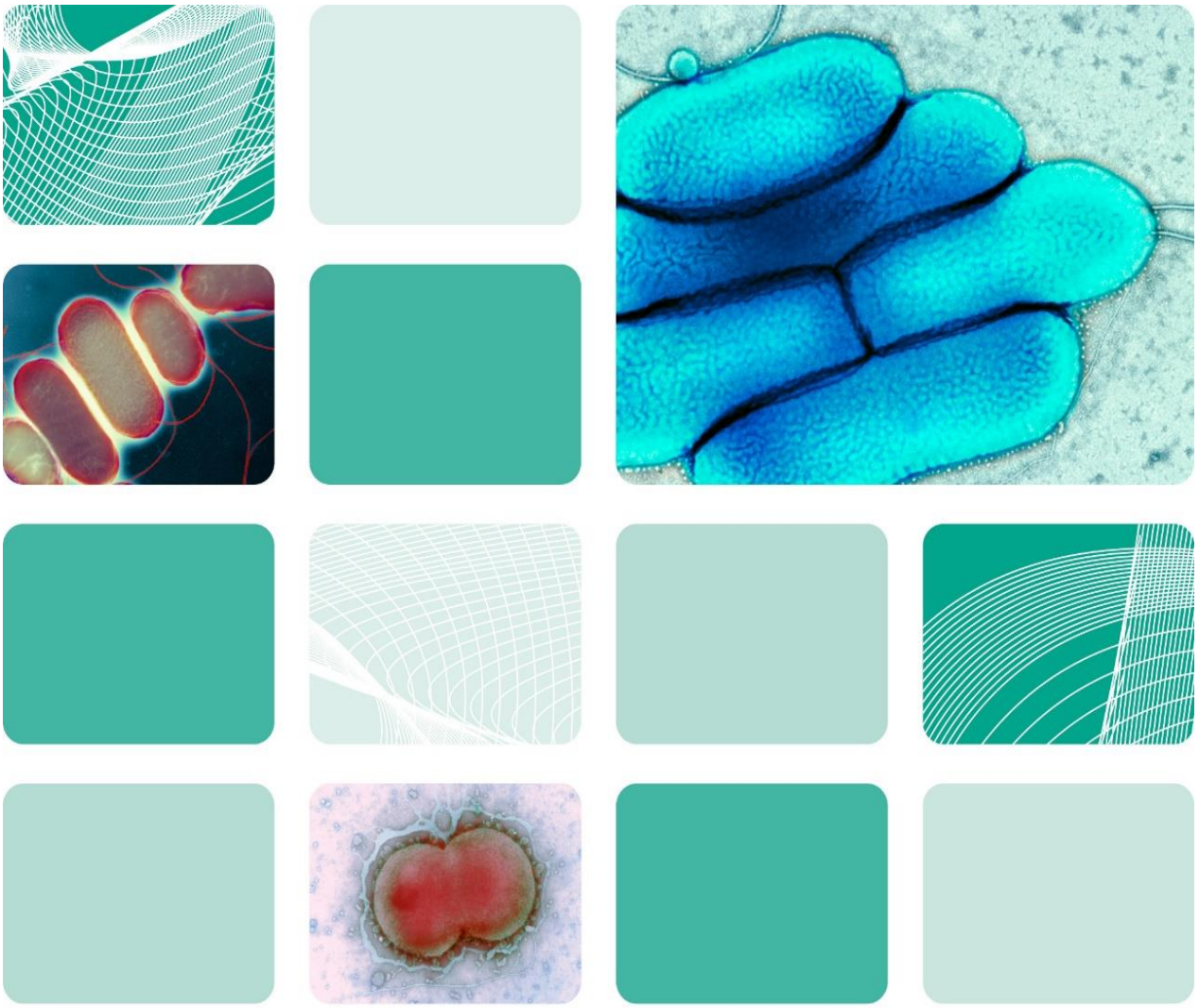
UK Health Security Agency

UK Standards for Microbiology Investigations

Oxidation/fermentation of glucose 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.

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UK SMIs are produced in association with:

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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 16/01/2019.** 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	6/16.01.19
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 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

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 used[1]. 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.

4 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 indicator[3]. 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 motility[4]. Staphylococci and micrococci are tested with the Baird-Parker modification of the medium[1].

5 Technical information/limitations

5.1 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 exchange[5].

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 extract[4].

5.2 Incubation

Prolonged incubation may be required by some organisms before acid production is visible[4]. The delayed reaction is attributed to inability of a carbohydrate to penetrate the bacterial cell[6].

5.3 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 reaction[7].

5.4 Sealant used

Mineral oil is not recommended for use because it is a heavy liquid petroleum and therefore increases air diffusion[7].

6 Safety considerations[8-25]

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.

7 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 medium[1]
- Gram negative rods require Hugh and Leifson's OF basal medium[7]

Bacteriological straight wire/loop or disposable alternative

Soft Paraffin Oil

8 Quality control organisms[7]

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

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

9 Procedure and results[1,3,4,7]

9.1 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

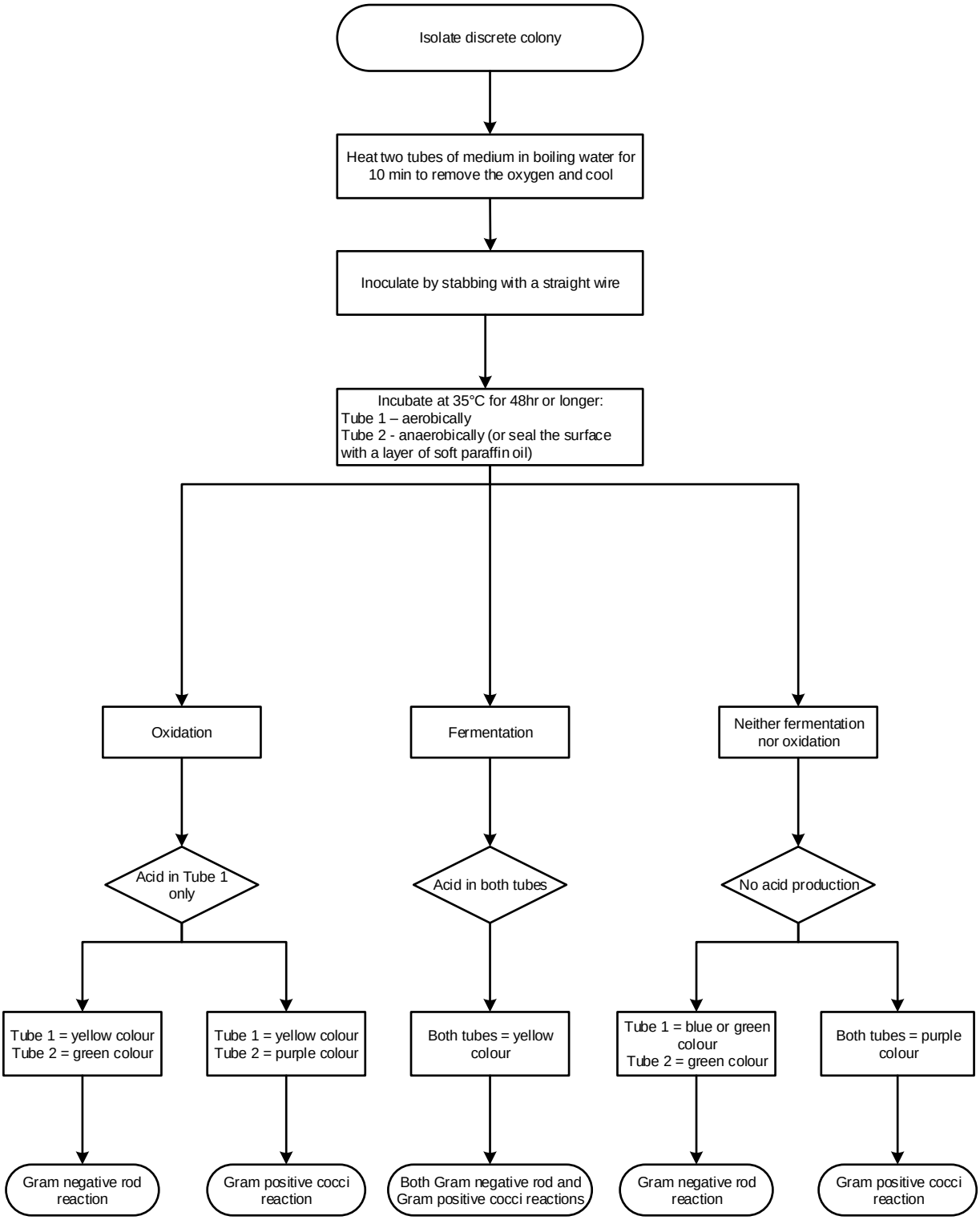
Acid in both tubes (yellow colour)

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.

Algorithm: Oxidation/fermentation of glucose test



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

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