

NATIONAL STANDARD METHOD

NAGLER TEST

BSOP TP 22

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



NAGLER 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 22
Controlled document title	Nagler 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	1.1	2		Whole document	Document reviewed, reference strains updated
3/ 09.12.10	2	2.1	1	Front page	The Association of Medical Microbiologist logo replaced with new British Infection Association logo.
			9	Appendix	Flowchart amended

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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.

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 agar². 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*³. Lipolytic organisms also produce an opalescence on egg yolk agar which is often accompanied by a distinctive “pearly layer” or iridescent film².

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-reaction².

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.

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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.

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 6801.

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

NB Strains not validated by NCTC to give this result

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4 PROCEDURE AND RESULTS

4.1 NAGLER/LECITHINASE PROCEDURE

- Inoculate half the egg yolk agar plate with 60 µL antitoxin. Spread with a 'hockey stick' spreader or 10 µL 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 24-48 hours
- Examine the plate for an opalescent 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.

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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.

For further information please contact us at:

Standards Unit
Department for Evaluations, Standards and Training
Centre for Infections
Health Protection Agency
Colindale
London
NW9 5EQ

E-mail: standards@hpa.org.uk

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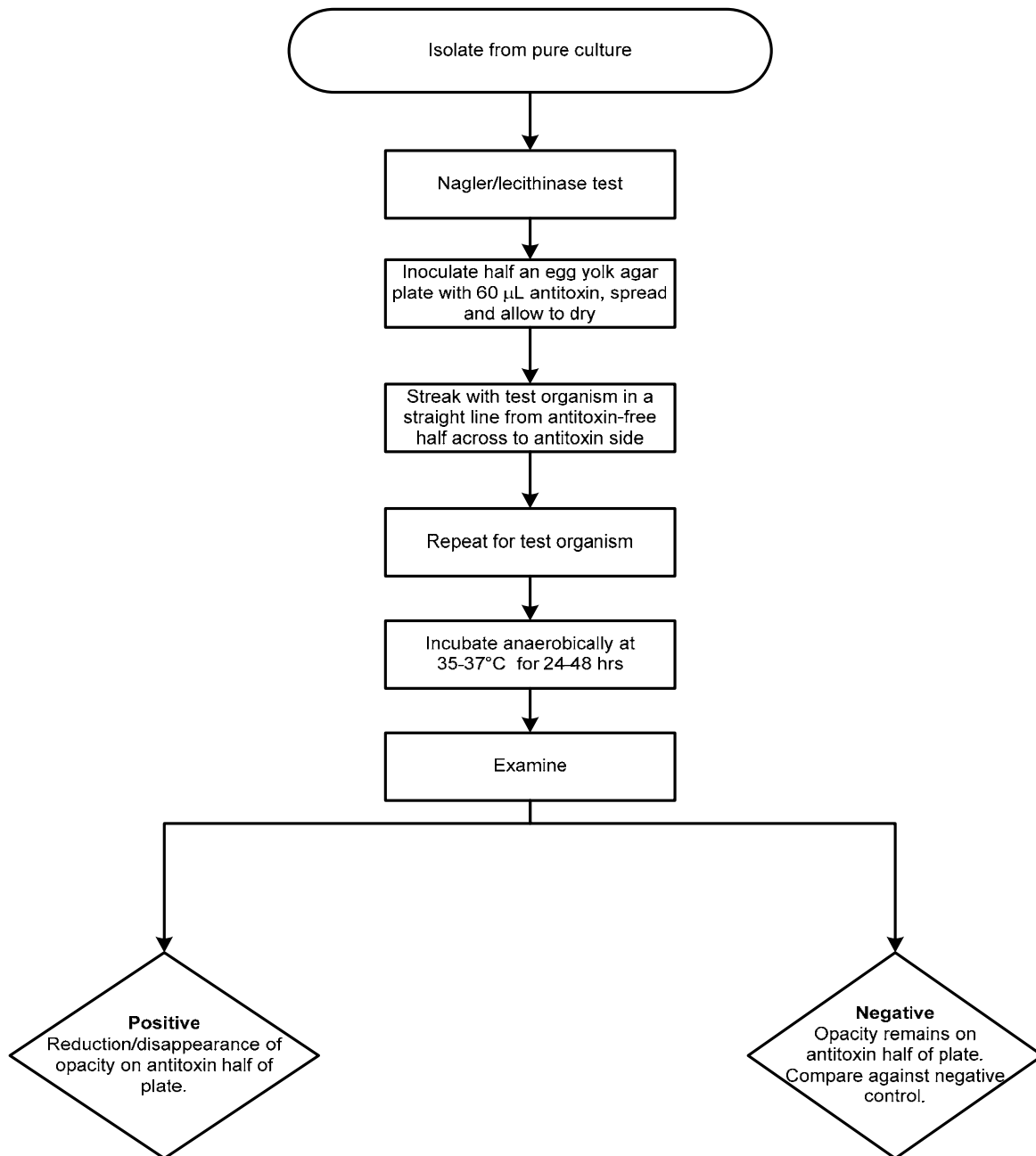
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APPENDIX: NAGLER TEST FLOWCHART



Note:

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

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