedure; the IM HCl is bactericidal for staphylococci in either isolated colonies or in heavier, more confluent growth. Once the HCl has been applied, the test must be read within 5 minutes and cannot be continued by reincubation^{1,5}.

Optimum expression of DNase activity depends upon an exact concentration of toluidine blue O (TBO) in the TBO flooding solutions. Therefore, strict attention must be paid to the dye content of commercially available TBO powders; TBO concentrations must reflect actual dye concentrations. Calculations must include a conversion factor that accounts for the true dye content of commercial preparations^{2,3}.

The DNase test should be used in conjunction with other tests for the identification of *S. aureus*.

1 Safety Considerations⁶⁻²²

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.

Note: Hydrochloric acid is a highly corrosive substance. The hazards of solutions of hydrochloric acid depend on the concentration.

Personal protective equipment such as rubber or PVC gloves, protective eye goggles, and protective clothing and shoes are used to minimize risks when handling hydrochloric acid.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Reagents and Equipment

Discrete pure bacterial colonies growing on solid medium.

DNase test agar.

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

1M hydrochloric acid^{1,3}

OR

0.01% to 0.05% toluidine blue O solution²

3 Quality Control Organisms

Positive Control

Staphylococcus aureus NCTC 6571

Negative Control

Staphylococcus haemolyticus NCTC 11042

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

4 Procedure and Results

For all methods the surface moisture from the DNase test agar plates must be dried and each plate divided into sections by drawing lines on the bottom of the plate. There are two types of inoculation that can be done. They are as follows: spot inoculations or the band (line) streak inoculations³.

4.1 Spot Inoculation³

- Touch a colony of the organism under test with a loop and inoculate it onto a small area of the DNase test agar plate, in the middle of one of the marked

sections to form a thick plaque of growth 5-10mm in diameter after incubation. It also helps to stab the agar as well as plate out on the surface

- Incubate the plate at 37°C for 18-24hr

4.2 Band or line streak inoculation³

- Use a heavy inoculum and draw a line 3-4cm long from the rim to the centre of the DNase test agar plate
- Incubate the plate at 37°C for 18-24hr

5 Detection of DNase Activity by Flooding with Hydrochloric Acid¹

- Flood the plate to a depth of a few millimetres of 1M hydrochloric acid to precipitate unhydrolysed DNA
- Leave the plate to stand for a few minutes to allow the reagent to absorb into the DNase test agar plate
- Decant excess hydrochloric acid and then examine against a dark background
- Always compare the zone around the test strain with the control zones
- Unhydrolysed DNA is precipitated and produces a white cloudy area in the agar because of the reaction of HCl with DNA salts in the DNase test agar plate

Positive Result

Colonies surrounded by clear zones comparable in width to that around the DNase positive control.

Negative Result

No zone of clearing or a zone narrower than the DNase positive control.

OR

Cloudy precipitate around colony and throughout DNase test agar plate.

6 Detection of DNase Activity by Flooding with Toluidine blue O (TBO) Solution^{2,3}

- Flood the plate to a depth of a few millimetres of TBO to complex with either hydrolysed or unhydrolysed DNA
- Leave the DNase test agar plate to stand for 3-5 minutes
- Decant excess TBO and examine immediately
- Always compare the zone around the test strain with the control zones
- Read at 5 minutes intervals for up to 30 minutes

- TBO forms a complex with hydrolysed DNA to produce bright pink zones surrounding colonies on a royal blue background. DNase-negative organisms produce no change in the background colour

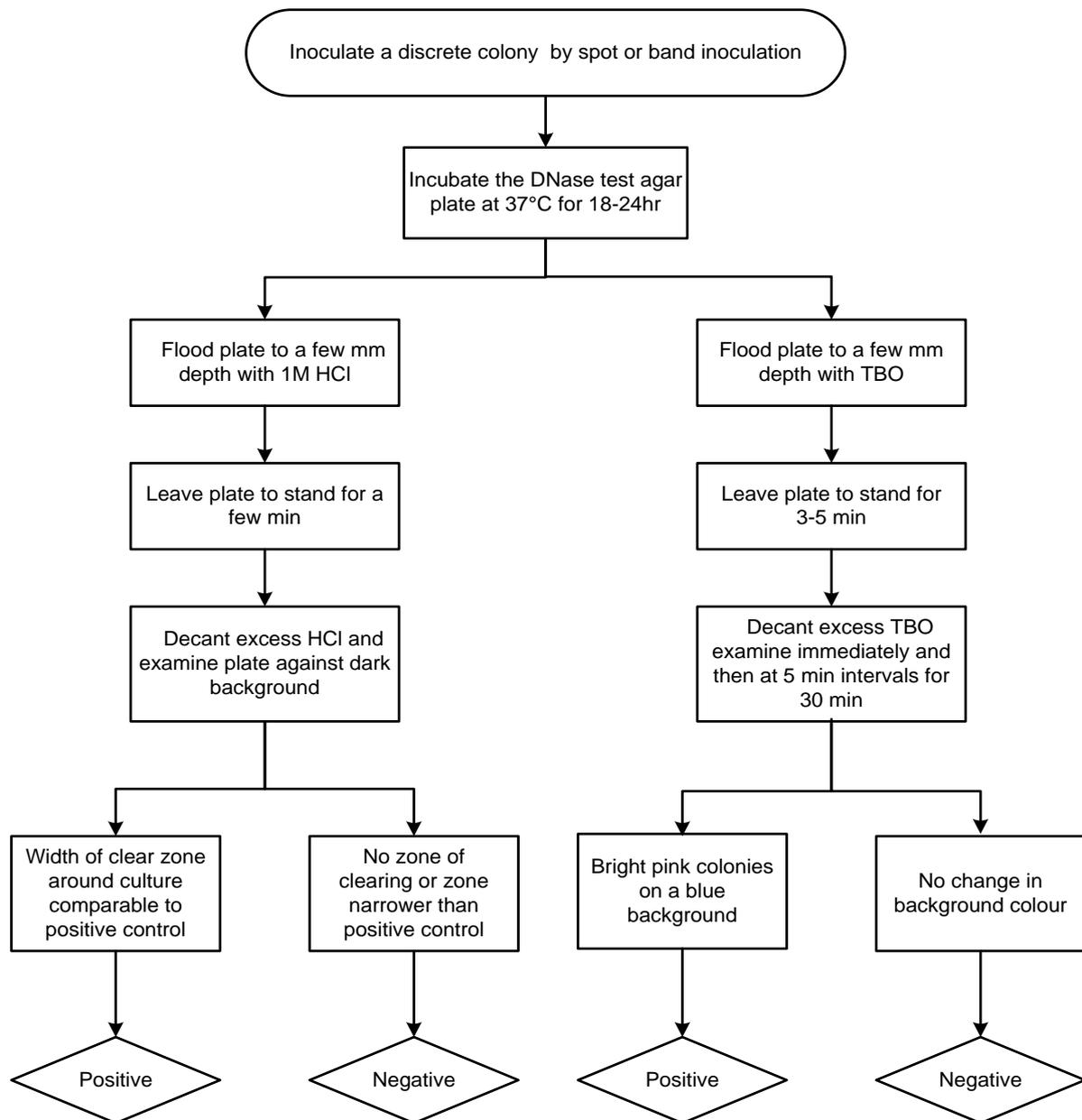
Positive Result

Bright pink zones surrounding colonies on a royal blue background comparable to that around the DNase positive control.

Negative Result

No change in background colour.

Appendix: Deoxyribonuclease Test



Note:

Positive control: *Staphylococcus aureus* NCTC 6571

Negative control: *Staphylococcus haemolyticus* NCTC 11042

The flowchart is for guidance only.

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