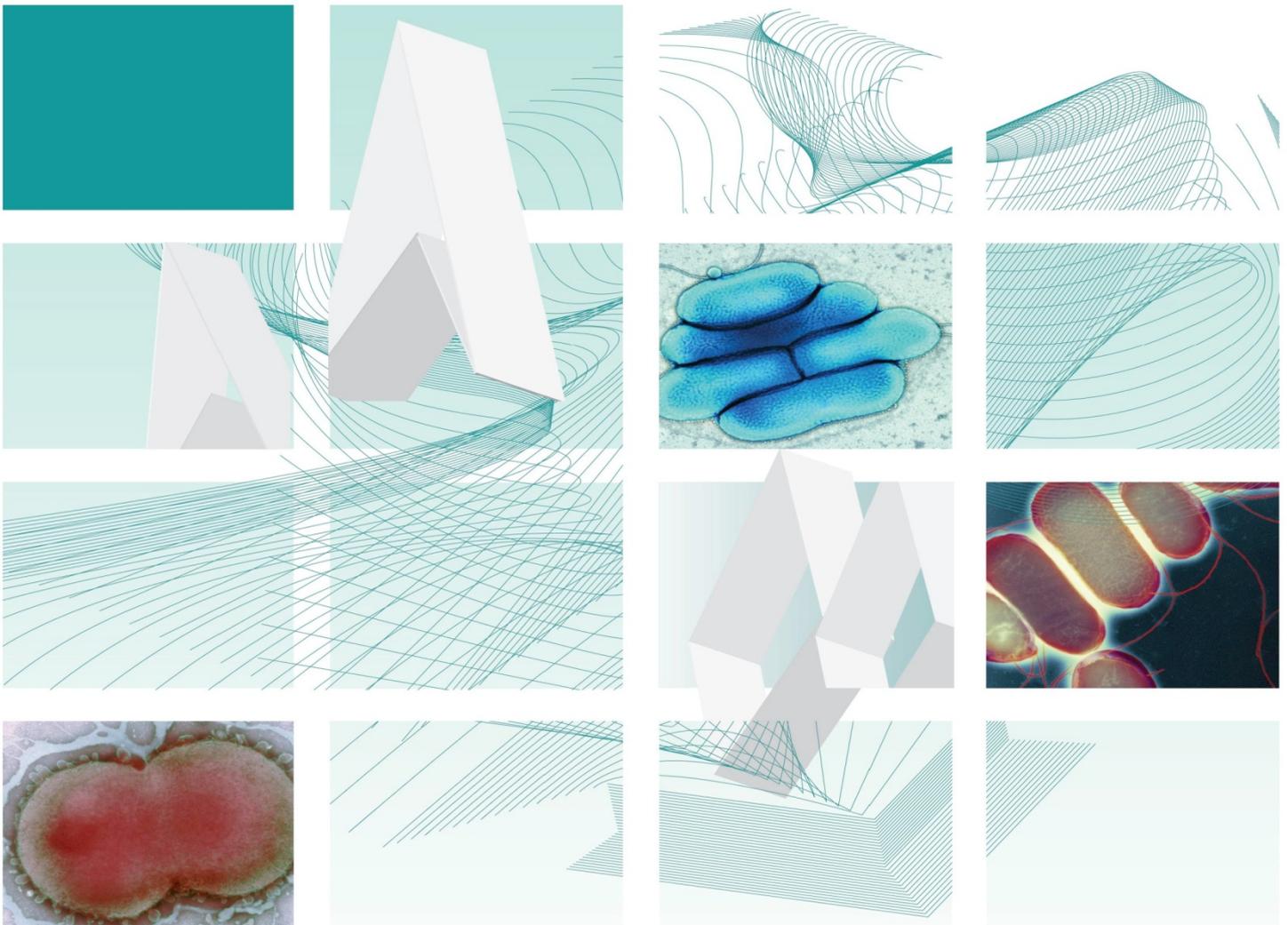




UK Standards for Microbiology Investigations

Indole Test



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 <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee>).

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:



Logos correct at time of publishing.

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NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

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.	6/21.11.14
Issue no. discarded.	2.3
Insert Issue no.	3
Section(s) involved	Amendment
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Introduction.	Updated to include the diagram of the degradation of the amino acid tryptophan with references.
Technical information/Limitations.	This section has been updated and references added.
Safety Considerations.	Section updated.
Reagents/Equipment.	Updated with references.
Procedures and Results.	This has been updated with references.
Flowchart.	This has been amended for easy guidance.
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 <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>.

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 <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>. 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.

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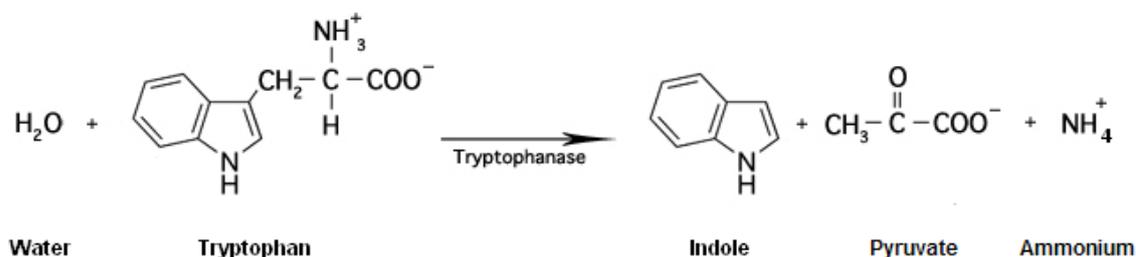
Scope of Document

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

This SMI should be used in conjunction with other SMIs.

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 ammonium¹.



A coloured product is produced when the indole is combined with certain aldehydes².

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 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 media on the market, and some are unsuitable for indole production because they contain too little tryptophan.

Organisms to be tested by the spot indole method must be taken from a tryptophan - containing medium (eg 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 difficult¹. The test can also be carried out from some chromogenic agars³.

Peptone media with added glucose should not be used because acid production may inhibit indole production due to a change in pH⁴.

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

Cultures to be tested for indole must be incubated aerobically because a decrease in oxygen tension decreases indole production¹.

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

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 nonfermentative 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 laboratories⁵.

1 Safety Considerations⁶⁻²²

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.

Extreme care should be taken by staff when the Kovac'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.

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

Discrete bacterial colonies on solid medium.

Tube method

1% tryptophan or peptone broth.

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

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

Spot Indole test

Whatman no. 1 Filter paper.

Use commercial kit and follow manufacturer's instructions.

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

Petri dish.

3 Quality Control Organisms

Positive Control

Escherichia coli NCTC 10418

Negative Control

Proteus mirabilis NCTC 10975

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

4 Procedure and Results

4.1 Tube Method (Broth cultures)^{1,23}

- Inoculate the tryptophan (or peptone) broth with the test organism and incubate at 37°C for 24 - 48hr
- Add 0.5mL of the Kovac's reagent and agitate 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

4.2 Spot Indole Test²⁴

- Place a piece of filter paper (Whatman no.1) into a sterile Petri dish and moisten with the 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

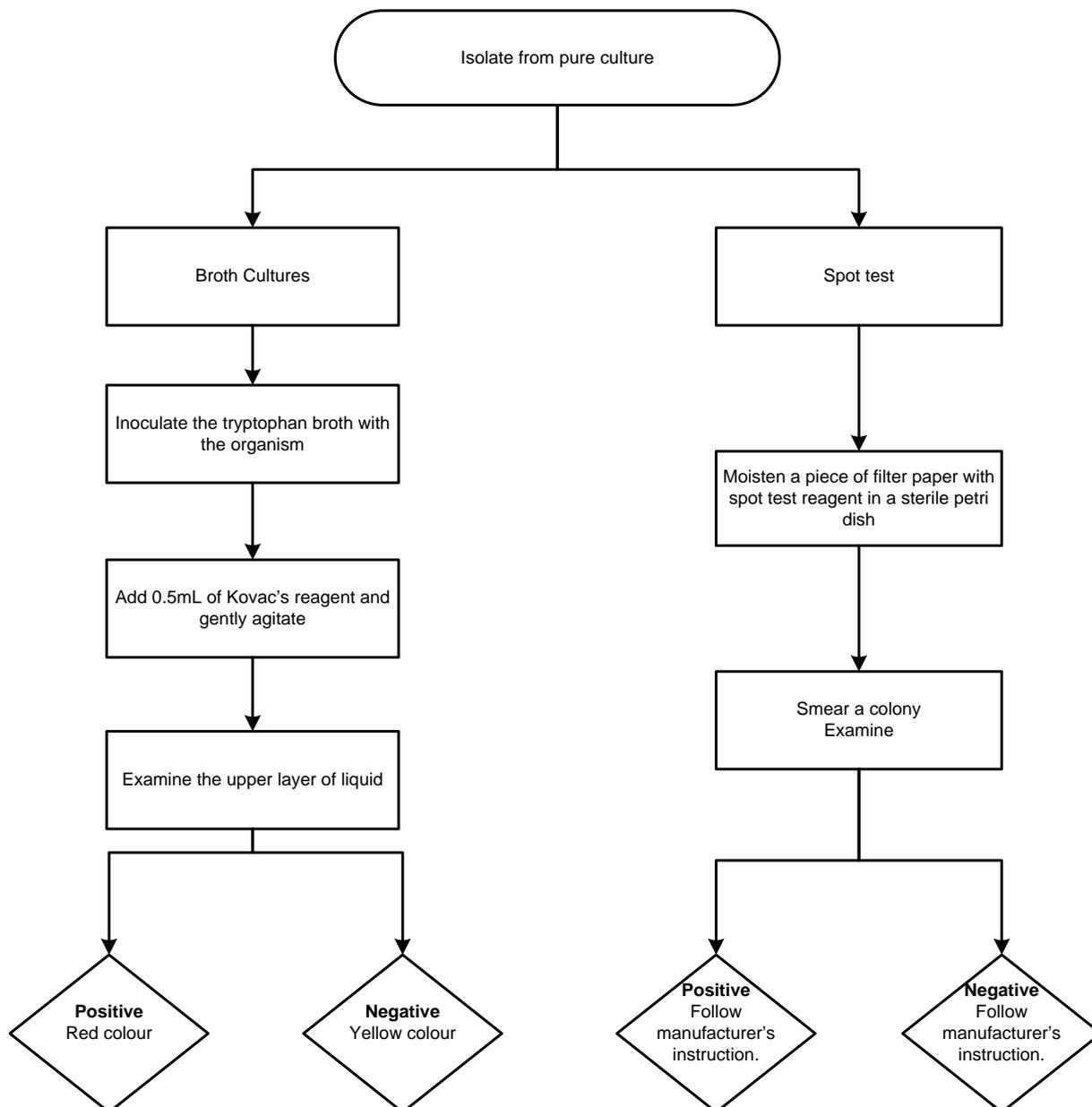
Follow manufacturer's instructions and interpretations.

Negative Result

Follow manufacturer's instructions and interpretations.

Note: The API commercial kits can also be used to determine whether an organism is Indole positive or negative.

Appendix: Indole Test



Note:

Positive control: *Escherichia coli* NCTC 10418

Negative control: *Proteus mirabilis* NCTC 10975

The flowchart is for guidance only.

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