



UK Standards for Microbiology Investigations

Potassium Hydroxide 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 <http://www.hpa.org.uk/SMI/Partnerships>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <http://www.hpa.org.uk/SMI/WorkingGroups>).

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
Microbiology Services
Public Health England
61 Colindale Avenue
London NW9 5EQ

E-mail: standards@phe.gov.uk

Website: <http://www.hpa.org.uk/SMI>

UK Standards for Microbiology Investigations are produced in association with:



Contents

ACKNOWLEDGMENTS	2
CONTENTS	3
AMENDMENT TABLE	4
UK STANDARDS FOR MICROBIOLOGY INVESTIGATIONS: SCOPE AND PURPOSE.....	5
SCOPE OF DOCUMENT	8
INTRODUCTION	8
TECHNICAL INFORMATION/LIMITATIONS.....	8
1 SAFETY CONSIDERATIONS	9
2 REAGENTS AND EQUIPMENT	9
3 QUALITY CONTROL ORGANISMS	9
4 PROCEDURE AND RESULTS.....	9
APPENDIX: POTASSIUM HYDROXIDE TEST.....	10
REFERENCES	11



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.	4/14.03.14
Issue no. discarded.	2.1
Insert Issue no.	2.2
Section(s) involved	Amendment
Whole document.	<p>Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England.</p> <p>Front page has been redesigned.</p> <p>Status page has been renamed as Scope and Purpose and updated as appropriate.</p> <p>Professional body logos have been reviewed and updated.</p> <p>Standard safety and notification references have been reviewed and updated.</p> <p>Scientific content remains unchanged.</p>

Amendment No/Date.	3/21.10.11
Issue no. discarded.	2
Insert Issue no.	2.1
Section(s) involved	Amendment
Whole document.	Document presented in a new format.
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

<http://www.npa.gov.uk/SMI/Partnerships>. 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 http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1317133470313. 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.

SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested Citation for this Document

Public Health England. (2014). Potassium Hydroxide Test. UK Standards for Microbiology Investigations. TP 30 Issue 2.2. <http://www.hpa.org.uk/SMI/pdf>.

UNDER REVIEW

Scope of Document

Many *Bacillus* and *Clostridium* species, organisms 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 differentiation between Gram positive and Gram negative organisms¹.

This SMI should be used in conjunction with other SMIs.

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'.

Technical Information/Limitations

Although useful, a negative test does not prove conclusively that an organism is Gram positive².

1 Safety Considerations³⁻¹³

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.

Potassium hydroxide 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 Equipment¹

Discrete colonies growing on solid medium.

3% potassium hydroxide in water.

Bacteriological straight wire/loop (preferably nichrome, or disposable alternative).

3 Quality Control Organisms^{1,14}

Positive Control

Escherichia coli NCTC 10418

Negative Control

Staphylococcus aureus NCTC 6571

Note: These strains are not validated by NCTC to give this result.

4 Procedure and Results¹

4.1 Potassium Hydroxide Procedure

- Place one drop of potassium hydroxide solution on a clean microscope slide
- Take a loop of growth of the suspect organism and make a dense suspension in the potassium hydroxide
- Mix continuously for 60s and then gently pull the loop away from the suspension

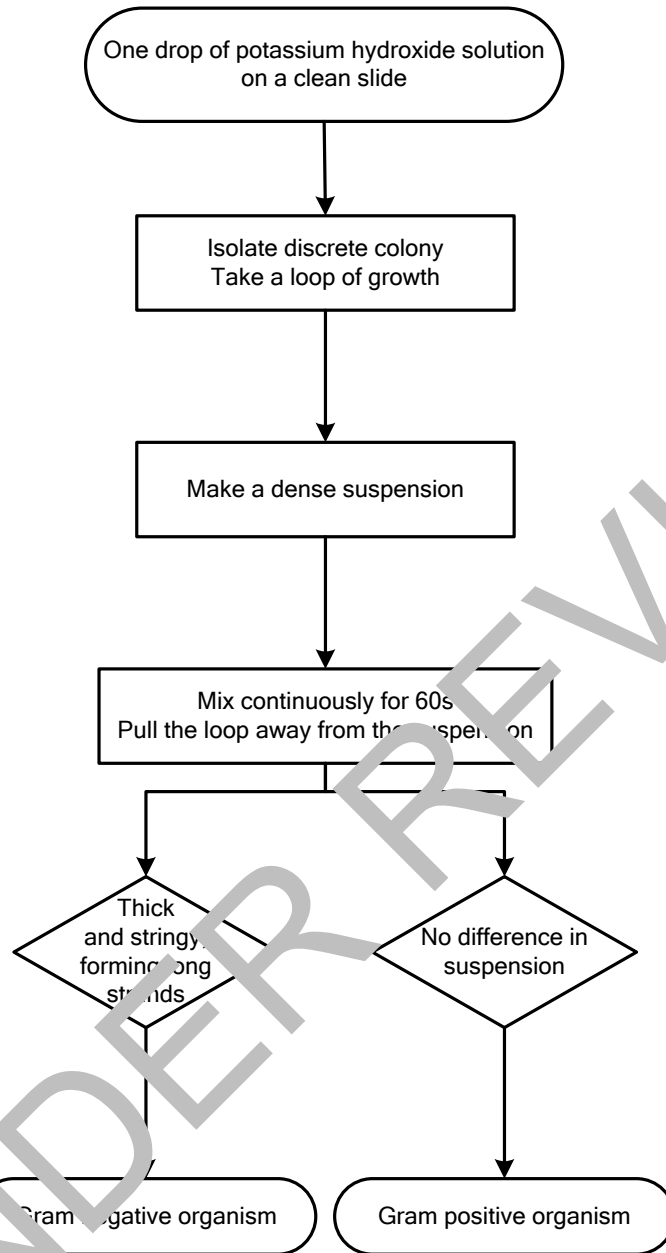
Positive Result

Gram negative organisms become thick and stringy and form long strands.

Negative Result

Gram positive organisms leave the suspension unaltered.

Appendix: Potassium Hydroxide Test



Note:

Positive control

Escherichia coli NCTC 10418

Negative control

Staphylococcus aureus NCTC 6571

This flowchart is for guidance only.

References

1. Halebian S, Harris B, Finegold SM, Rolfe RD. Rapid method that aids in distinguishing Gram-positive from Gram-negative anaerobic bacteria. *J Clin Microbiol* 1981;13:444-8.
2. von Graevenitz A, Bucher C. Accuracy of the KOH and vancomycin tests in determining the Gram reaction of non-enterobacterial rods. *J Clin Microbiol* 1983;18:983-5.
3. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Her Majesty's Stationery Office. Norwich. 2004. p. 1-21
4. Advisory Committee on Dangerous Pathogens. Infections at work: Controlling the risks. Her Majesty's Stationery Office. 2003.
5. Advisory Committee on Dangerous Pathogens. Biological agents: Managing the risks in laboratories and healthcare premises. Health and Safety Executive. 2005.
6. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002. 5th ed. HSE Books; 2002.
7. Health and Safety Executive. Five Steps to Risk Assessment: A Step by Step Guide to a Safer and Healthier Workplace. HSE Books. 2002.
8. Health and Safety Executive. A Guide to Risk Assessment requirements: Common Provisions in Health and Safety Law. HSE Books. 2002.
9. British Standards Institution (BSI). BS EN12669 - Photobiology - performance criteria for microbiological safety cabinets. 2000.
10. British Standards Institution (BSI). Part 2: Recommendations for information to be exchanged between purchaser, vendor and installer and recommendations for installation. BS 5726 - Microbiological safety cabinets. 1992.
11. British Standards Institution (BSI). Part 4: Recommendations for the selection, use and maintenance. BS 5726 - Microbiological safety cabinets. 1992.
12. Health Services Advisory Committee. Safe Working and the Prevention of Infection in Clinical Laboratories and Similar Facilities. HSE Books. 2003.
13. Department for transport. Transport of Infectious Substances, 2011 Revision 5. 2011.
14. Snell JS, Broome FJ, Roberts C, editors. Quality Assurance Principles and Practice in the Microbiology Laboratory. London: Public Health Laboratory Service; 1999. p. 147-8