

NATIONAL STANDARD METHOD

OXIDATION/FERMENTATION OF GLUCOSE TEST

BSOP TP 27

Issued by Standards Unit, Department for Evaluations, Standards and Training
Centre for Infections



OXIDATION/FERMENTATION OF GLUCOSE TEST

Issue no: 2.1 Issue date: 09.12.10 Issued by: Standards Unit, Department for Evaluations, Standards and Training Page no: 1 of 10
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STATUS OF NATIONAL STANDARD METHODS

National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

National Standard Methods are developed, reviewed and updated through an open and wide consultation process where the views of all participants are considered and the resulting documents reflect the majority agreement of contributors.

Representatives of several professional organisations, including those whose logos appear on the front cover, are members of the working groups which develop National Standard Methods. Inclusion of an organisation's logo on the front cover implies support for the objectives and process of preparing standard methods. The representatives participate in the development of the National Standard Methods but their views are not necessarily those of the entire organisation of which they are a member. The current list of participating organisations can be obtained by emailing standards@hpa.org.uk.

The performance of standard methods depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

Whereas every care has been taken in the preparation of this publication, the Health Protection Agency or any supporting organisation cannot be responsible for the accuracy of any statement or representation made or the consequences arising from the use of or alteration to any information contained in it. These procedures are intended solely as a general resource for practising professionals in the field, operating in the UK, and specialist advice should be obtained where necessary. If you make any changes to this publication, it must be made clear where changes have been made to the original document. The Health Protection Agency (HPA) should at all times be acknowledged.

The HPA is an independent organisation dedicated to protecting people's health. It brings together the expertise formerly in a number of official organisations. More information about the HPA can be found at www.hpa.org.uk.

The HPA aims to be a fully 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 conditions¹.

More details can be found on the website at www.evaluations-standards.org.uk. Contributions to the development of the documents can be made by contacting standards@hpa.org.uk.

The reader is informed that all taxonomy in this document was correct at time of issue.

Please note the references are now formatted using Reference Manager software . If you alter or delete text without Reference Manager installed on your computer, the references will not be updated automatically.

Suggested citation for this document:

Health Protection Agency (2010). *Oxidation/Fermentation of Glucose Test*. National Standard Method BSOP TP 27 Issue 2.1. http://www.hpa-standardmethods.org.uk/pdf_sops.asp.

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AMENDMENT PROCEDURE

Controlled document reference	BSOP TP 27
Controlled document title	Oxidation/Fermentation of Glucose Test

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
2/ 23.04.10	1.1	2	All	Whole document	Document reviewed, no updates required
3/ 9.12.10	2	2.1	1	Front page	The Association of Medical Microbiologist logo replaced with new British Infection Association logo.
			6	Quality control organisms	Positive control for Gram Negative rods, amended from <i>Serratia marcescens</i> NCTC 11935 to <i>Escherichia coli</i> NCTC 10418.
			9	Appendix	Flowchart amended

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SCOPE OF DOCUMENT

Bacteria utilise glucose and other carbohydrates various metabolic pathways. Some are oxidative routes but others involve fermentation reactions. The oxidation-fermentation test, also known as the "oxferm" test, is used to determine which route is used. The test is used to differentiate between species, particularly Gram-negative rods.

INTRODUCTION

Oxidative organisms can only metabolise glucose or other carbohydrates under aerobic conditions i.e. 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 described², 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. Two tubes are inoculated and one is immediately sealed to produce anaerobic conditions. Oxidising organisms, eg *Pseudomonas* species, produce an acid reaction in the open tube only. Fermenting organisms, eg *Enterobacteriaceae*, produce an acid reaction throughout the medium in both tubes. Organisms that cannot break down the carbohydrate aerobically or anaerobically, eg *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. Staphylococci and micrococci are tested with the Baird-Parker modification of the medium³.

TECHNICAL INFORMATION/LIMITATIONS

The colour change produced by oxidative organisms starts 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 reaction⁴.

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.

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1 SAFETY CONSIDERATIONS⁵⁻¹¹

Refer to current guidance on the safe handling of all organisms and reagents documented in this National Standard Method.

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 under test. Staphylococci and micrococci require Baird Parker's medium, and Gram-negative rods Hugh and Leifson's medium.

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

3 QUALITY CONTROL ORGANISMS¹²

Gram-negative rods			
Positive control	Oxidation	<i>Pseudomonas aeruginosa</i>	NCTC 10662
	Fermentation	<i>Escherichia coli</i>	NCTC 10418
Negative control	No action	<i>Acinetobacter lwoffii</i>	NCTC 5866
Gram-Positive cocci			
Positive control	Oxidation	<i>Micrococcus luteus</i>	NCTC 4351
	Fermentation	<i>Staphylococcus aureus</i>	NCTC 8532
Negative control	No action	OF basal medium without carbohydrate	

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4 PROCEDURE AND RESULTS²⁻⁴

Quality control should be carried out on each batch of medium.

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.

4.1 OXIDATION FERMENTATION TEST METHOD

- Heat two tubes of medium in boiling water for 10 minutes to drive off the oxygen, cool and inoculate by inserting a straight wire vertically
- Incubate one tube aerobically and either incubate the second tube anaerobically or seal the surface with a layer of sterile liquid paraffin oil to create anaerobic conditions
- Incubate at 35-37°C for 72 hours. Longer incubation may be required for slowly growing species
- Examine tubes daily for colour change.

RESULTS

Gram-negative rod reactions:

Oxidation acid in aerobic tube only (yellow colour in aerobic tube, green in anaerobic)

Fermentation acid in both tubes (yellow colour)

Neither fermentation nor oxidation no acid production (green colour in aerobic tube, purple in anaerobic)

Gram-positive cocci reactions:

Oxidation acid in aerobic tube only (yellow colour in aerobic tube, purple in anaerobic)

Fermentation acid in both tubes (yellow colour)

Neither fermentation nor oxidation no acid production (purple colour in both tubes)

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5 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the National Standard Methods Working Group for Clinical Bacteriology (http://www.hpa-standardmethods.org.uk/wg_bacteriology.asp). The contributions of many individuals in clinical bacteriology laboratories and specialist organisations who have provided information and comment during the development of this document, and final editing by the Medical Editor are acknowledged.

The National Standard Methods are issued by Standards Unit, Department for Evaluations, Standards and Training, Centre for Infections, Health Protection Agency, London.

For further information please contact us at:

Standards Unit
Department for Evaluations, Standards and Training
Centre for Infections
Health Protection Agency
Colindale
London
NW9 5EQ

E-mail: standards@hpa.org.uk

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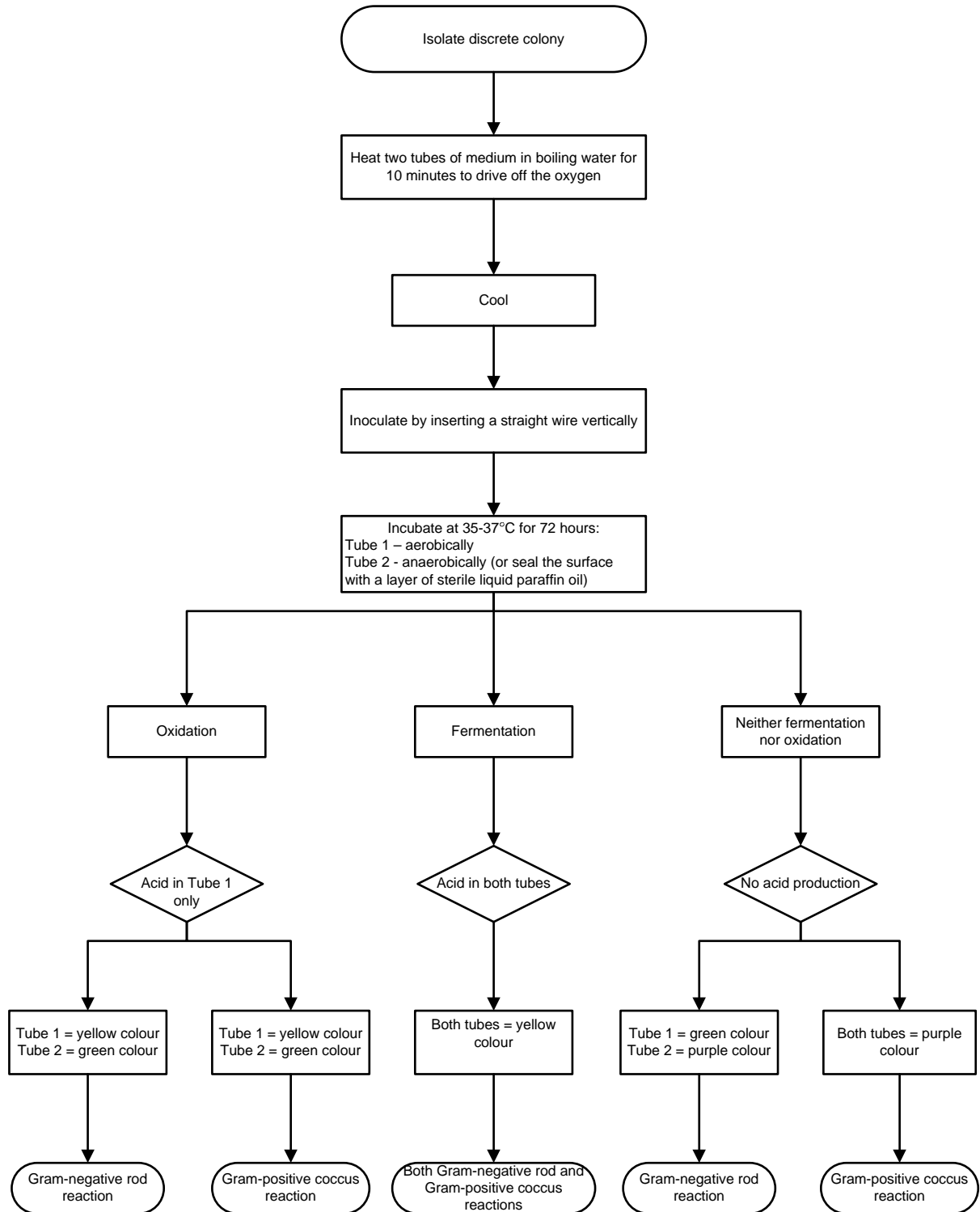
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APPENDIX: OXIDATION/FERMENTATION OF GLUCOSE TEST



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