INDOLE TEST

BSOP TP 19

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

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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.

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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.
1 SAFETY CONSIDERATIONS\textsuperscript{6-12}

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 method\textsuperscript{2}

1% tryptophan or peptone broth.

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

Spot test reagent (for use with plate cultures)\textsuperscript{13}

Use commercial kit and follow manufacturer’s instructions.

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

3 QUALITY CONTROL ORGANISMS\textsuperscript{14}

Positive control: \textit{Providencia rettgeri} NCTC 7475

Negative control: \textit{Serratia marcescens} NCTC 11935
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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APPENDIX: INDOLE TEST FLOWCHART

Isolate from pure culture

**Broth Cultures**

Inoculate the tryptophan broth with the organism

Add 0.5 mL of Kovac’s reagent and gently agitate

Examine the upper layer of liquid

Positive
Red colour

Negative
Yellow colour

**Spot test**

Moisten a piece of filter paper with spot test reagent

Smear a colony
Examine

Positive
Green/blue colour

Negative
No colour change

**Note:**

Positive control: *Providencia rettgeri* NCTC 7475
Negative control: *Serratia marcescens* NCTC 11935
REFERENCES


