

NATIONAL STANDARD METHOD

INDOLE TEST

BSOP TP 19

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



INDOLE TEST

Issue no: 2.1 Issue date: 09.12.10 Issued by: Standards Unit, Department for Evaluations, Standards and Training Page: 1 of 9
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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 19
Controlled document title	Indole 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/ 23.04.10	1.1	2	All	Whole document	Document reviewed, no updates required
3/ 09.12.10	2	2.1	1	Front page	The Association of Medical Microbiologist logo replaced with new British Infection Association logo.
			6	Quality control organisms	Positive control amended from <i>Providencia rettgeri</i> NCTC 7475 to <i>Escherichia coli</i> NCTC 10418. Negative control corrected from <i>Serratia marcescens</i> NCTC 11935 to <i>Proteus mirabilis</i> NCTC 10975
			8	Appendix	Flowchart amended

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INDOLE TEST

SCOPE OF DOCUMENT

The indole test detects tryptophanase production, and is an aid in differentiation of the Enterobacteriaceae and other genera.

INTRODUCTION

The indole test determines the ability of an organism to produce indole from the degradation of the amino acid tryptophan. Tryptophan is hydrolysed by tryptophanase to produce three possible end products – one of which is indole².

A coloured product is produced when the indole is combined with certain aldehydes³.

Two methods are described; a spot indole test, which detects rapid indole producing organisms and a conventional tube method requiring overnight incubation, which identifies weak indole producing organisms.

TECHNICAL INFORMATION/LIMITATIONS

If peptone is used instead of tryptophan broth, the batch should be checked with a positive control to ensure the peptone is adequate for indole production.

Organisms to be tested by the spot indole method must be taken from a tryptophan - containing medium (eg blood agar).

Do not use peptone media with added glucose because acid production may inhibit indole production due to a change in pH⁴.

Anaerobes, particularly *Clostridium* species, can rapidly break down indole.

Cultures to be tested for indole must be incubated aerobically (as far as possible)⁵ because a decrease in oxygen tension decreases indole production.

Indole is a diffusible product. To prevent indole diffusion select a well isolated colony for the spot indole test.

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1 SAFETY CONSIDERATIONS⁶⁻¹²

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.

Hydrochloric acid is corrosive.

Kovac's indole reagent is an irritant.

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

Compliance with postal and transport regulations is essential

2 REAGENTS AND EQUIPMENT

Tube method²

1% tryptophan or peptone broth.

Kovac's reagent (for use with broth cultures).

Spot test reagent (for use with plate cultures)¹³

Use commercial kit and follow manufacturer's instructions.

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

3 QUALITY CONTROL ORGANISMS¹⁴

Positive control: *Escherichia coli* NCTC 10418

Negative control: *Proteus mirabilis* NCTC 10975

INDOLE TEST

4 PROCEDURE AND RESULTS

4.1 BROTH CULTURES

- Inoculate the tryptophan (or peptone) broth with the test organism and incubate at 37°C for 24-28 hours
- Add 0.5 mL of the Kovac's reagent and gently agitate
- Examine the upper layer of liquid

Positive result red colour (occurring within a few seconds)

Negative result yellow colour

4.2 SPOT TEST

- Moisten a piece of filter paper with the spot test reagent and smear a colony on to the surface
- Examine immediately

Positive result: Green/blue colour

Negative result: No colour change

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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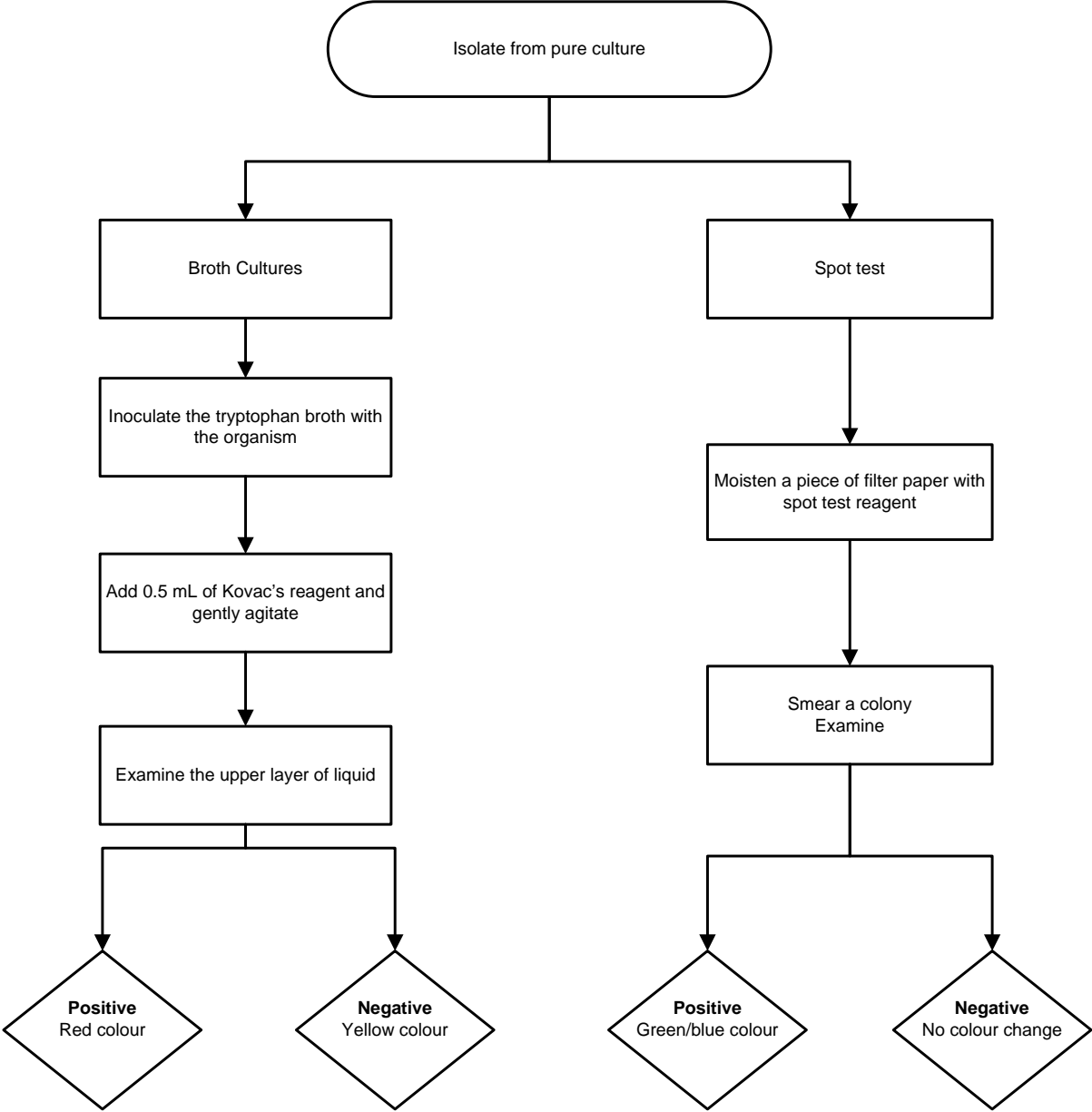
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APPENDIX: INDOLE TEST FLOWCHART



Note:

Positive control: *Escherichia coli* NCTC 10418

Negative control: *Proteus mirabilis* NCTC 10975

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