NATIONAL STANDARD METHOD

OXIDATION/FERMENTATION OF GLUCOSE TEST

BSOP TP 27

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

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

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Suggested citation for this document:

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### AMENDMENT PROCEDURE

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**OXIDATION/FERMENTATION OF GLUCOSE TEST**

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OXIDATION/FERMENTATION OF GLUCOSE TEST

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 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 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.
1 SAFETY CONSIDERATIONS\textsuperscript{5-11}

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 ORGANISMS\textsuperscript{12}

<table>
<thead>
<tr>
<th>Gram-negative rods</th>
<th>Positive control</th>
<th>Oxidation</th>
<th>Fermentation</th>
<th>Pseudomonas aeruginosa</th>
<th>NCTC 10662</th>
<th>Serratia marcescens</th>
<th>NCTC 11935</th>
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<tbody>
<tr>
<td>Negative control</td>
<td>No action</td>
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<td></td>
<td>Acinetobacter Iwoffii</td>
<td>NCTC 5866</td>
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<table>
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<tr>
<th>Gram-Positive Cocci</th>
<th>Positive control</th>
<th>Oxidation</th>
<th>Fermentation</th>
<th>Micrococcus luteus</th>
<th>NCTC 4351</th>
<th>Staphylococcus aureus Subsp. Aureus</th>
<th>NCTC 8532</th>
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<tbody>
<tr>
<td>Negative control</td>
<td>No action</td>
<td></td>
<td></td>
<td>OF basal medium</td>
<td></td>
<td>without carbohydrate</td>
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</tr>
</tbody>
</table>
4 PROCEDURE AND RESULTS

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)
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.

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APPENDIX: OXIDATION/FERMENTATION OF GLUCOSE TEST

1. Isolate discrete colony

2. Heat two tubes of medium in boiling water for 10 minutes to drive off the oxygen

3. Cool

4. Inoculate by inserting a straight wire vertically

5. Incubate at 35-37°C for 72 hours:
   - Tube 1 – aerobically
   - Tube 2 - anaerobically (or seal the surface with a layer of sterile liquid paraffin oil)

6. Oxidation
   - Acid in Tube 1 only
     - Tube 1 = yellow colour
     - Tube 2 = green colour
     - Gram-negative rod reaction
   - Acid in both tubes
     - Both tubes = yellow colour
     - Both Gram-negative rod and Gram-positive coccus reactions

7. Fermentation
   - Acid in both tubes
     - Both tubes = purple colour
     - Gram-negative rod reaction

8. Neither fermentation nor oxidation
   - No acid production
     - Tube 1 = green colour
     - Tube 2 = purple colour
     - Gram-positive coccus reaction
REFERENCES


