

NATIONAL STANDARD METHOD

DEOXYRIBONUCLEASE TEST

BSOP TP 12

Issued by Standards Unit, Department for Evaluations, Standards and Training
Centre for Infections







DEOXYRIBONUCLEASE TEST

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STATUS OF NATIONAL STANDARD METHODS

National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

National Standard Methods are developed, reviewed and updated through an open and wide consultation process where the views of all participants are considered and the resulting documents reflect the majority agreement of contributors.

Representatives of several professional organisations, including those whose logos appear on the front cover, are members of the working groups which develop National Standard Methods. Inclusion of an organisation's logo on the front cover implies support for the objectives and process of preparing standard methods. The representatives participate in the development of the National Standard Methods but their views are not necessarily those of the entire organisation of which they are a member. The current list of participating organisations can be obtained by emailing standards@hpa.org.uk.

The performance of standard methods depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

Whereas every care has been taken in the preparation of this publication, the Health Protection Agency or any supporting organisation cannot be responsible for the accuracy of any statement or representation made or the consequences arising from the use of or alteration to any information contained in it. These procedures are intended solely as a general resource for practising professionals in the field, operating in the UK, and specialist advice should be obtained where necessary. If you make any changes to this publication, it must be made clear where changes have been made to the original document. The Health Protection Agency (HPA) should at all times be acknowledged.

The HPA is an independent organisation dedicated to protecting people's health. It brings together the expertise formerly in a number of official organisations. More information about the HPA can be found at www.hpa.org.uk.

The HPA aims to be a fully 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 conditions¹.

More details can be found on the website at www.evaluations-standards.org.uk. Contributions to the development of the documents can be made by contacting standards@hpa.org.uk.

The reader is informed that all taxonomy in this document was correct at time of issue.

Please note the references are now formatted using Reference Manager software. If you alter or delete text without Reference Manager installed on your computer, the references will not be updated automatically.

Suggested citation for this document:

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AMENDMENT PROCEDURE

Controlled document reference	BSOP TP 12
Controlled document title	Deoxyribonuclease Test

Each National Standard 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.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
2/ 06.07.10	1	2		Whole document	Document reviewed, reference strains updated

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DEOXYRIBONUCLEASE TEST

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 [BSOPTP 34 - Thermonuclease Activity Test](#)

INTRODUCTION²

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 activity³.

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* 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 epidermidis* 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^{2,3}.

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1 SAFETY CONSIDERATIONS⁴⁻¹⁰

Refer to current guidance on the safe handling of all organisms and reagents documented in this NSM.

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^{2,3,10,11}

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 epidermidis* NCTC 4276

NB Strains not validated by NCTC to give this result

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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 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 18-24 hour(s)

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.

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5 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the National Standard Methods Working Group for Clinical Bacteriology (http://www.hpa-standardmethods.org.uk/wg_bacteriology.asp). The contributions of many individuals in clinical bacteriology laboratories and specialist organisations who have provided information and comment during the development of this document, and final editing by the Medical Editor are acknowledged.

The National Standard Methods are issued by Standards Unit, Department for Evaluations, Standards and Training, Centre for Infections, Health Protection Agency, London.

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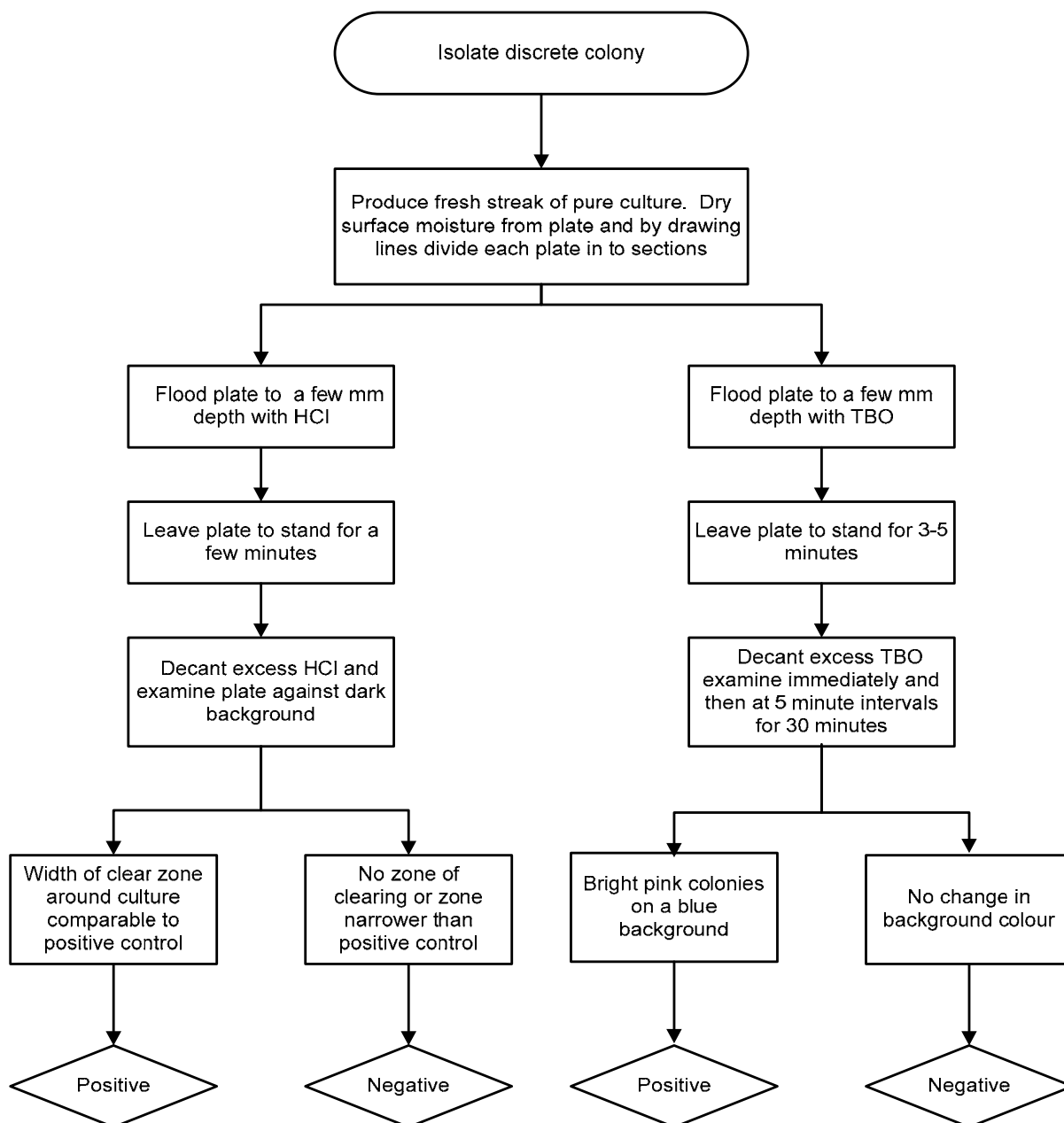
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APPENDIX: DEOXYRIBONUCLEASE TEST FLOWCHART



Note:

Positive control: *Staphylococcus aureus* NCTC 8532
Negative control: *Staphylococcus epidermis* NCTC 4276

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