

UK Standards for Microbiology Investigations

Deoxyribonuclease Test



Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of the Health Protection Agency (HPA) 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 <http://www.hpa.org.uk/SMI/Partnerships>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <http://www.hpa.org.uk/SMI/WorkingGroups>).

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:



UK Standards for Microbiology Investigations[#]: Status

Users of SMIs

Three groups of users have been identified for whom SMIs are especially relevant:

- SMIs are primarily intended as a general resource for practising professionals in the field operating in the field of laboratory medicine in the UK. Specialist advice should be obtained where necessary.
- SMIs provide clinicians with information about the standard of laboratory services they should expect for the investigation of infection in their patients and the documents provide information that aids the electronic ordering of appropriate tests from hospital wards.
- SMIs also provide commissioners of healthcare services with the 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 essential laboratory methodologies which underpin quality, for example assay validation, quality assurance, and understanding uncertainty of measurement.

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 interventions, surveillance, and research and development activities. SMIs align advice on testing strategies with the UK diagnostic and public health agendas.

Involvement of Professional Organisations

The development of SMIs is undertaken within the HPA in partnership with the NHS, Public Health Wales and with professional organisations.

The list of participating organisations may be found at <http://www.hpa.org.uk/SMI/Partnerships>. Inclusion of an organisation's logo in an SMI implies support for the objectives and process of preparing SMIs. Representatives of professional organisations are members of the steering committee and working groups which develop SMIs, although the views of participants are not necessarily those of the entire organisation they represent.

SMIs are developed, reviewed and updated through a wide consultation process. The resulting documents reflect the majority view of contributors. SMIs are freely available to view at <http://www.hpa.org.uk/SMI> as controlled documents in Adobe PDF format.

[#] UK Standards for Microbiology Investigations were formerly known as National Standard Methods.

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

The process for the development of SMIs is certified to ISO 9001:2008.

NHS Evidence has accredited the process used by the HPA to produce SMIs. Accreditation is valid for three years from July 2011. The accreditation is applicable to all guidance produced since October 2009 using the processes described in the HPA's Standard Operating Procedure SW3026 (2009) version 6.

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 well referenced 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. SMIs should be used in conjunction with other SMIs.

UK microbiology laboratories that do not use SMIs should be able to demonstrate at least equivalence in their testing methodologies.

The performance of SMIs depends on well trained staff and the quality of reagents and equipment used. 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.

Whilst every care has been taken in the preparation of SMIs, the HPA, its successor organisation(s) 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.

SMIs are the copyright of the HPA which should be acknowledged where appropriate.

Microbial taxonomy is up to date at the time of full review.

Equality and Information Governance

An Equality Impact Assessment on SMIs is available at <http://www.hpa.org.uk/SMI>.

The HPA 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.

Suggested Citation for this Document

Health Protection Agency. (2011). Deoxyribonuclease Test. UK Standards for Microbiology Investigations. TP 12 Issue 2.2. <http://www.hpa.org.uk/SMI/pdf>.

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ISO 9001:2008

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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@hpa.org.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date	4/20.07.11
Issue no. discarded	2.1
Insert Issue no.	2.2
Section(s) involved/ Page no.	Amendment
Whole document/-	Document presented in a new format. Amended to incorporate <i>Moraxella</i> species for which DNase can be used to differentiate <i>Moraxella catarrhalis</i> from other <i>Moraxella species</i> .
Introduction	<i>Staphylococcus epidermidis</i> amended to <i>capitis</i> .
Hyperlinks/-	Within the document there are hyperlinks that have been removed pending launch of a new website.
References/-	Some references updated.

Amendment No/Date	3/09.12.10
Issue no. discarded	2
Insert Issue no.	2.1
Section(s) involved/ Page no.	Amendment
Front page/1	The Association of Medical Microbiologist logo replaced with new British Infection Association logo.
Quality Control Organisms/6	Negative control amended from <i>Staphylococcus epidermidis</i> to <i>Staphylococcus haemolyticus</i> .
Appendix/9	Flowchart amended.

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). The thermonuclease test is described in TP 34 - Thermonuclease Activity Test.

Introduction¹

The test is used primarily to distinguish pathogenic staphylococci which produce large quantities of extracellular DNase. It 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. Subspecies of *Staphylococcus schleiferi* are DNase positive and produce heat stable nucleases. Some other organisms such as *Serratia* and *Moraxella* species also produce deoxyribonuclease.

Technical Information/Limitations

Spot-inoculate strains, including controls, so as not to overlap. Always compare the zone around the test strain with the control zones.

Some strains of *Staphylococcus intermedius* are DNase positive.

Some strains of MRSA are DNase negative.

The subspecies of *Staphylococci schleiferi* are DNase positive and produce heat stable nucleases.

Some coagulase negative staphylococci such as *Staphylococcus capitis* give weak reactions.

This test should always be used in conjunction with another test for confirmation of identification of staphylococcal isolates.

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^{1,2}.

1 Safety Considerations³⁻¹³

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 corrosive substance.

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

2 Reagents and Equipment^{1,2,12,14}

Discrete bacterial colonies growing on solid medium.

DNase test agar.

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

1M (3.6%) hydrochloric acid or

0.01% to 0.05% toluidine blue O solution.

3 Quality Control Organisms

Positive Control

Staphylococcus aureus NCTC 6571

Negative Control

Staphylococcus haemolyticus NCTC 4276

NB: These strains are not validated by NCTC to give this result.

4 Procedure and Results

For all methods the surface moisture from the plates must be dried and each plate divided into sections by drawing lines on the bottom of the plate.

4.1 Spot Inoculation

- Touch a colony of the *Staphylococcus* or *Moraxella* species under test with a loop and inoculate it onto a small area of the medium plate, in the middle of one of the marked sections to form a thick plaque of growth 5-10 mm in diameter after incubation

4.2 Band or line streak inoculation

- Use a heavy inoculum and draw a line 3-4 cm long from the rim to the centre of the plate.
- Incubate the plate at 37°C for a minimum of 15 hours and a maximum of 24 hours.

4.3 Detection of DNase activity by flooding with hydrochloric acid

- 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, decant excess hydrochloric acid and then examine against a dark background.
- Unhydrolysed DNA is precipitated and produces a white opacity in the agar.

Positive Result

Cultures 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.

4.4 Detection of DNase activity by flooding with Toluidine blue O (TBO) solution

- Flood the plate with a few millimetres of TBO to complex with either hydrolysed or Unhydrolysed DNA.
- Leave the plate to stand for 3-5 minutes, decant excess TBO and examine immediately. Examine at 5 minutes intervals for 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.

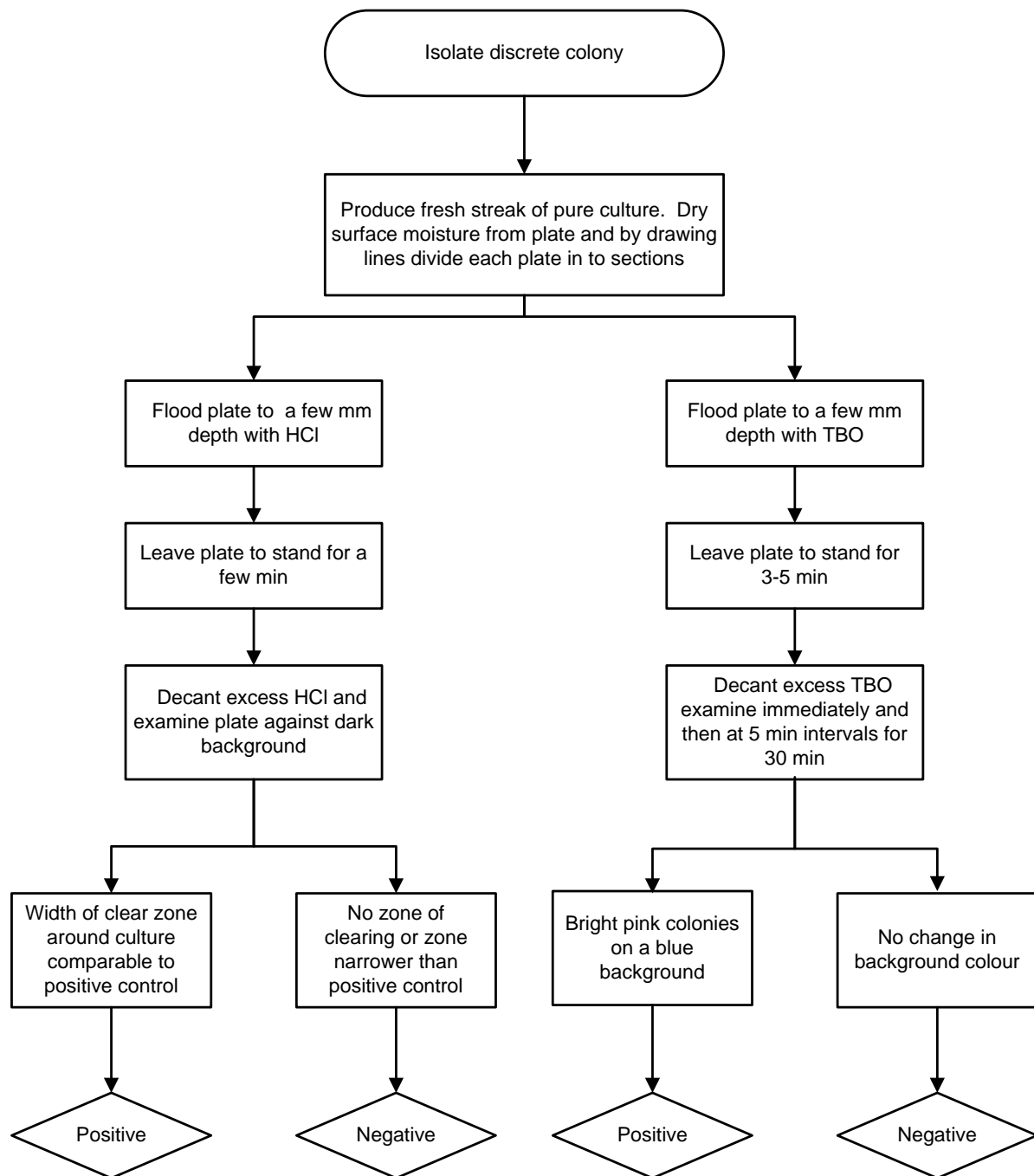
Positive Result

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 Flowchart



Note:

Positive control: *Staphylococcus aureus* NCTC 6571

Negative control: *Staphylococcus haemolyticus* NCTC 4276

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