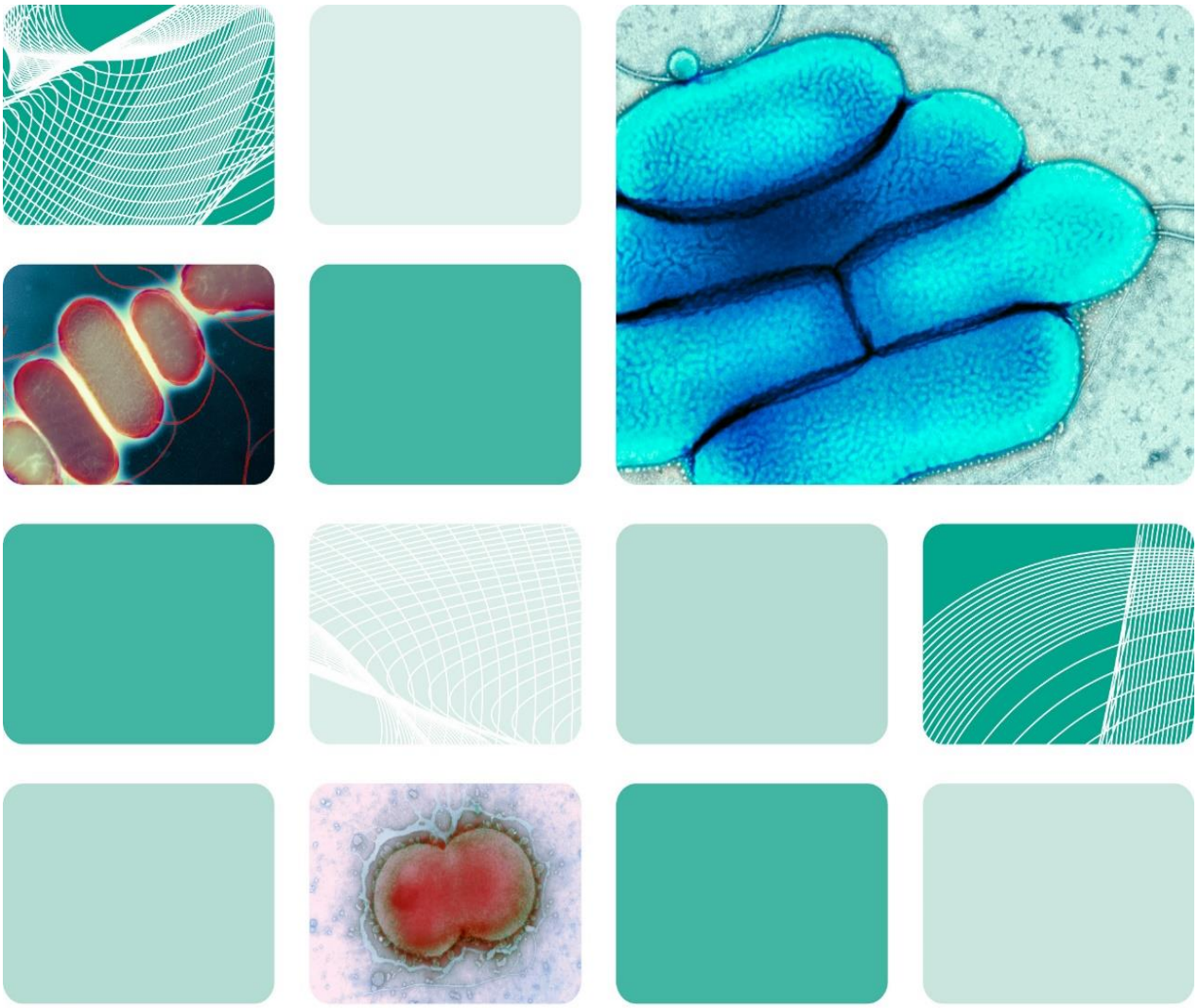
UK Health Security Agency

UK Standards for Microbiology Investigations

Indole 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	8/28.02.25
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 03/12/2018.** 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	7/03.12.18
Issue number discarded	3
Insert issue number	4
Anticipated next review date*	03.12.21
Section(s) involved	**Amendment**
Whole document.	Document and flowchart updated.

	Technical limitations updated with subheadings. References updated with grades.
Quality control organisms.	Alternative positive bacterial NCTC strain tested and validated for this test.

*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.

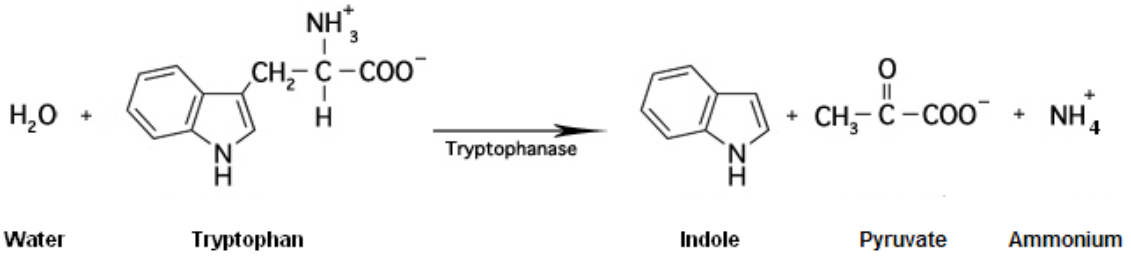
3 Scope of document

This document covers the procedure for indole test. The indole test detects tryptophanase production and is an aid in the differentiation of the Enterobacterales and other genera.

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

4 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 indole, the others are pyruvate and ammonium ion as shown by the following reaction[1]:



A coloured product is produced when the indole is combined with certain aldehydes[2].

Two indole test 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.

5 Technical information/limitations

5.1 Peptone broth varieties

If peptone broth is used instead of tryptophan broth, the batch should be checked with a positive control to ensure the peptone is adequate for indole production. This is because there are varieties of peptone broth media on the market, and some are unsuitable for indole production because they contain too little tryptophan.

5.2 Spot indole method

Organisms to be tested by the spot indole method must be taken from a tryptophan - containing medium (for example blood agar) and never from MacConkey agar as they have pH indicators and pigmentation of lactose-positive colonies which will make interpretation of colour reaction difficult[1]. The test can be carried out from some chromogenic agars[3,4].

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

5.3 Inhibition of indole production

Peptone media with added glucose should not be used because acid production may inhibit indole production due to a change in pH[1,5].

5.4 False reactions

Anaerobes, particularly *Clostridium* species, form indole but can rapidly break it down as it is produced; therefore, false negative reactions may occur[1].

False positive reactions may occur with the spot indole test if the inoculum is a mixed culture of indole positive and indole negative organisms[1,4,6].

5.5 Aerobic incubation

Cultures to be tested for indole must be incubated aerobically because a decrease in oxygen tension decreases indole production[1].

5.6 Alternative reagent

Ehrlich's reagent, an alternative to Kovács reagent, also contains Dimethylamino-benzaldehyde (DMAB), which reacts with indole to produce a red product. The Ehrlich formulation is more sensitive but contains additional toxic or flammable solvents; it is recommended when testing bacterial groups that produce little indole such as non-fermentative bacilli or anaerobes. Kovács reagent is more stable and the absence of the additional organic extraction (required with Ehrlich's) makes Kovács formulation more suitable for laboratories[7].

6 Safety considerations[8-25]

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.

Extreme care should be taken by staff when the Kovác's reagent has to be made up before use, as one of the key ingredients used is the concentrated Hydrochloric acid and it is highly corrosive.

Kovác'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.

7 Reagents and equipment

Discrete bacterial colonies on solid medium.

Tube method

1% tryptophan or peptone broth.

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

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

Spot indole test

Whatman no. 1 Filter paper.

Spot indole reagent (1% or 5% *p*-methylaminobenzaldehyde OR 1% *p*-dimethylaminocinnamaledhyde)[4].

If using commercial kit, follow manufacturer's instructions.

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

Petri dish.

8 Quality control organisms

Positive control:

Escherichia coli NCTC 10418 or NCTC 12241

Negative control:

Proteus mirabilis NCTC 10975

Note: The reference strains are validated by NCTC for the test shown.

9 Procedure and results

9.1 Tube method (broth cultures)[1,26]

- inoculate the tryptophan (or peptone) broth with the test organism and incubate at 37°C for 24 - 48hr
- add 0.5mL of the Kovác's reagent and shake gently
- examine the upper layer of liquid after about 1min

Positive result

Formation of a pink to red colour (occurring within a few seconds)

Negative result

No colour change, the reagent layer remains yellow or slightly cloudy

9.2 Spot indole test[4,27]

- place a piece of filter paper (Whatman no.1) into a sterile Petri dish and moisten with 1 -1.5mL Indole reagent or if using commercial pre-prepared filter paper containing the indole reagent, to equilibrate to room temperature before use
- smear an isolated pure colony (from an 18 -24hr culture) onto the saturated surface of the filter paper using a sterile loop
- examine immediately

Positive result

Follow manufacturer's instructions and interpretations.

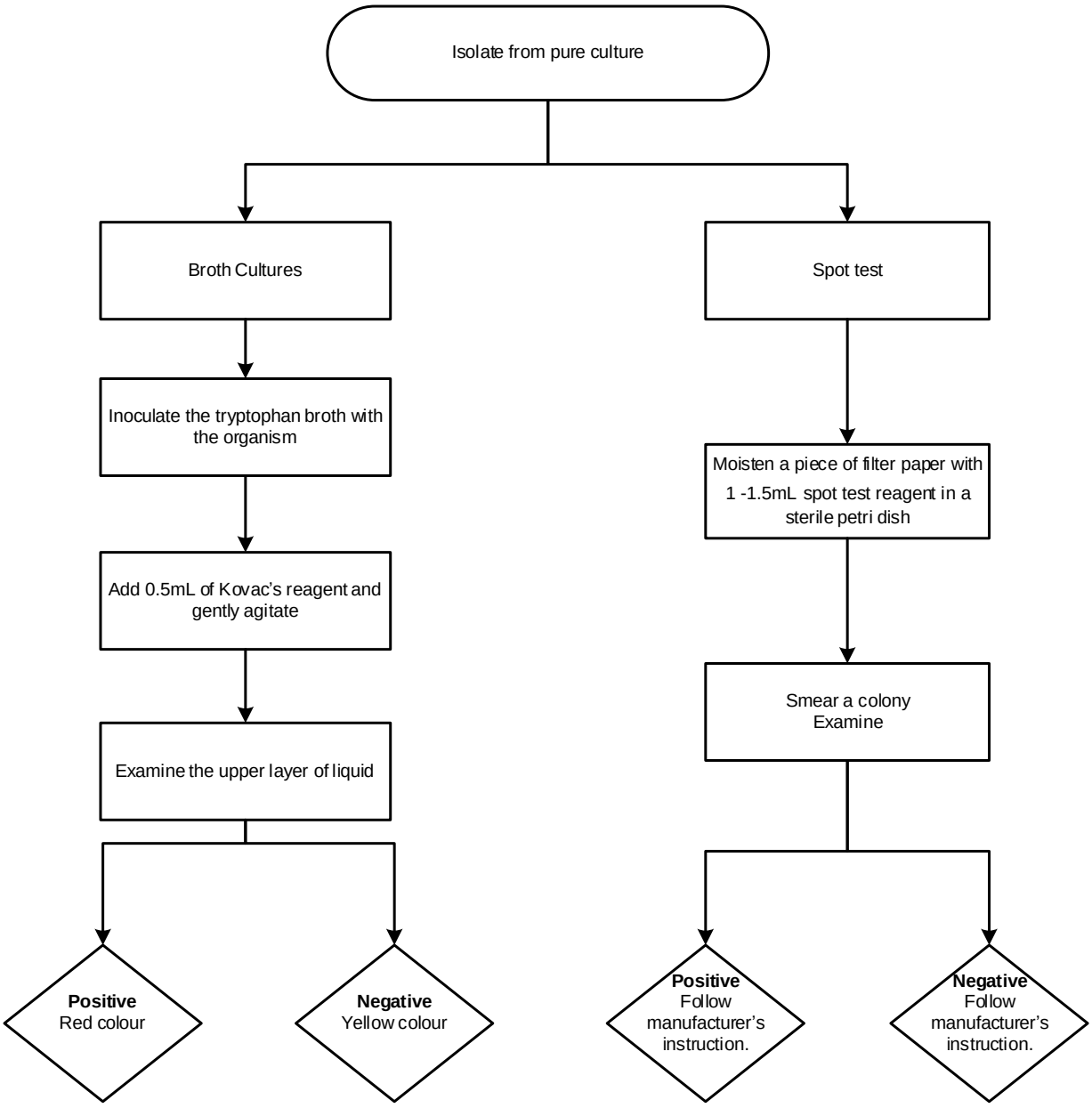
Negative result

Follow manufacturer's instructions and interpretations.

Note:

1. The API commercial kits can also be used to determine whether an organism is Indole positive or negative.
2. Depending on the spot indole reagent used for the spot indole test, the resulting colours differ. If using *p*-methylaminobenzaldehyde, the presence of indole is indicated by a red colour and if using *p*-dimethylaminocinnamaledhyde, a bluish-green colour is observed.

Algorithm: Indole test



Note:

Positive control: *Escherichia coli* NCTC 10418 or NCTC 12241
Negative control: *Proteus mirabilis* NCTC 10975

References

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

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