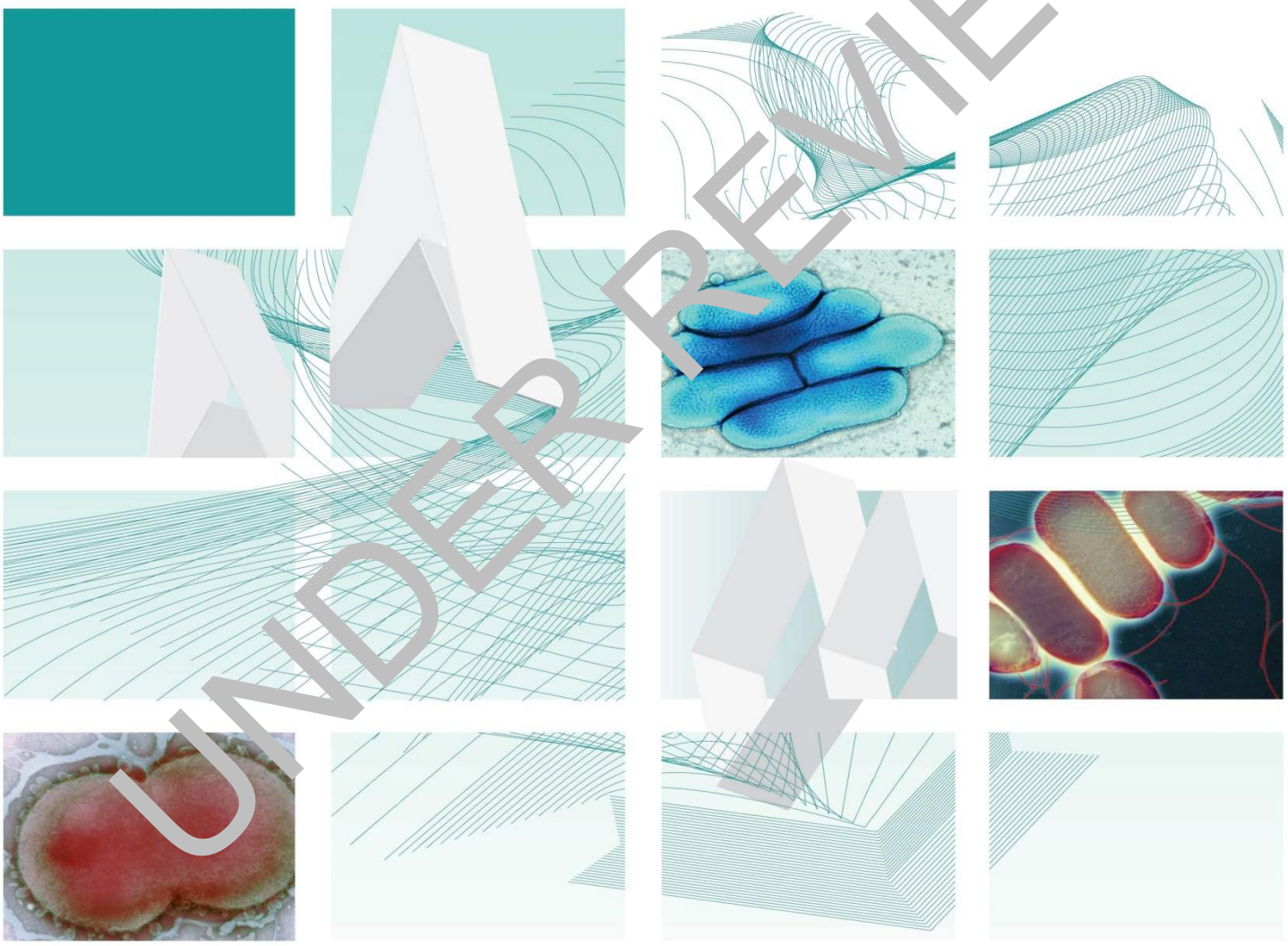
Public Health England

NHS

UK Standards for Microbiology Investigations

Nagler 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.	5/14.03.14
Issue no. discarded.	2.2
Insert Issue no.	2.3
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.	4/21.10.11
Issue no. discarded.	2.1
Insert Issue no.	2.2
Section(s) involved	**Amendment**
Whole document.	Document presented in a new format.
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 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). Nagler Test. UK Standards for Microbiology Investigations. TP 22 Issue 2.3. http://www.hpa.org.uk/SMI/pdf.

Scope of Document

To determine the ability of microorganisms to produce the enzyme lecithinase this is shown by the appearance of egg yolk opacity. Commonly found in *Clostridium perfringens, Bacillus cereus, Pseudomonas fluorescens* and some others.

This SMI should be used in conjunction with other SMIs.

Introduction

Bacterial lecithinase breaks down lecithin (a normal component of egg yolk) to insoluble diglycerides, resulting in an opaque halo surrounding the colony when grown on egg yolk agar[1]. Although the test is mainly used for the differentiation of the *Clostridium* species, the demonstration of lecithinase production is useful for the division of the genus *Bacillus*[2]. Lipolytic organisms also produce an opalescence on egg yolk agar which is often accompanied by a distinctive "pearly layer" or iridescent film[1].

The Nagler test is principally used for the differentiation of *Clostridium perfringens* from other members of the genus *Clostridium* by neutralisation of lecithinase C by a specific antitoxin. *Clostridium baratii, Clostridium absonum, Clostridium bifermentans, Clostridium sordelli and Clostridium novyi* also produce lecithinase. *Clostridium baratii and Clostridium absonum* may produce a partial cross reaction with the antitoxin if a heavy inoculum of the organism is used. *C. sordelli and C. bifermentans* produce enzymes that are also closely related to *C. perfringens* alpha toxin (leathinase) and can produce a partial cross-reaction[1].

Technical Information/Limitations

New batches of antitoxin should be tested before use.

C. baratii and *C. absonum* may produce a partial cross reaction with the antitoxin, if a heavy inoculum of the organism is used.

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.

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

Note: Dangerous Pathogens and Toxins

Part 7 of the Anti-Terrorism, Crime and Security Act 2001 (and subsequent amendments) requires holders of hazardous pathogens (and toxins) in the UK, listed in Schedule 5 of the Act, to be registered with the Home Office. The term 'holder' means retaining the organism for control purposes/future study; it does not apply if the organism is identified from a diagnostic specimen or QA sample and is not retained further than is necessary for diagnostic purposes. *Clostridium perfringens* is now included on Schedule 5.

Failure to comply with the legislation may result in prosecution.

Those unaware of this legislation and who need to register, can do so by e-mail to Pathogens@homeoffice.gsi.gov.uk or by post to Pathogens Notifications, 5th Floor, 2 Marsham Street, London SW1P 4DF or by fax to 0870 336 9057. Any enquiries may be addressed to the duty officer on 020 7035 3331.

2 Reagents and Equipment

Egg Yolk Agar

Clostridium perfringens type A antitoxin.

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

3 Quality Control Organisms

Positive Control

Clostridium perfringens	NCTC 8359

Negative Control

Clostridium difficile	NCTC 11204

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

4 Procedure and Results

4.1 Nagler/Lecithinase Procedure

- Inoculate half the egg yolk agar plate with 60µl antitoxin. Spread with a 'hockey stick' spreader or 10µl loop
- Allow to absorb and dry

- Mark which side of the plate has been inoculated with the antitoxin
- Streak the test organism in a straight line from the antitoxin-free half, across to the antitoxin side of the plate
- Inoculate the control organisms in the same manner on the same plate
- Incubate anaerobically at 35-37°C for 24-48hr
- Examine the plate for an opalscent halo around the inoculum and inhibition by antitoxin

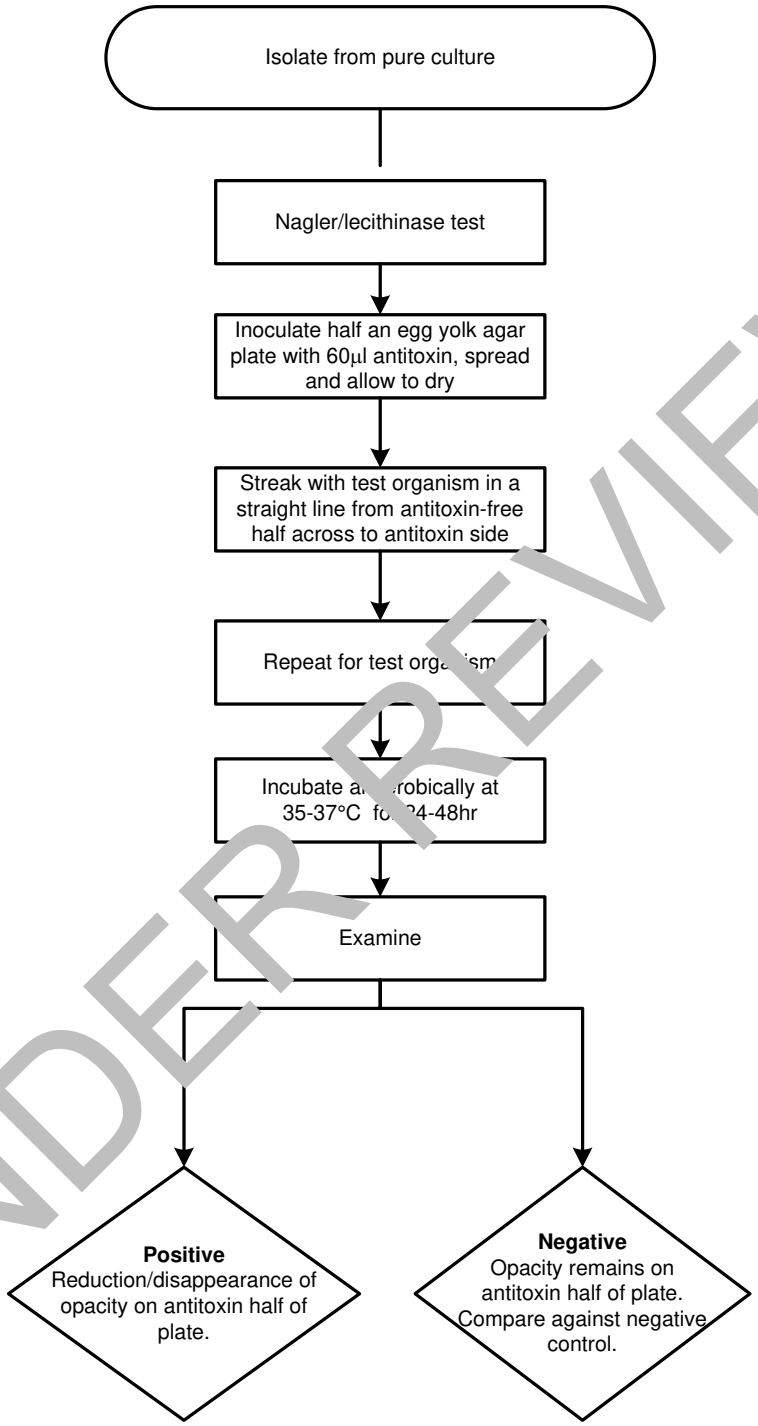
Positive Result

Disappearance or marked reduction of the opacity on the antitoxin half of the plate (denoting neutralisation of the lecithinase).

Negative Result

No disappearance of the opacity on the antitoxin half of the plate. Compare negative plate with uninoculated plate, because lecithinase can diffuse through out the agar and make interpretation difficult.

Appendix: Nagler Test



Note:

Positive control:	*Clostridium perfringens*	NCTC 8359
Negative control:	*Clostridium difficile*	NCTC 11204

The flowchart is for guidance only

References

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2. Barrow GI, Feltham RKA, editors. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd ed. Cambridge: Cambridge University Press; 1993. p. 36-7

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".

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