Public Health England

NHS

UK Standards for Microbiology Investigations

Deoxyribonuclease Test

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Issued by the Standards Unit, Microbiology Services, PHE

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 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:

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.	7/13.03.14
Issue no. discarded.	2.4
Insert Issue no.	2.5
Section(s) involved	**Amendment**
Whole document.	Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England. Front page has been redesigned. Status page has been renamed as Scope and Purpose and updated as appropriate. Professional body logos have been reviewed and updated. Standard safety and notification references have been reviewed and updated. Scientific content remains unchanged.

Amendment No/Date.	6/05.04.12
Issue no. discarded.	2.3
Insert Issue no.	2.4
Section(s) involved	**Amendment**
Whole document	Document updated to a revised format.

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 http://www.hpa.org.uk/SMI/Partnerships. 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 http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133470313. 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.

Suggested Citation for this Document

Public Health England. (2014). Deoxyribonuclease Test. UK Standards for Microbiology Investigations. TP 12 Issue 2.5. http://www.hpa.org.uk/SMI/pdf.

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.

This SMI should be used in conjunction with other SMIs.

Introduction[1]

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 activity[2].

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 *Staphylococcus 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 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.

Note: Hydrochloric acid is a corrosive substance.

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

2 Reagents and Equipment[1,2,17,20]

Discrete bacterial colonies growing on solid medium.

DNase test agar.

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

1 M (3.6%) hydrochloric acid or 0.01%-0.05% toluidine blue O solution.

3 Quality Control Organisms

Positive Control

Staphylococcus aureus NCTC 6571

Negative Control

Staphylococcus haemolyticus NCTC 4276

Note: 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 5-10mm in diameter after incubation

4.2 Band or Line Streak Inoculation

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

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 3-5min, decant excess TBO and examine immediately. Examine at 5min intervals for 30min
- 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

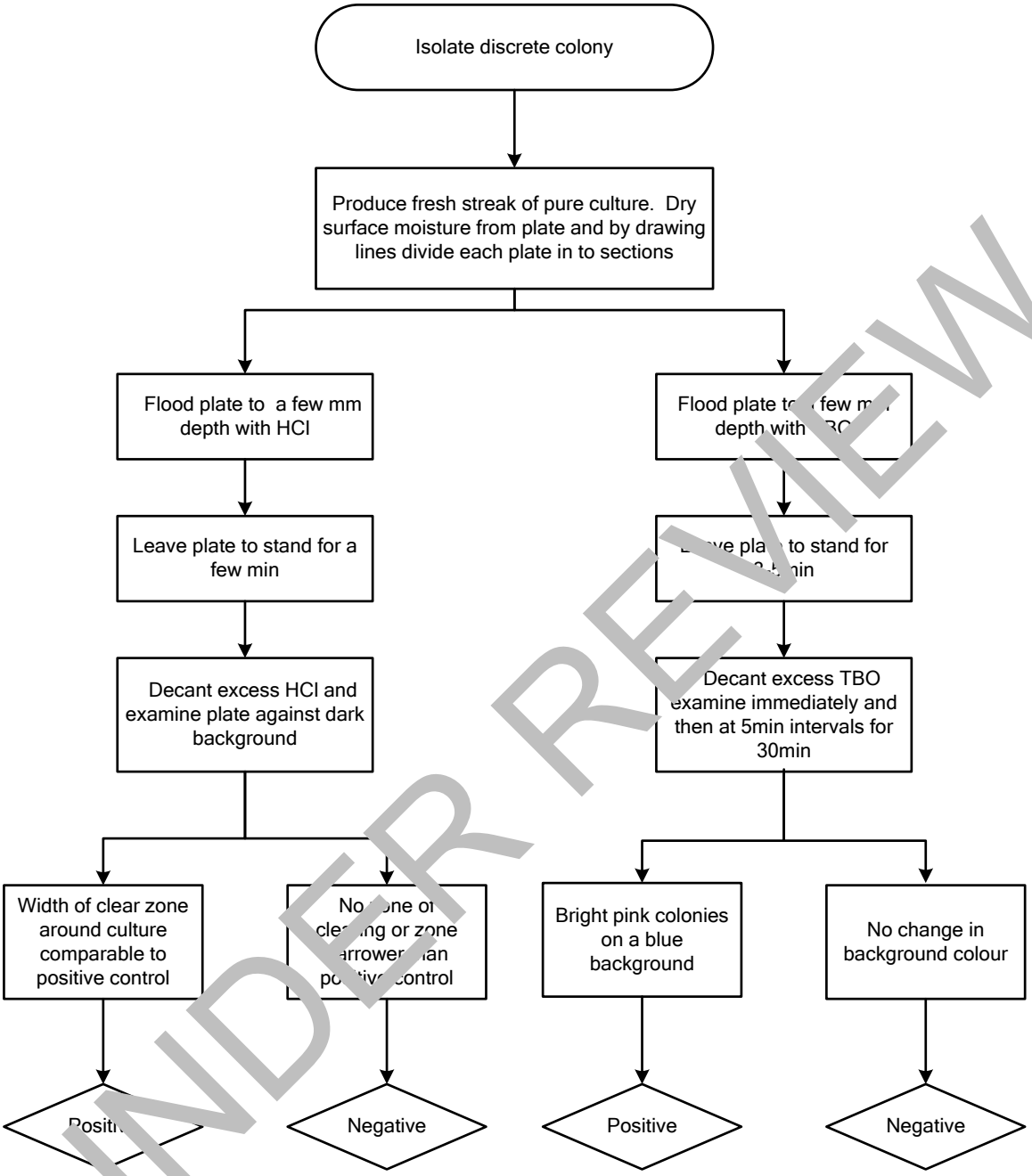
Positive Result

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 4276

References

1. MacFaddin JF. Optochin Disk Test. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Philadelphia: Lippincott Williams and Wilkins; 2000. p. 363-7.

2. Waller JR, Hodel SL, Nuti RN. Improvement of two toluidine blue O-mediated techniques for DNase detection. J Clin Microbiol 1985;21:195-9.

3. European Parliament. UK Standards for Microbiology Investigations (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".

4. Official Journal of the European Communities. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices. 7-12-1998. p. 1-37.

5. Health and Safety Executive. Safe use of pneumatic air tube transport systems for pathology specimens. 9/99.

6. Department for transport. Transport of Infectious Substances, 2011 Revision 5. 2011.

7. World Health Organization. Guidance on regulations for the Transport of Infectious Substances 2013-2014. 2012.

8. Home Office. Anti-terrorism, Crime and Security Act. 2001 (as amended).

9. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive. 2013. p. 1-32.

10. Advisory Committee on Dangerous Pathogens. Infections at work: Controlling the risks. Her Majesty's Stationery Office. 2003.

11. Advisory Committee on Dangerous Pathogens. Biological agents: Managing the risks in laboratories and healthcare premises. Health and Safety Executive. 2005.

12. Advisory Committee on Dangerous Pathogens. Biological Agents: Managing the Risks in Laboratories and Healthcare Premises. Appendix 1.2 Transport of Infectious Substances - Revised. Health and Safety Executive. 2008.

13. Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. MMWR Surveill Summ 2012;61:1-102.

14. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002. 5th ed. HSE Books; 2002.

15. Health and Safety Executive. Five Steps to Risk Assessment: A Step by Step Guide to a Safer and Healthier Workplace. HSE Books. 2002.

16. Health and Safety Executive. A Guide to Risk Assessment Requirements: Common Provisions in Health and Safety Law. HSE Books. 2002.

17. Health Services Advisory Committee. Safe Working and the Prevention of Infection in Clinical Laboratories and Similar Facilities. HSE Books. 2003.

18. British Standards Institution (BSI). BS EN12469 - Biotechnology - performance criteria for microbiological safety cabinets. 2000.

19. British Standards Institution (BSI). BS 5726:2005 - Microbiological safety cabinets. Information to be supplied by the purchaser and to the vendor and to the installer, and siting and use of cabinets. Recommendations and guidance. 24-3-2005. p. 1-14

20. Snell JJS, Brown DFJ, Roberts C, editors. Quality Assurance Principles and Practice in the Microbiology Laboratory. London: Public Health Laboratory Service; 1999. p. 147-8