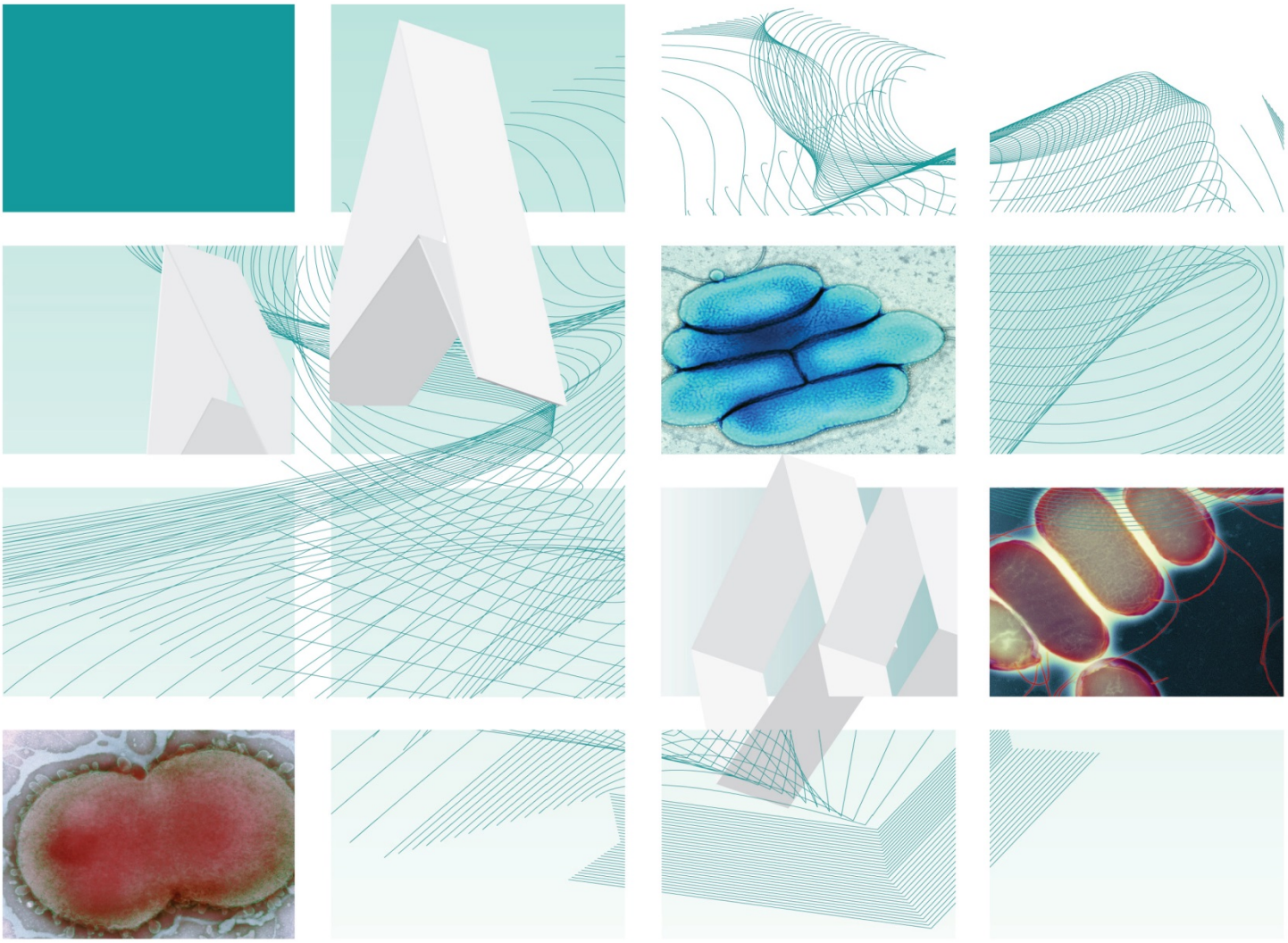
Public Health England

NHS

UK Standards for Microbiology Investigations

Urease test



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"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, S9365', 2016**. The original accreditation term began in **July 2011.**"

Issued by the Standards Unit, National Infection Service, PHE

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.

For further information please contact us at:

Standards Unit
National Infection Service
Public Health England
61 Colindale Avenue
London NW9 5EQ

E-mail: standards@phe.gov.uk

Website: https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

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Logos correct at time of publishing.

Contents

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	6/02.04.19
Issue number discarded	3
Insert issue number	4
Anticipated next review date*	02.04.22
Section(s) involved	**Amendment**
Whole document.	Document updated. Technical limitations updated with some information. References updated with grades. Flowchart updated to include commercial alternatives.
Quality control organisms.	Alternative negative bacterial NCTC strain (NCTC 12241) tested and validated for this test and EUCAST susceptibility tests. Fungal NCPF strains added.

*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. 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

[#] 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.

accredited and represent neither minimum standards of practice nor the highest level 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.

Suggested citation for this document

Public Health England. (2019). Urease test. UK Standards for Microbiology Investigations. TP 36 Issue 4. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

Scope of document

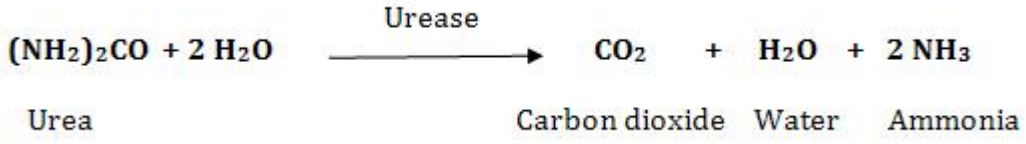
The urease test is used to differentiate urease-positive organisms (eg *Proteus*) from other organisms. It can also be used to detect the presence of *Helicobacter pylori*[1].

This test can be used for differentiation between the yeasts, *Candida albicans* and *Cryptococcus neoformans*. A presumptive identification of *C. neoformans* is based on rapid urease production, whilst *Candida albicans* do not produce urease[2,3].

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

Introduction

The urease test is used to determine the ability of an organism to split urea, through the production of the enzyme urease. Two units of ammonia are formed with resulting alkalinity in the presence of the enzyme, and the increased pH is detected by a pH indicator[4]. This is shown in the reaction below:



Adapted from Brink et al[5].

Christensen's urea agar contains 2% urea and phenol red as a pH indicator. An increase in pH due to the production of ammonia results in a colour change from yellow (pH 6.8) to bright pink (pH 8.2). Urea agar (Christensen's urea agar) has reduced buffer content and contains more peptones and glucose than Rustigian and Stuart's urea broth. This medium detects all rapidly urease-positive *Proteus* species and supports the growth of many enterobacteria allowing for the observation of urease activity[6].

Christensen's urea broth is a nutrient broth to which 2% urea is added. The pH indicator is phenol red, which is red at neutral pH but turns yellow at pH < 6.8 and changes to magenta or bright pink at pH >8.4.

Rustigian and Stuart's urea broth is a highly buffered medium requiring large quantities of ammonia to raise the pH above 8.0 resulting in a colour change. This differential medium provides all essential nutrients for *Proteus* [7]. The medium also give positive results for most *Morganella* species and a few *Providencia stuartii* strains.

Technical information/limitations

Urease test on cultures

Helicobacter pylori split urea rapidly, usually within 30 seconds. Its rapidity is a key distinguishing factor for *H. pylori* from other *Helicobacter* species.

Bacteroides ureolyticus splits urea rapidly, usually within a few minutes, and this is a quick way of identifying this organism.

The urease test may be used to distinguish *Psychrobacter phenylpyruvicus* from *Moraxella* species and *Corynebacterium diphtheriae* which is urease negative from the urease positive *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis*.

Some strains of *Enterobacter* and *Klebsiella* species are also urease positive.

Other species of *Cryptococcus*, *Trichosporon* and *Rhodotorula* can give a positive result for the urease test. Occasionally, *Candida krusei* can give a positive result[2].

Rapid urease test

The rapid urease test also known as the CLO test (*Campylobacter*-like organism test), is a rapid test for diagnosis of *Helicobacter pylori* infection on a biopsy sample. The principle of the test is the ability of *H. pylori* to secrete the urease enzyme, which catalyses the conversion of urea to ammonia and carbon dioxide. Results can be interpreted within 1 minute up to 3 hours, although the CLO test may be held up to 24 hours in some cases[1].

Note: Several commercial varieties of the Rapid Urease tests are available and users should follow manufacturers' instructions when using these.

Gastric biopsy specimen

It should be noted that when a drop of gastric biopsy specimen is added into the Christensen's urea broth, it changes colour very rapidly within an hour, if positive[1,8].

Quality control of media

Quality control should be carried out on each batch of urea media (agar or slant).

Urea media if exposed to light may develop peroxide, which can interfere with the urease test. Urea is also known to undergo auto-hydrolysis; and so, it is advisable to store media in the refrigerator 4-8°C.

All identification tests should be performed, where possible, from a non-selective medium. However, if the test is performed from a selective agar, a purity plate must still be included to check for purity of the organism.

1 Safety considerations[9-26]

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. For the urease test, a urea slope is considered safer than a liquid medium. Eye protection must be used where there is a known or potential risk of exposure to splashes.

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 colonies growing on solid medium

Two media types are commonly used to detect urease activity. Both media types are commercially available as prepared tubes or as a powder[4]. They are:

- Christensen's urea agar slant. This could be used in liquid form if desired
- Rustigian and Stuart's urea broth

Alternatively, a commercially available reagent/kit should be used according to the manufacturer's instructions.

Rapid urease test (agar gel-based tests or paper based strips, Christensen's urea broth) used for *H. pylori*

Bacteriological straight wire/loop or disposable alternative

3 Quality control organisms

Bacteria

Positive control

Proteus mirabilis NCTC 10975

Negative control

Escherichia coli NCTC 10418 or NCTC 12241

The bacterial reference strains have been validated by NCTC for the test.

Fungi

Positive control

Cryptococcus neoformans NCPF 3168

Negative control

Candida albicans NCPF 3281

The fungal reference strains have not been validated by NCTC for the test at the time of publication.

Note:

Negative and positive controls must be run simultaneously with the test specimens.

4 Procedure and results

4.1 Christensen's urea agar slant procedure[4,6]

- inoculate slope heavily (from an 18 - 24hr pure culture) over the entire surface by streaking the surface of the agar in a zigzag manner. Do not stab the butt; it serves as a colour control
- incubate inoculated slope with loosened caps at 35-37°C for 24-48 hours
- examine slopes for colour change after 6hr and after overnight incubation. Longer periods may be necessary

Note:

1. Rapidly urease-positive *Proteeae* (*Proteus* sp., *Morganella morganii*, and some *Providencia stuartii* strains) will produce a strong positive reaction within 1 to 6 hours of incubation.
2. Delayed-positive organisms (eg, *Klebsiella* or *Enterobacter*) will typically produce a weak positive reaction on the slant after 6 hours, but the reaction will intensify and spread to the butt on prolonged incubation (up to 6 days).
3. For yeasts, the inoculated slope is further incubated for up 4-5 days before it is considered negative.

4.2 Rustigian and Stuart's urea broth procedure[5,7]

- inoculate the broth heavily from an 18 - 24hr pure culture
- shake the tube gently to suspend the colonies
- incubate inoculated slope with loosened caps at 35°C in an incubator or water bath for 24-48 hours
- examine broths for colour change at 8,12, 24, and 48 hours of incubation

Note:

1. Rapidly urease-positive *Proteeae* (*Proteus* sp., *Morganella morganii*, and some *Providencia stuartii* strains) for which this medium is differential, will produce a strong positive reaction as early as 8 hours, but always within 48 hours of incubation.
2. Delayed-positive organisms (eg, *Enterobacter*) will not produce a positive reaction due to the high buffering capacity of this medium.

Interpretation

Positive result

Bright pink (fuchsia) colour on the slant that may extend into the butt

OR

Bright pink (fuchsia) colour throughout the broth

Note that any degree of pink is considered a positive reaction - See Appendix 2.

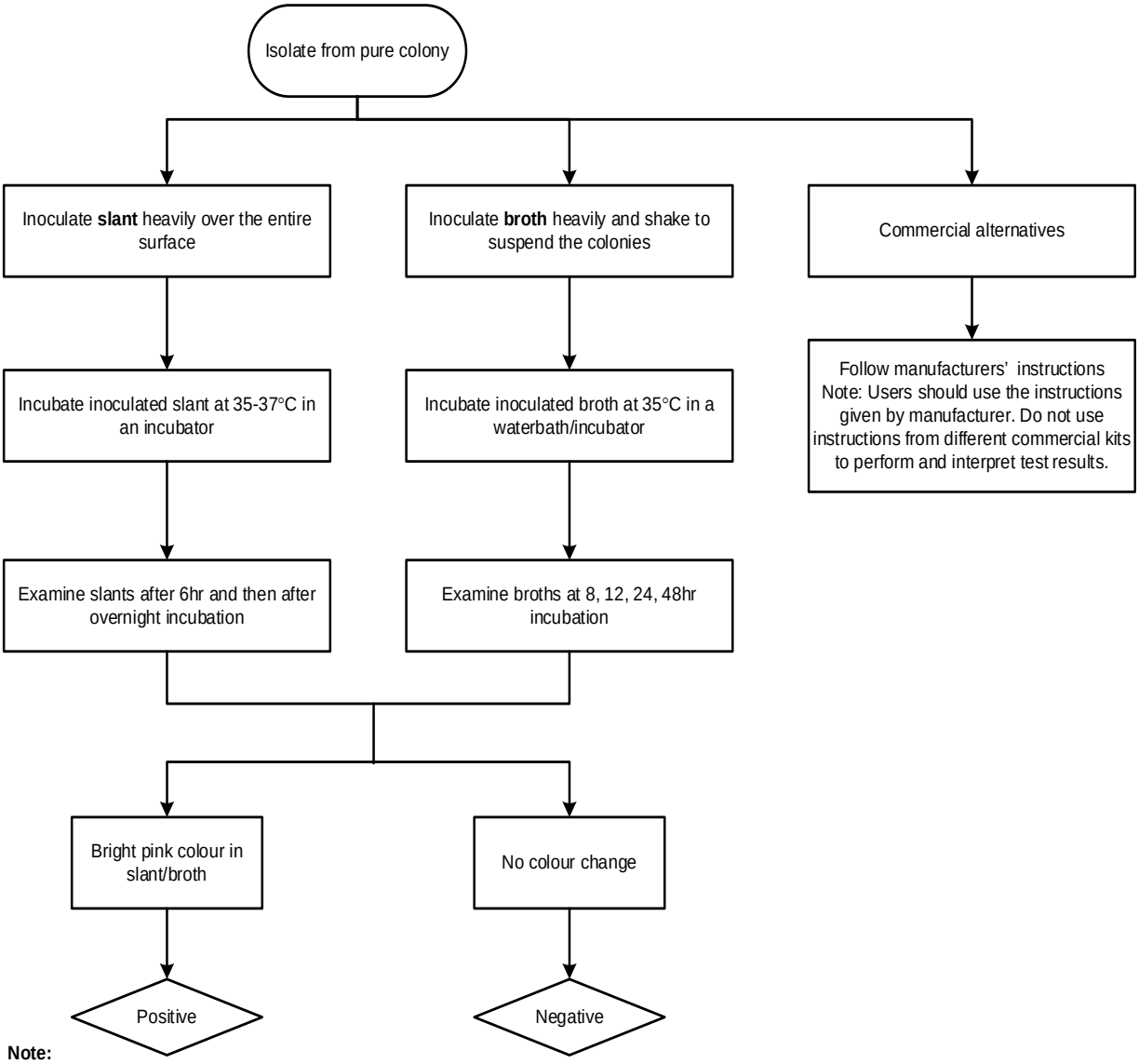
Negative result

No colour change in agar slant/broth

4.3 Rapid urease test using dry strips

Rapid urease test kits are available commercially and should be used following the manufacturer's instructions.

Appendix 1: Urease test



Note:
Bacteria

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

Fungi

Positive control	*Cryptococcus neoformans* NCPF 3168
Negative control	*Candida albicans* NCPF 3281

The flowchart is for guidance only.

Appendix 2: Urea agar and Urea slant test results Adapted from Brink, B., 2013[5]

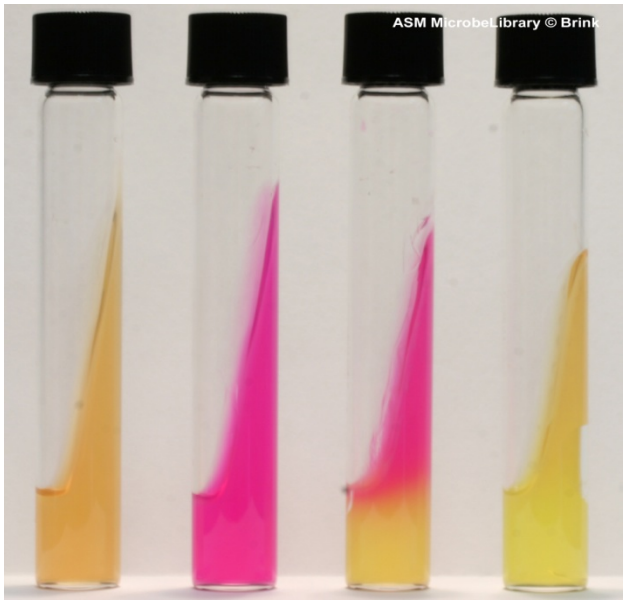


Figure 1. Urea agar test results. Urea agar slants were inoculated as follows: (a) uninoculated, (b) *Proteus mirabilis* (rapidly urease positive), (c) *Klebsiella pneumoniae* (delayed urease positive), (d) *Escherichia coli* (urease negative).

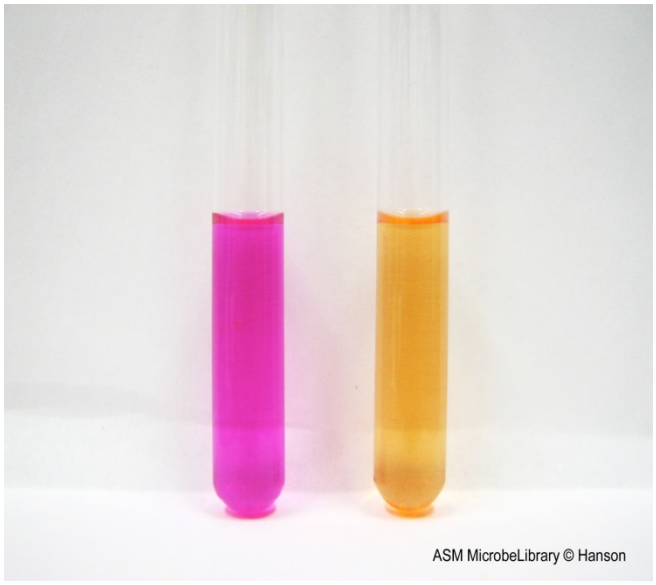


Figure 2. Urea broth test results. Urea broth test tubes were inoculated as follows: (a) *Proteus vulgaris* (urease positive) and (b) *Escherichia coli* (urease negative).

References

Modified GRADE table used by UK SMIs 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 SMIs 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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