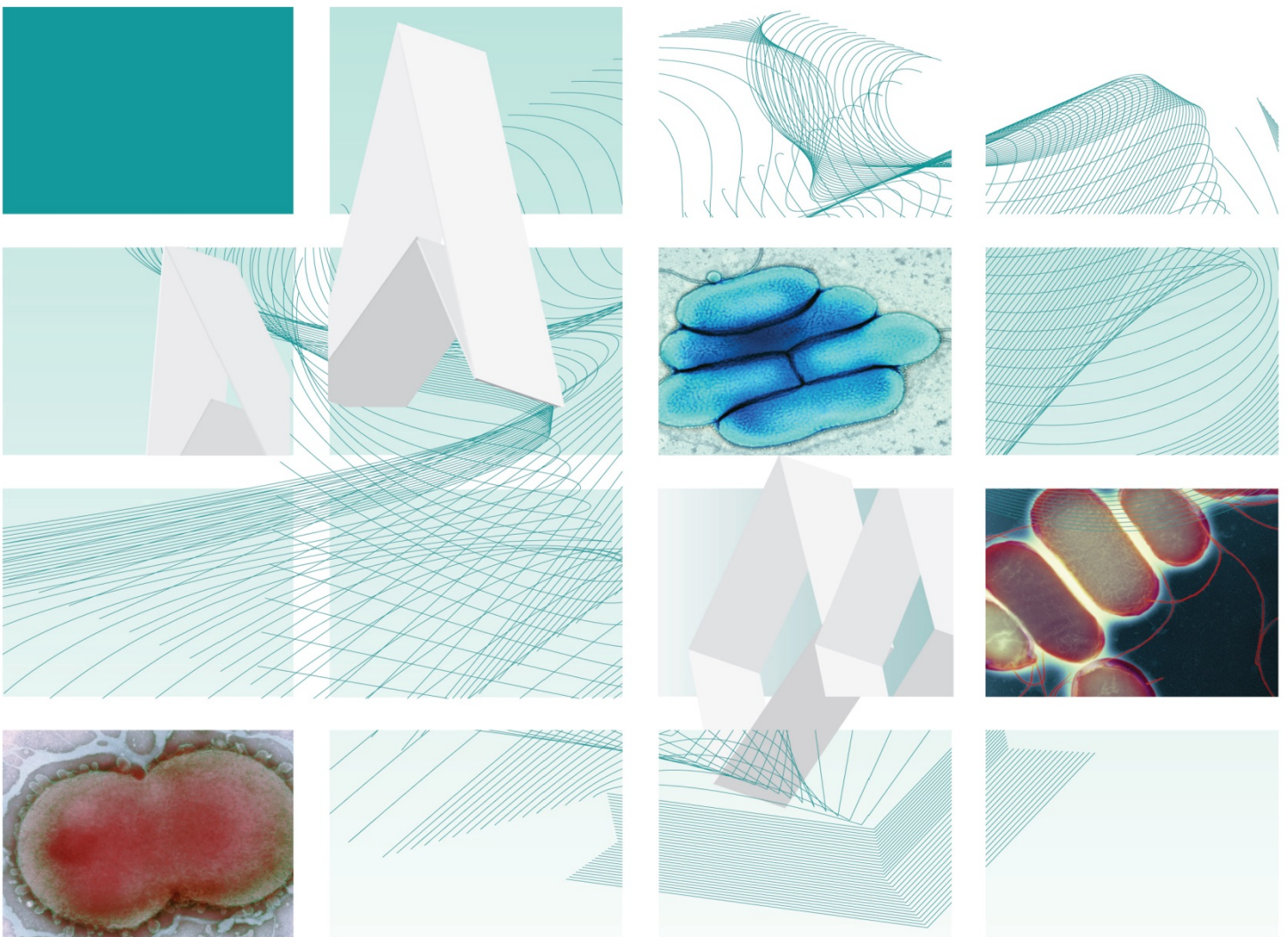




UK Standards for Microbiology Investigations

Indole test



"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365¹, 2016**. The original accreditation term began in **July 2011**."

Acknowledgments

UK Standards for Microbiology Investigations (UK 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>. UK 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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PHE publications gateway number: 2018381

UK Standards for Microbiology Investigations are produced in association with:



Logos correct at time of publishing.

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Amendment table

Each UK 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 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 five years subject to resources available.

UK SMI[#]: scope and purpose

Users of UK SMIs

Primarily, UK SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. UK SMIs also 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. The documents also 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 UK SMIs

UK 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. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of UK 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

UK 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><http://www.hpa-standardmethods.org.uk/>.

Inclusion of a logo in an UK SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing UK SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop UK 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. UK SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the UK SMI working groups to produce UK SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of UK SMIs is certified to ISO 9001:2008. UK SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. UK SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level

[#] 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.

of complex laboratory investigation possible. In using UK SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. UK SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. UK SMIs also provide a reference point for method development. The performance of UK 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 UK SMI working groups are committed to patient and public involvement in the development of UK SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting UK 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 UK SMIs is subject to PHE Equality objectives <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>.

The UK 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

While every care has been taken in the preparation of UK SMIs, PHE and the partner organisations, 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 UK SMI or any information contained therein. If alterations are made by an end user to an UK SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as UK SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

The evidence base and microbial taxonomy for the UK SMI is as complete as possible at the date 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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Suggested citation for this document

Public Health England. (2018). Indole test. UK Standards for Microbiology Investigations. TP 19 Issue 4. <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>

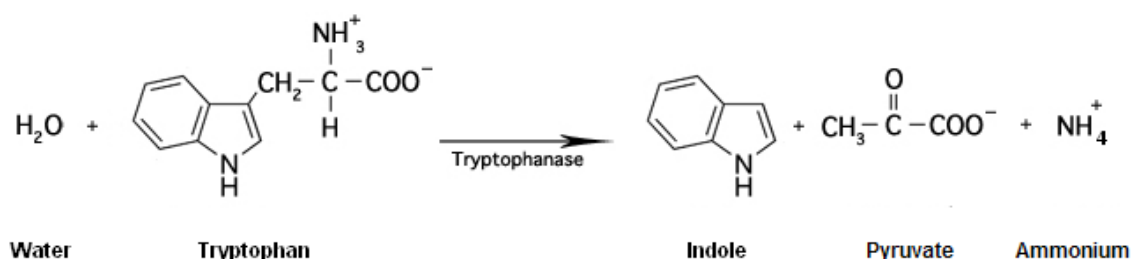
Scope of document

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

This UK SMI should be used in conjunction with other UK 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 ammonium ion as shown by the following reaction¹:



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

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.

Technical information/limitations

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.

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 difficult¹. 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.

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}.

False reactions

Anaerobes, particularly *Clostridium* species, form indole but can rapidly break it down as it is produced; therefore, false negative reactions may occur¹.

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}.

Aerobic incubation

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

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 laboratories⁷.

1 Safety considerations⁸⁻²⁵

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.

Kováč'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.

Kováč'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*-dimethylaminocinnamaledehyde)⁴.

If using commercial kit, 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 or NCTC 12241

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)^{1,26}

- 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

4.2 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.

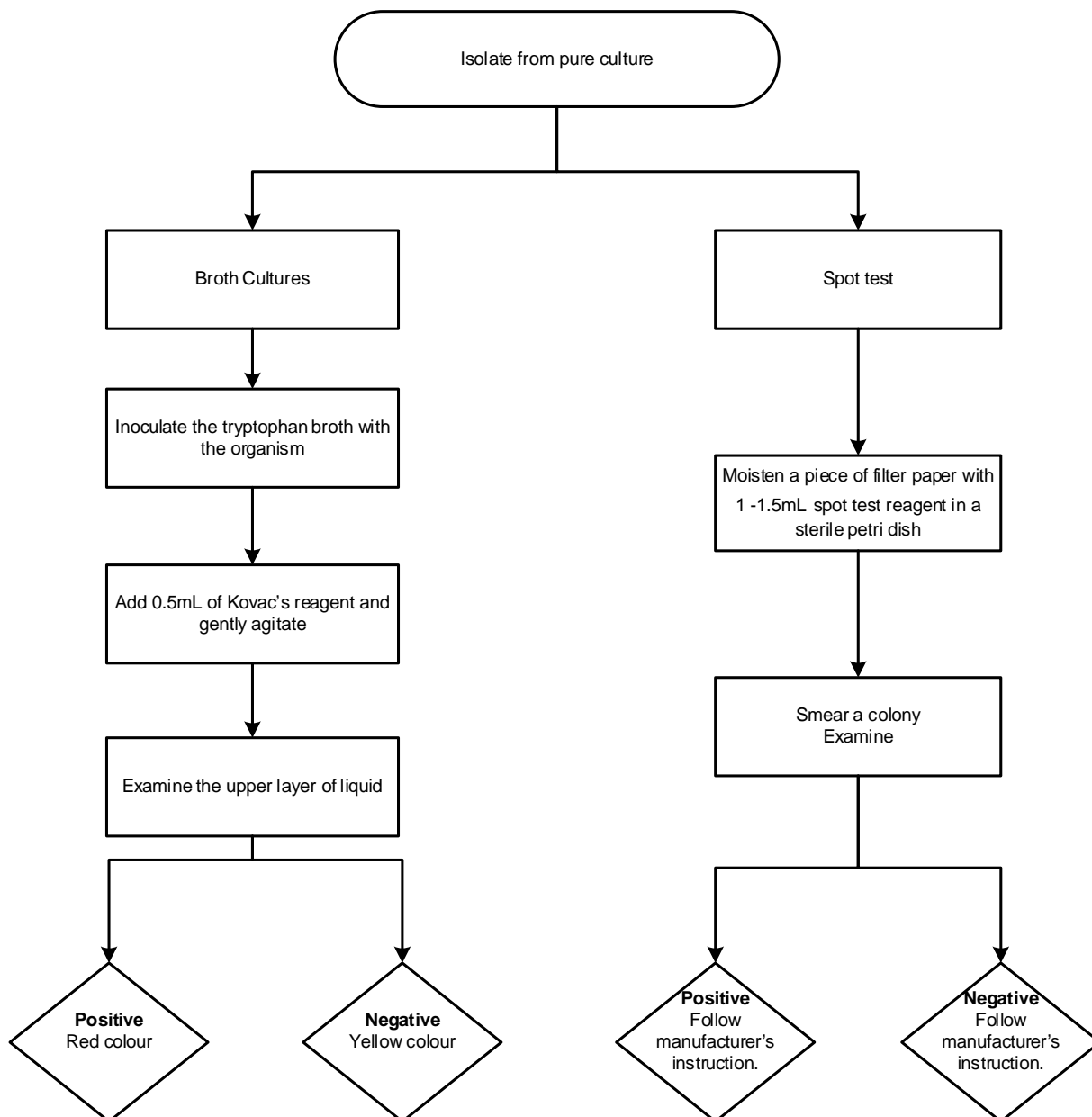
Negative result

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.

Appendix: Indole test



Note:

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

The flowchart is for guidance only.

References

Modified GRADE table used by UK SMI's when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMI's for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VIII). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Quality/certainty of evidence	Types of evidence
A Strongly recommended	I Evidence from randomised controlled trials, meta-analysis and systematic reviews
B* Recommended but other alternatives may be acceptable	II Evidence from non-randomised studies
	III Evidence from documents describing techniques, methods or protocols
C* Weakly recommended: seek alternatives	IV Non-analytical studies, eg case reports, reviews, case series
D Never recommended	V Expert opinion and wide acceptance as good practice but with no study evidence
	VI Required by legislation, code of practice or national standard/ guideline
	VII Letter /short communication /editorials /conference communication
	VIII Electronic citation

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