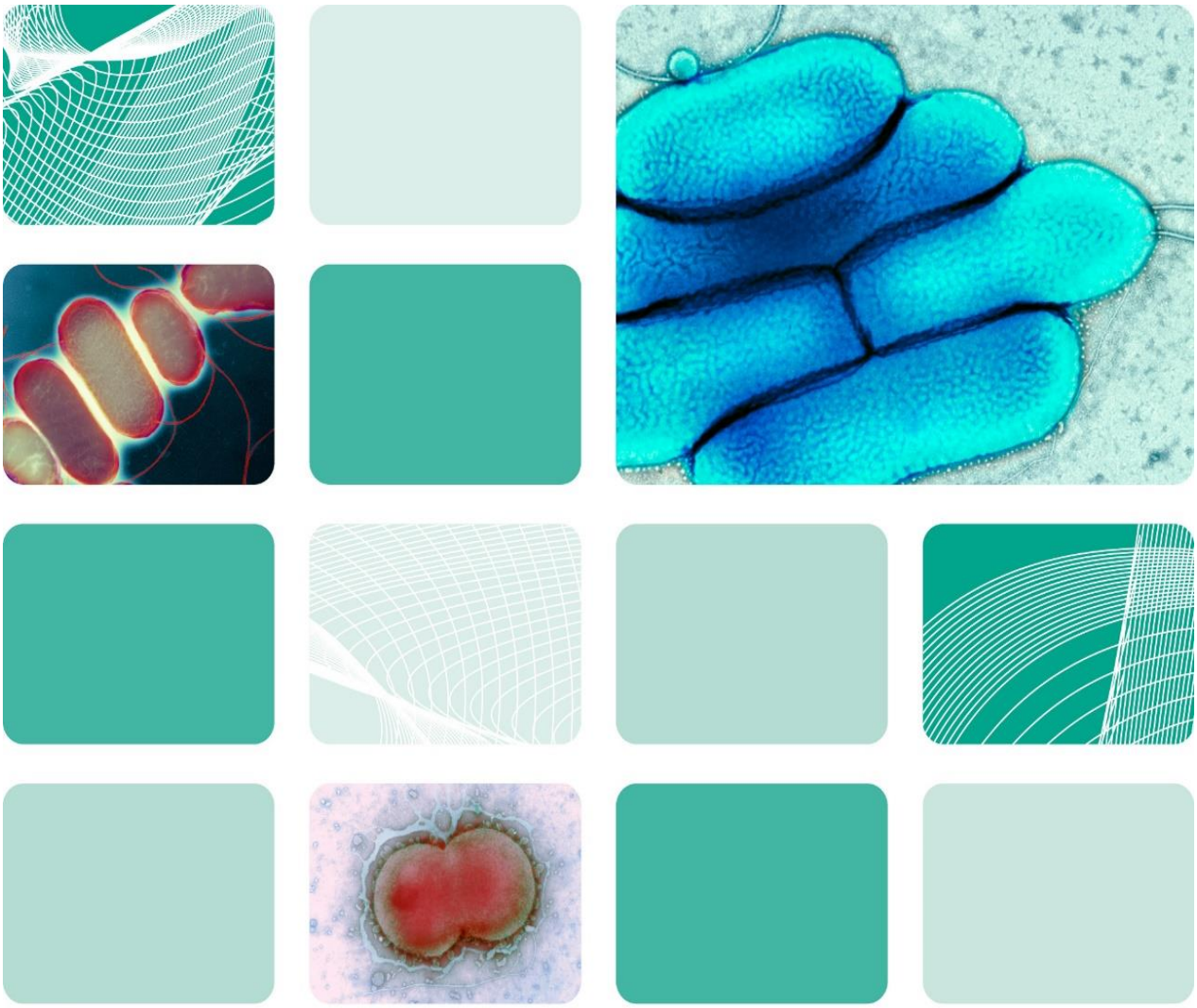
UK Health Security Agency

UK Standards for Microbiology Investigations

Potassium hydroxide 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	7/06.03.25
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 subheadings. References updated with grades. Modified GRADE table added in the references section to explain the grades given to references.
Quality control organisms.	Alternative NCTC control strains that could be used have been added and updated in the document. These have also been validated by NCTC.

*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

Many organisms such as *Bacillus* and *Clostridium* species that have lost some of the integrity of their cell wall appear Gram negative on staining resulting in possible misidentification.

The potassium hydroxide test may aid in differentiation between Gram positive and Gram negative organisms and is a useful complement to the Gram stain and the antibiotic disc test[1,2]. Like the Gram stain reaction, the test is based on differences in the chemistry of the bacterial cell wall.

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

4 Introduction

In the presence of potassium hydroxide, Gram negative cell walls are broken down, releasing viscid chromosomal material which causes the bacterial suspension to become thick and stringy. Gram positive organisms remain unaffected hence the alternative name for this procedure, the “String Test”.

5 Technical information/limitations

5.1 Benefits of using potassium hydroxide test

The potassium hydroxide test has its advantages; it is simple and easy to use, rapid and inexpensive. In laboratories where large numbers of cultures have to be processed, the above test may be used in addition to Gram stain for preliminary differentiation.

Although useful, a negative test does not prove conclusively that an organism is Gram positive[3].

5.2 Misidentification of older cultures

Older cultures (>48hr) may give unreliable results after mixing the bacteria in the KOH solution. This is common with certain species such as *Achromobacter* species, *Brucella melitensis, Pseudomonas paucimobilis*, *Moraxella* species, etc[3].

5.3 Quality control

Potassium hydroxide solution should be freshly prepared and any bottle containing a white precipitate must be discarded.

6 Safety considerations[4-21]

Refer to current guidance on the safe handling of all organisms and reagents documented in this UK SMI.

Potassium hydroxide solution is an irritant.

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.

7 Reagents and equipment[1]

Discrete colonies growing on solid medium

3% potassium hydroxide in water

Microscope slide

Bacteriological straight wire/loop or disposable alternative

8 Quality control organisms

Positive control:

Escherichia coli	NCTC 10418 or NCTC 12241

Negative control:

Staphylococcus aureus	NCTC 6571 or NCTC 12973

Note: These strains have been validated by NCTC to give this result.

9 Procedure and results[1,22,23]

9.1 Potassium hydroxide procedure

1. place one drop of 3% potassium hydroxide solution on a clean microscope slide
2. emulsify a few colonies of the suspect organism in the drop of potassium hydroxide to make a dense suspension
3. stir continuously for 60sec and then gently pull the loop away from the suspension

4. observe any changes – if positive, a string of the suspension will follow the loop when it is raised

Interpretation

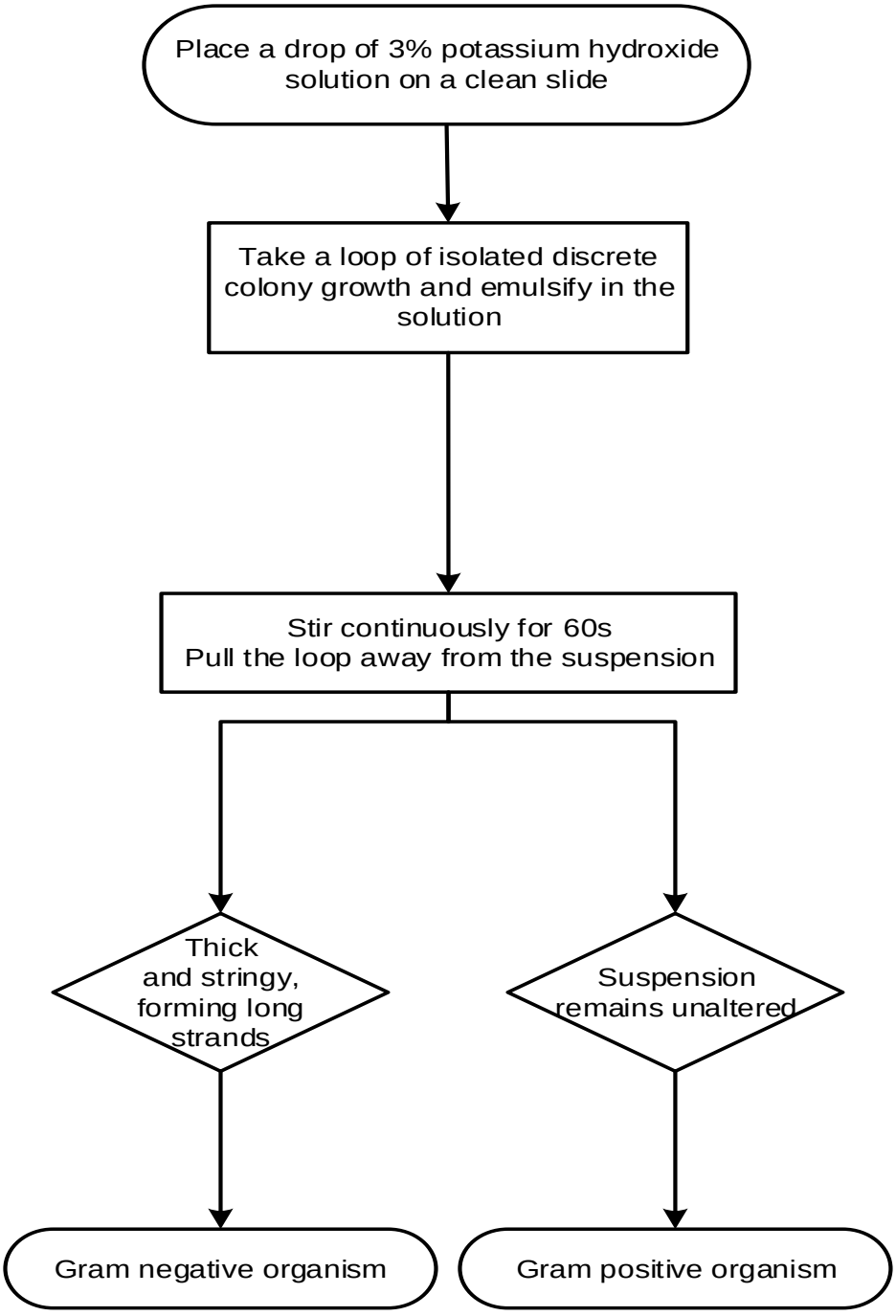
Positive result:

Organisms become thick, stringy and form long strands within the first 30sec. This is seen in Gram negative bacteria.

Negative result:

Organisms leave the suspension unaltered or there is absence of stringing. This is seen in Gram positive bacteria.

Algorithm: Potassium hydroxide test



Note:
Positive control *Escherichia coli* NCTC 10418 or NCTC 12241

Negative control *Staphylococcus aureus* NCTC 6571 or NCTC 12973

References

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

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13. European Parliament. UK Standards for Microbiology Investigations (UK SMIs) use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes". 1998. **A, VI**

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