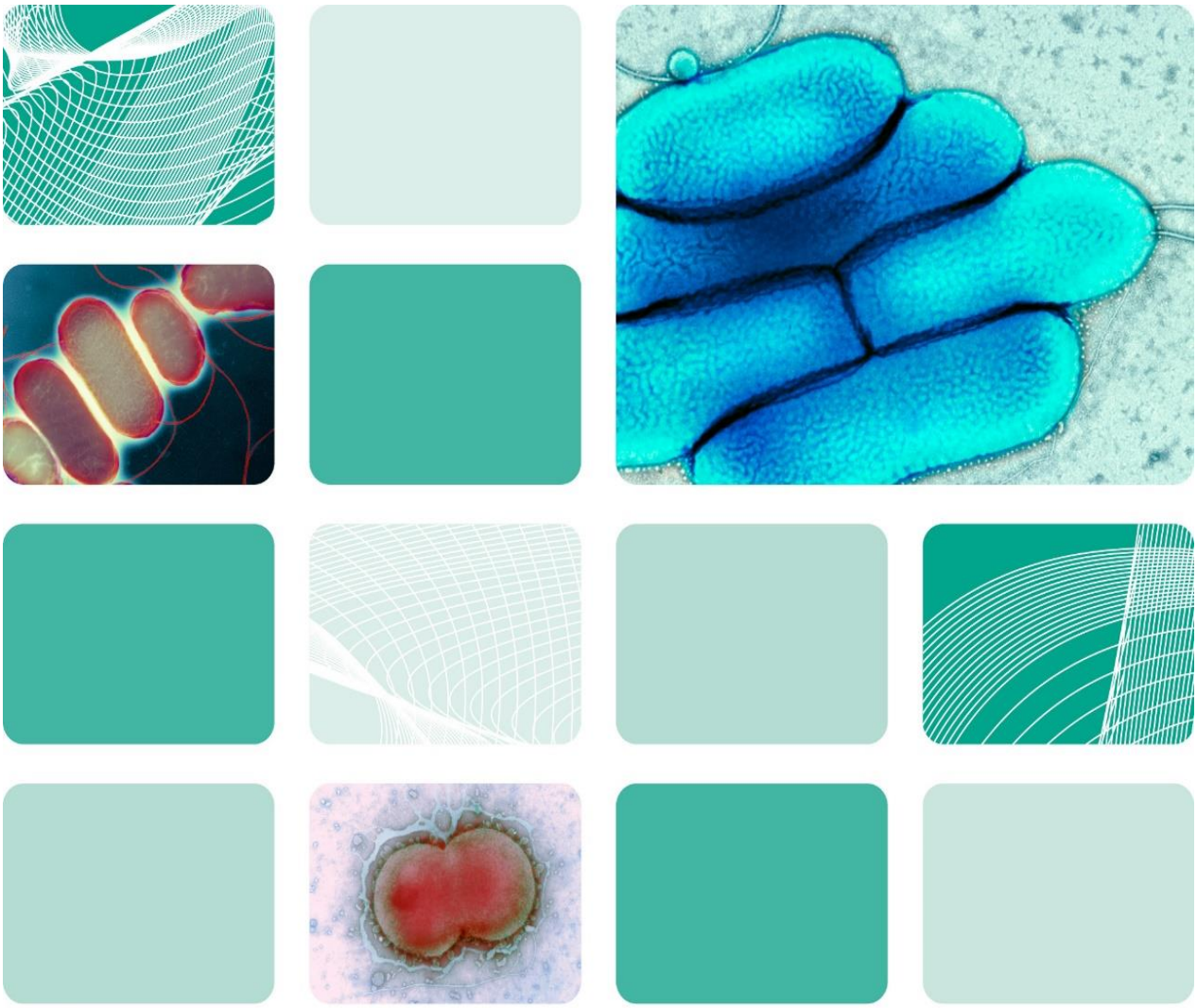
UK Health Security Agency

UK Standards for Microbiology Investigations

Deoxyribonuclease test



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

Acknowledgments

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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	10/28.02.25
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 11/09/2018.** 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	9/11.09.18
Issue number discarded	3
Insert issue number	4
Anticipated next review date*	11.09.21
Section(s) involved	**Amendment**

Whole document.	Document updated. Technical limitations/information updated with subheadings. References updated with grades. Flowchart updated. Quality control organisms updated.

*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

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

The thermonuclease test is described in UK SMI TP 34 - Thermonuclease test.

This UK SMI should be used in conjunction with other UK SMIs.

4 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 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 such as *Staphylococcus capitis*. Some strains of *Staphylococcus intermedius* are DNase positive. Subspecies of *Staphylococcus schleiferi* are DNase positive and produce heat stable nucleases[3].

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.

5 Technical information/limitations

5.1 Spot Inoculation

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

5.2 1M Hydrochloric acid (HCl) procedure

There are some disadvantages that limit the usefulness of the 1M Hydrochloric acid (HCl) procedure; the 1M 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].

5.3 Concentration of toluidine blue O

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].

5.4 Alternative DNase test agar media

The Methyl green – DNase test agar is an improved, highly sensitive alternative agar medium that could be used in place of the traditional DNase test agar. The use of this agar media is based on the modification of Jefferies *et al* procedure[1]. It supports growth of both Gram positive and Gram negative bacteria. In this scenario, the DNase producing organisms depolymerises the DNA substrate in the medium followed by the methyl green fading into a colourless compound which is exhibited as distinct clear zones around growth against the green background. Fading does not happen instantly, it takes about 4 to 6hrs incubation for this to occur[3]. Its main advantage is that it does not require reagent addition as it is contained within the already prepared agar medium.

6 Safety considerations[6-23]

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.

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 minimise risks when handling hydrochloric acid.

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 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]

Note: Some manufacturers will refer to this acid as “1N hydrochloric acid”. They both mean the same thing.

OR

0.01% to 0.05% toluidine blue O solution[2]

8 Quality control organisms

Positive control:

Staphylococcus aureus NCTC 6571 or NCTC 12973

Negative control:

Staphylococcus haemolyticus NCTC 11042

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

9 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 inoculation or the band or line streak inoculation[3]. For the step by step procedure on how to perform these inoculations, refer to UK SMI Q 5: Inoculation of culture media for bacteriology.

Spot inoculation[3]

- 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

Band or line streak inoculation[3]

- 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

9.1 Detection of DNase activity by flooding with hydrochloric acid[1]

- 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.

9.2 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 minute 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

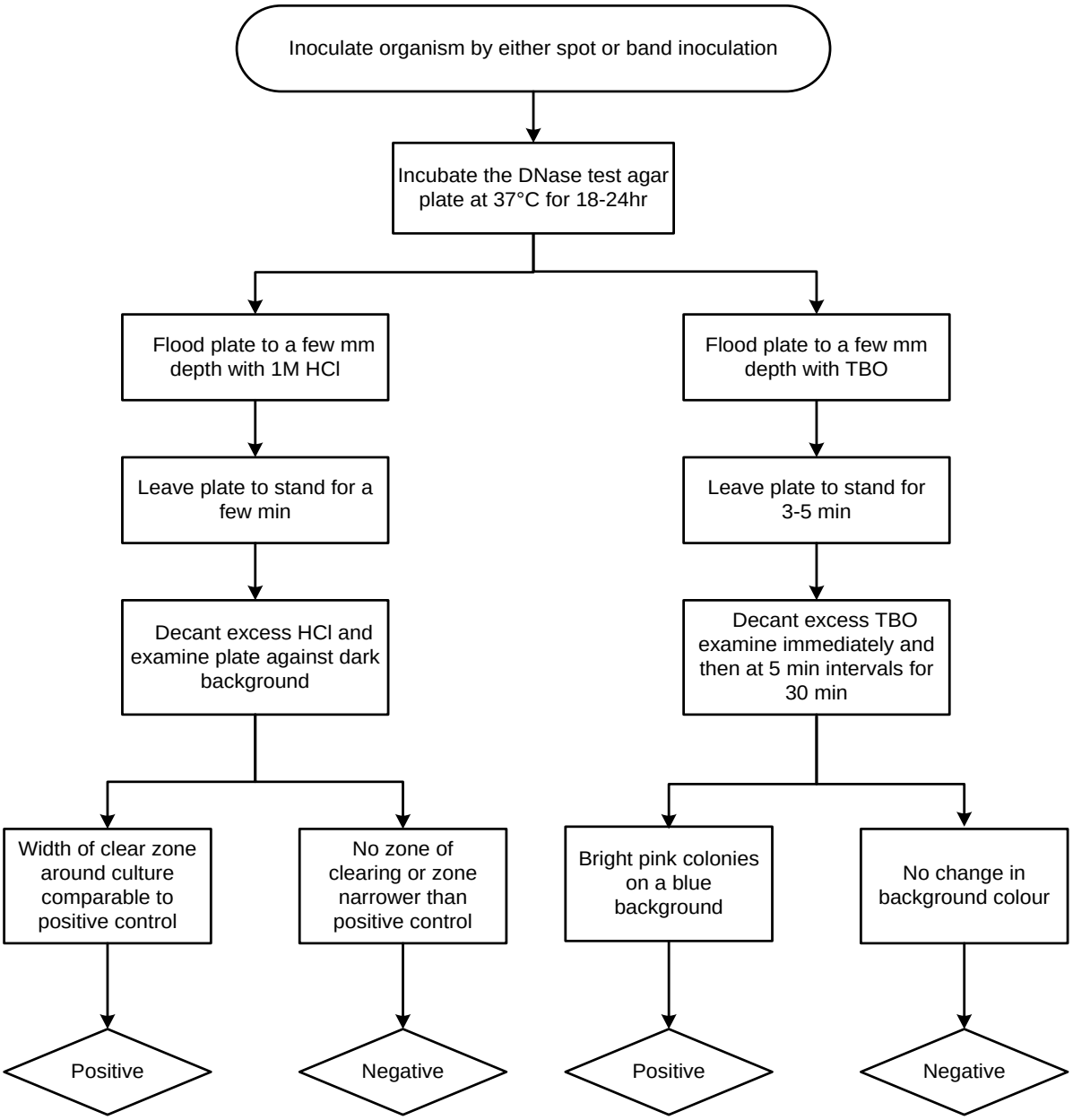
Positive result

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.

Algorithm: Deoxyribonuclease test



Note:

Positive control: *Staphylococcus aureus* NCTC 6571 or NCTC 12973

Negative control: *Staphylococcus haemolyticus* NCTC 11042

The flowchart is for guidance only.

References

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

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