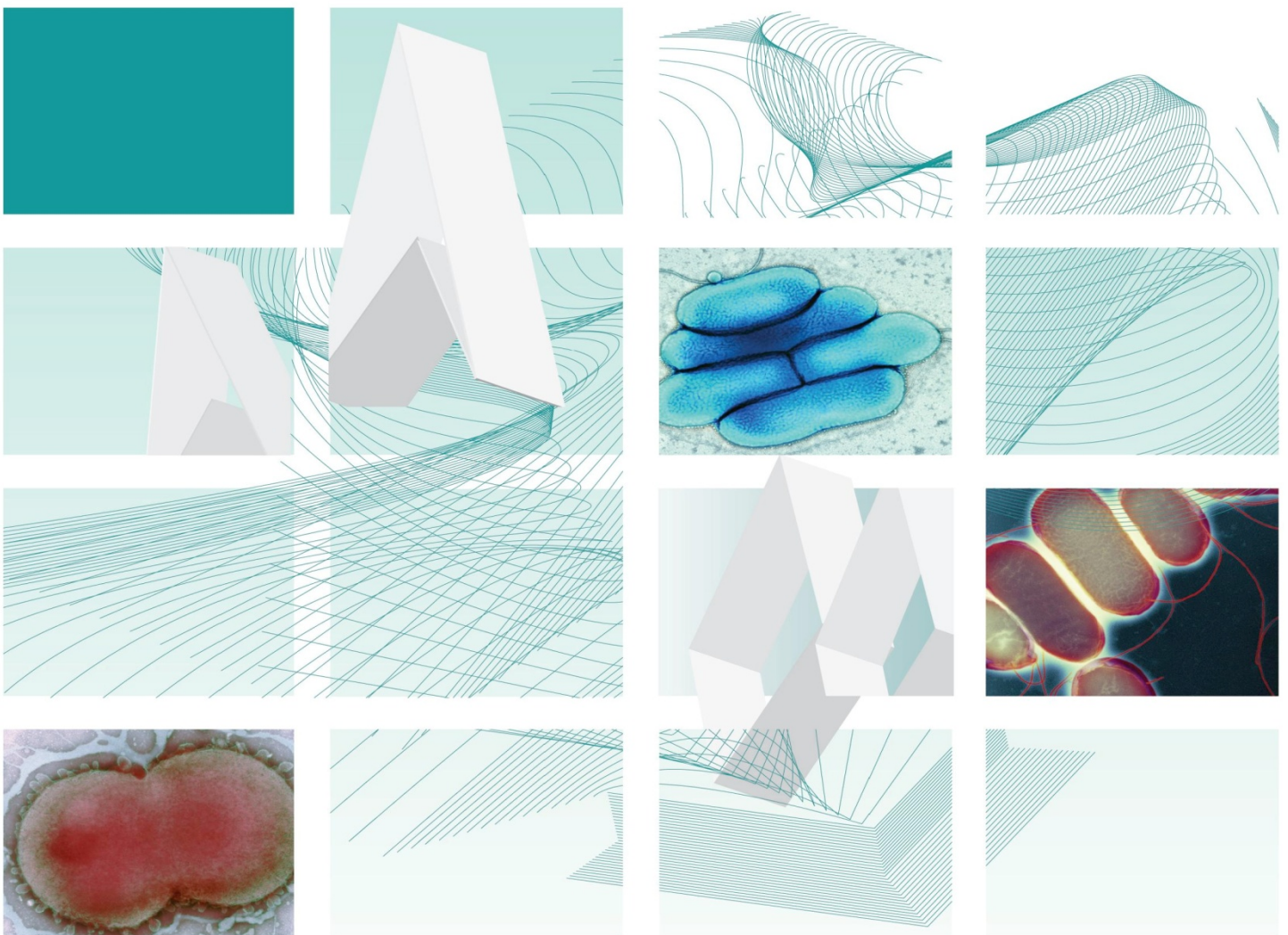




UK Standards for Microbiology Investigations

Urease 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.	5/14.01.15
Issue no. discarded.	2.2
Insert Issue no.	3
Section(s) involved	Amendment
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Introduction.	This section has been updated and references added.
Technical information/Limitations.	This section has been updated.
Safety Considerations.	References updated.
Reagents/Equipment.	This section has been updated and referenced.
Quality Control Organisms.	The quality control organisms have been validated by NCTC.
Procedures and Results.	Information and references updated.
Flowchart.	This flowchart has been modified for easy guidance. In Appendix 2, pictures of positive and negative urease tests are shown and referenced.
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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Suggested Citation for this Document

Public Health England. (2015). Urease Test. UK Standards for Microbiology Investigations. TP 36 Issue 3. <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>

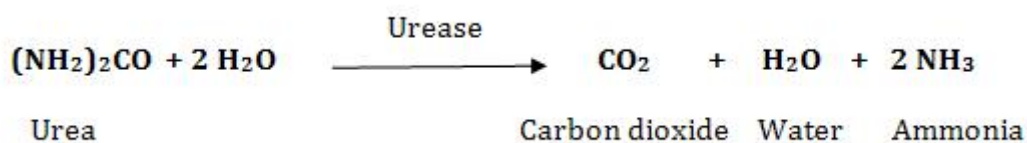
Scope of Document

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*.

This SMI should be used in conjunction with other 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 indicator¹. This is shown in the reaction below:



Adapted from Brink et al².

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 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 activity³.

Christensen's urea broth is a nutrient broth to which 2.0% urea is added. The pH indicator is phenol red, which is red at neutral pH but turns yellow at pH < 6.8. It also 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 medium provides all essential nutrients for *Proteus*, for which it is differential⁴. The medium also give positive results for most *Morganella* species and a few *Providencia stuartii* strains.

The urease test may be used to distinguish *Psychrobacter phenylpyruvicus* from *Moraxella* species, *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.

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

Technical Information/Limitations

Urease test

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

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*. 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 cases⁵.

Note: Several commercial varieties of the Rapid Urease tests are available and users should follow manufacturer's 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^{5,6}.

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. If the test is performed from selective agar, a purity plate must be included to check for purity of the organism.

1 Safety Considerations⁷⁻²³

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.

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 available commercially as prepared tubes or as a powder¹. 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 may 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

Positive Control

Proteus mirabilis NCTC 10975

Negative Control

Escherichia coli NCTC 10418

Note: The reference strain has been validated by NCTC for the test shown.

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

4 Procedure and Results

4.1 Christensen's Urea Agar Slant Procedure^{1,3}

- 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: Rapidly urease-positive *Proteaeae* (*Proteus* sp., *Morganella morganii*, and some *Providencia stuartii* strains) will produce a strong positive reaction within 1 to 6 hours of incubation. 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).

4.2 Rustigian and Stuart's Urea Broth Procedure^{2,4}

- 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: Rapidly urease-positive *Proteaeae* (*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. 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.

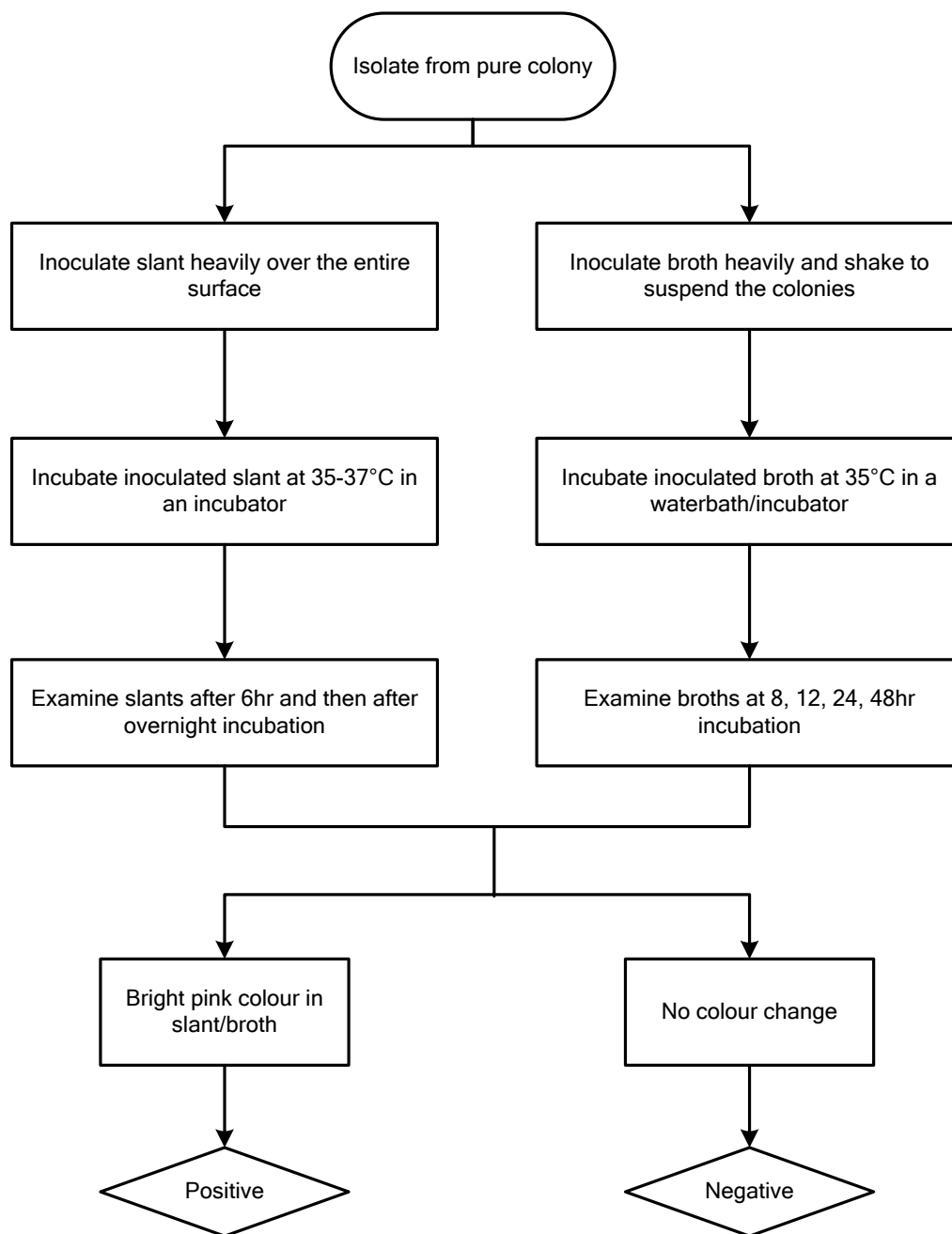
Negative Result

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: Positive control
Negative control

Proteus mirabilis NCTC 10975
Escherichia coli NCTC 10418

The flowchart is for guidance only.

Appendix 2: Urea agar and Urea slant test results

Adapted from Brink, B., 2013²

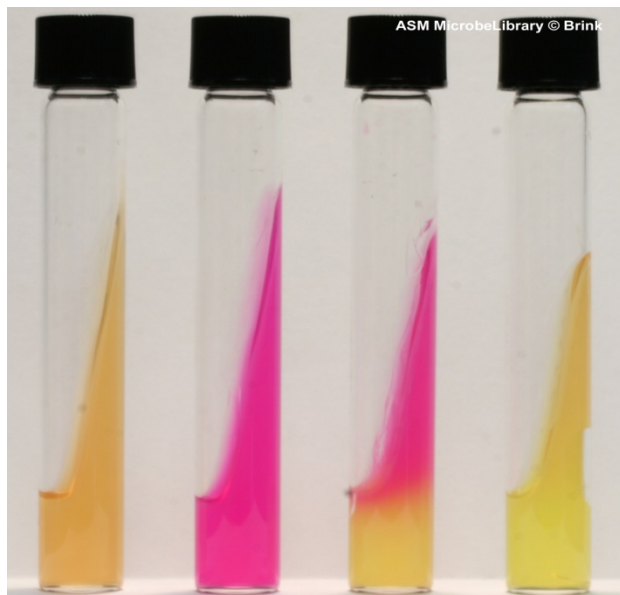


FIG. 1 Urea agar test results. Urea agar slants were inoculated as follows: (a) uninoculated, (b) *Proteus mirabilis* (rapidly urease positive), (c) *Klebsiella pneumoniae* (delayed urease positive), (d) *Escherichia coli* (urease negative).

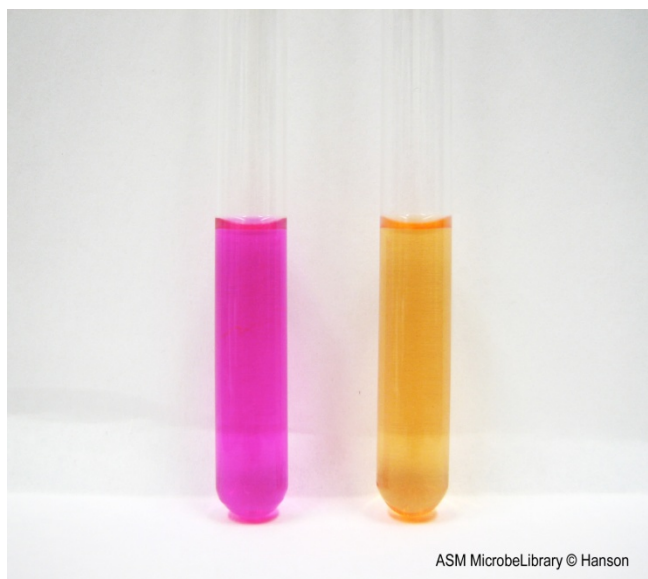


FIG. 2 Urea broth test results. Urea broth test tubes were inoculated as follows: (a) *Proteus vulgaris* (urease positive) and (b) *Escherichia coli* (urease negative).

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