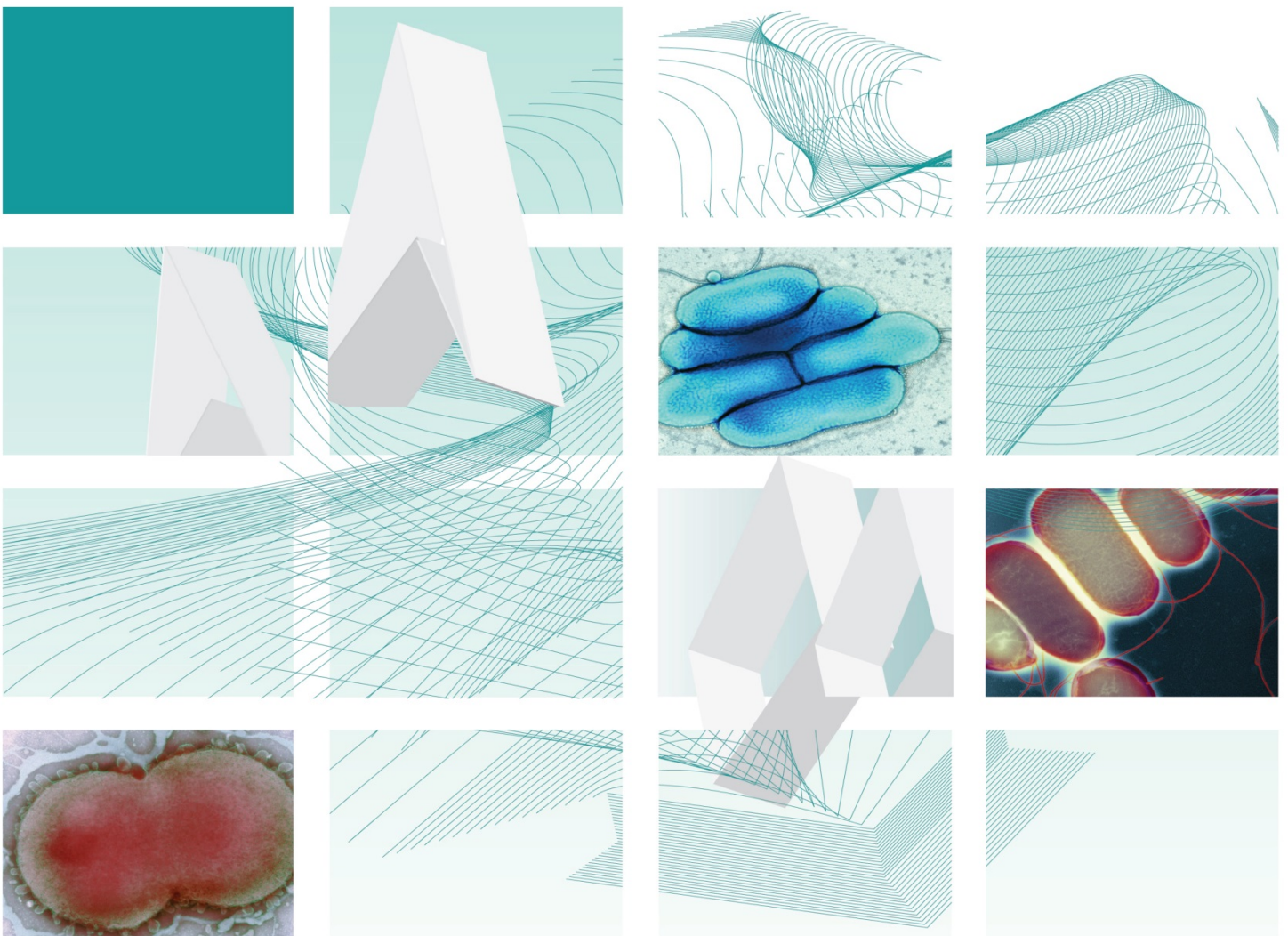




UK Standards for Microbiology Investigations

Oxidation/fermentation of glucose test



"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**."

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>).

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Logos correct at time of publishing.

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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	6/16.01.19
Issue number discarded	3
Insert issue number	4
Anticipated next review date*	16.01.22
Section(s) involved	Amendment
Whole document.	Document and flowchart updated. Technical limitations updated with subheadings. Quality control organisms updated. References updated with grades.

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

[#] 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.

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.

UK SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested citation for this document

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Scope of document

Bacteria utilise glucose and other carbohydrates through various metabolic pathways. Some are oxidative routes but others involve fermentation reactions. The oxidation-fermentation test, also known as the “oxferm”/ OF test, is used to determine which route is used¹. The test is used to differentiate between species, particularly Gram negative rods as well as between genera *Staphylococcus* and *Micrococcus*².

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

Introduction

The oxidative-fermentative test is used to determine if bacteria metabolise carbohydrates oxidatively, by fermentation, or are non-saccharolytic and therefore have no ability to use the carbohydrate in the media.

Oxidative organisms can only metabolise glucose or other carbohydrates under aerobic conditions ie oxygen is the ultimate hydrogen acceptor. Other organisms ferment glucose and the hydrogen acceptor is then another substance eg sulphur. This fermentative process is independent of oxygen and cultures of organisms may be aerobic or anaerobic. The end product of metabolising a carbohydrate is an acid.

The method described, sometimes referred to as the Hugh and Leifson test employs a semi-solid medium in tubes containing the carbohydrate under test (usually glucose) and a pH indicator³. Two tubes are inoculated and one is sealed immediately to produce anaerobic conditions. The *Enterobacteriaceae*, produce an acid reaction throughout the medium in both tubes. Organisms that cannot break down the carbohydrate aerobically or anaerobically, for example *Alcaligenes faecalis*, produce an alkaline reaction in the open tube and no change in the covered tube. Hugh and Leifson’s medium can also be used for recording gas production and motility⁴. Staphylococci and micrococci are tested with the Baird-Parker modification of the medium¹.

Technical information/limitations

Culture media

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.

If screw cap tubes are used, they should not be closed too tightly to permit air exchange⁵.

Some organisms are unable to grow in Hugh and Leifson’s medium. In this instance, repeat the test after enriching each tube with 2% serum or 0.1% yeast extract⁴.

Incubation

Prolonged incubation may be required by some organisms before acid production is visible⁴. The delayed reaction is attributed to inability of a carbohydrate to penetrate the bacterial cell⁶.

Interpretation of reactions

The colour change produced by oxidative organisms start at the surface of the medium. It may not be apparent for several days. Care must be taken not to mistake this for a negative reaction⁷.

Sealant used

Mineral oil is not recommended for use because it is a heavy liquid petroleum and therefore increases air diffusion⁷.

1 Safety considerations⁸⁻²⁵

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.

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 bacterial colonies growing on solid medium

Two different media are used depending on the organism being tested.

- Staphylococci and micrococci require Baird Parker's modification of the OF medium¹
- Gram negative rods require Hugh and Leifson's OF basal medium⁷

Bacteriological straight wire/loop or disposable alternative

Soft Paraffin Oil

3 Quality control organisms⁷

Gram negative rods			
Positive control	Oxidation	<i>Pseudomonas aeruginosa</i>	NCTC 10662 or NCTC 12903
	Fermentation	<i>Escherichia coli</i>	NCTC 10418 or NCTC 12241
Negative control	No reaction	<i>Acinetobacter lwoffii</i>	NCTC 5866
Gram positive cocci			
Positive control	Oxidation	<i>Micrococcus luteus</i>	NCTC 2665
	Fermentation	<i>Staphylococcus aureus</i>	NCTC 6571 or NCTC 12973
Negative control	No reaction	OF basal medium without carbohydrate	N/A

Note 1: Quality control should be carried out on every batch of media. These strains have been validated by NCTC to give this result.

4 Procedure and results^{1,3,4,7}

4.1 Oxidation fermentation test method

- heat 2 tubes of medium in boiling water for 10 minutes to remove the oxygen and allow cooling before use
- stab-inoculate both tubes by inserting a straight wire vertically to approximately 0.5cm from the bottom
- incubate one tube aerobically and either incubate the second tube anaerobically or seal the surface with a layer of melted soft paraffin to a depth of about 3cm above the medium to create anaerobic conditions. Set up the controls alongside the test organism
- incubate at 35°C for 48hr or longer. Longer incubation may be required for slow growing species
- examine tubes daily for colour change

Interpretation

Gram negative rod reactions

Positive result

Oxidation

Acid in aerobic tube only (yellow colour in aerobic tube, green in anaerobic tube)

Fermentation

Acid in both tubes (yellow colour)

Negative result (Neither fermentation nor oxidation)

No acid production (blue or green colour in aerobic tube, green in anaerobic tube)

Gram positive cocci reactions

Positive result

Oxidation

Acid in aerobic tube only (yellow colour in aerobic tube, purple in anaerobic tube)

Fermentation

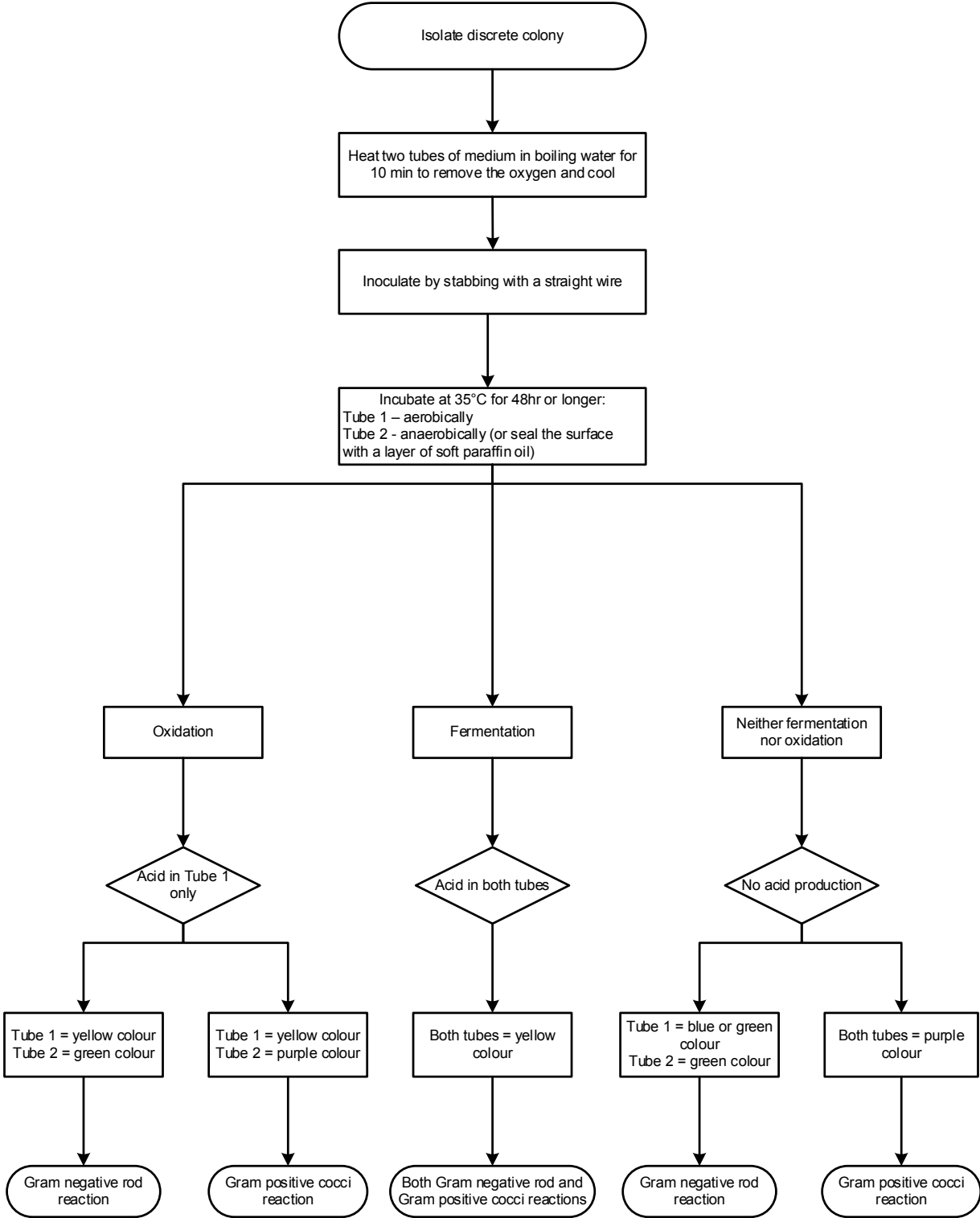
Acid in both tubes (yellow colour)

Negative result (Neither fermentation nor oxidation)

No acid production /No colour change (purple colour in both tubes)

Note: The semisolid consistency of the medium also allows for detection of motility. Hazy growth away from the stab line can also be noted.

Appendix: Oxidation/fermentation of glucose test



This flowchart is for guidance only.

References

Modified GRADE table used by UK SMI's 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 SMI's 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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