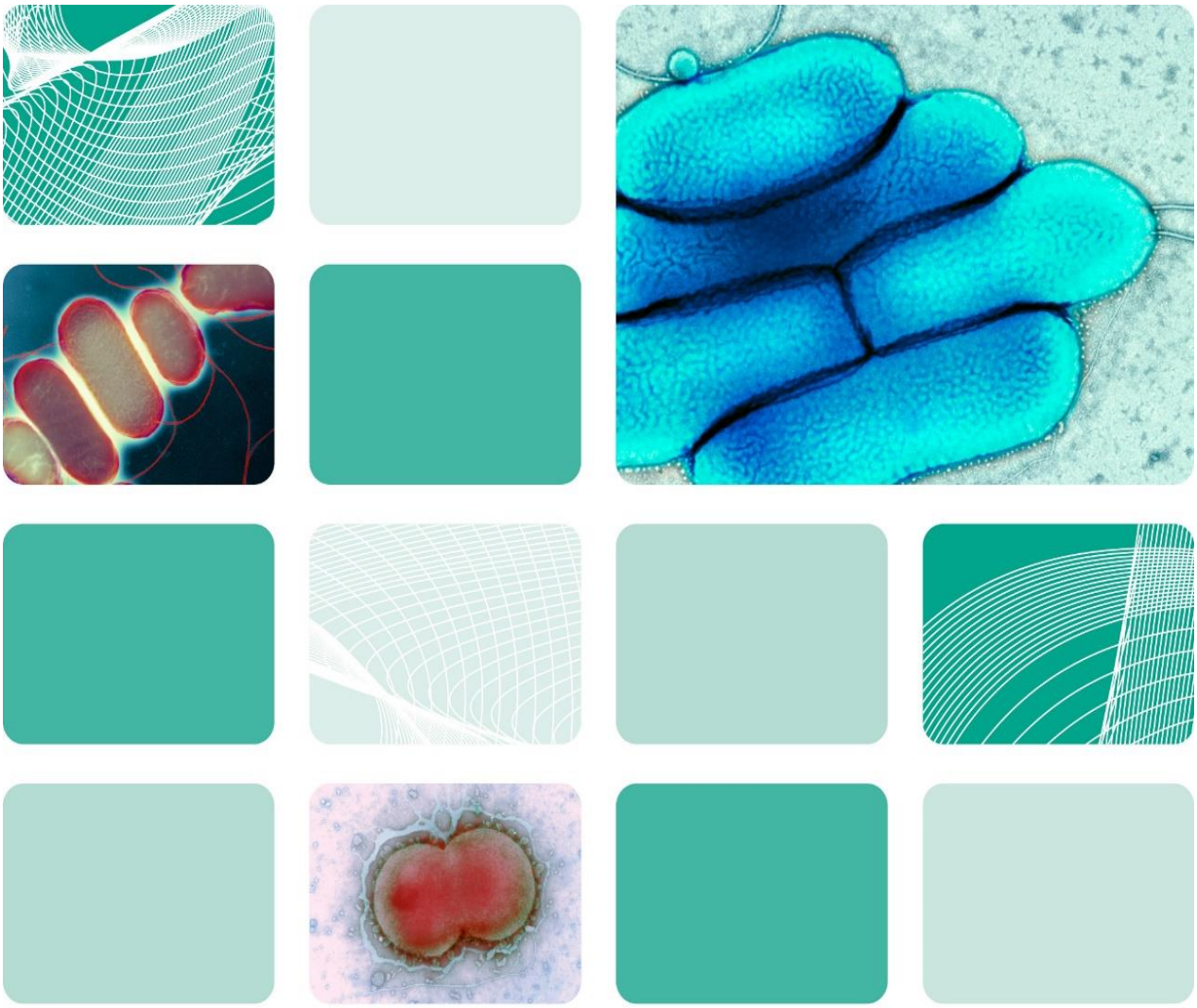
UK Health Security Agency

UK Standards for Microbiology Investigations

Urease test



Issued by the Standards Unit, UK Standards for Microbiology Investigations, UKHSA

Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on the UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a 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.

UK SMIs are produced in association with:

Displayed logos correct as of December 2024

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	7
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 02/04/2019** 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	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 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

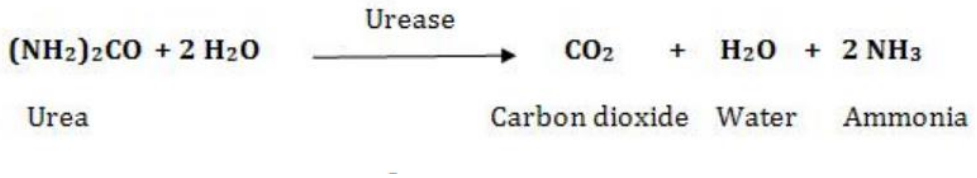
The urease test is used to differentiate urease-positive organisms (eg *Proteus*) from other organisms. It can also be used to detect the presence of *Helicobacter pylori*[1].

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.

4 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.

5 Technical information/limitations

5.1 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].

5.2 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.

5.3 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].

5.4 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.

6 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.

7 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

8 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.

9 Procedure and results

9.1 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.

9.2 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.

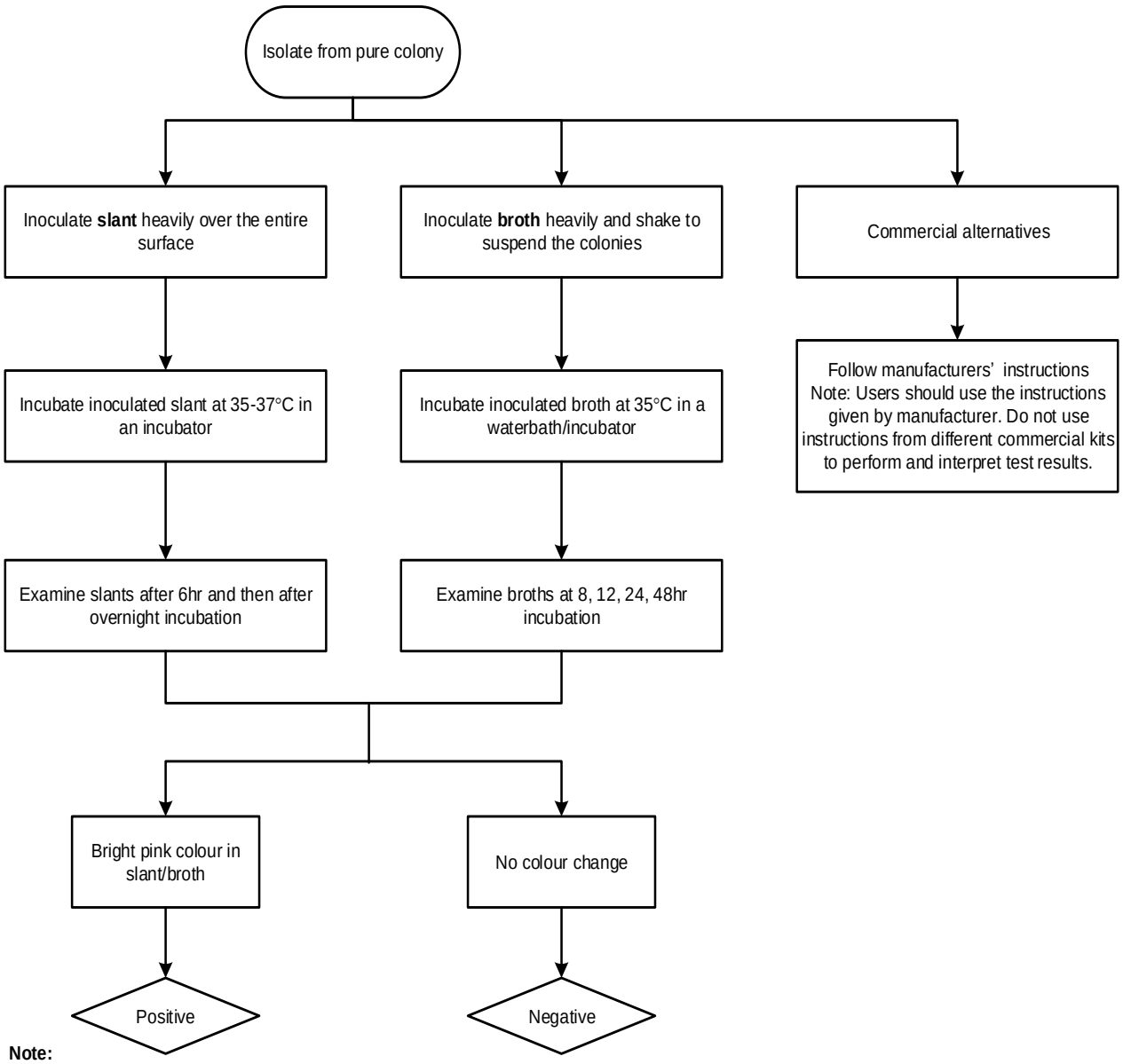
Negative result:

No colour change in agar slant/broth

9.3 Rapid urease test using dry strips

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

Algorithm: 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

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).

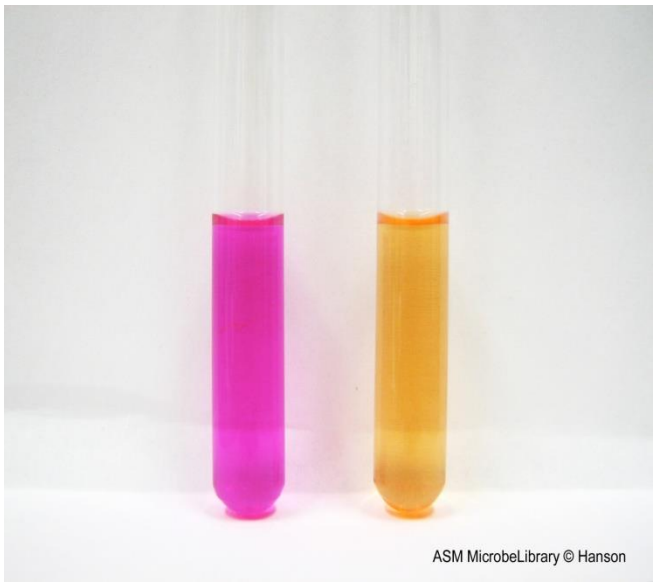


Figure 2. Urea broth test results. Urea broth test tubes were inoculated as follows: (a) *Proteus vulgaris* (urease positive) and (b) *Escherichia coli* (urease negative)

References

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

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