NATIONAL STANDARD METHOD

MOTILITY TEST

BSOP TP 21

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

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.

Suggested citation for this document:

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.
INDEX

STATUS OF NATIONAL STANDARD METHODS ........................................................................................................ 2
INDEX ........................................................................................................................................................................ 3
AMENDMENT PROCEDURE ....................................................................................................................................... 4
SCOPE OF DOCUMENT ............................................................................................................................................. 5
INTRODUCTION .......................................................................................................................................................... 5
TECHNICAL INFORMATION/LIMITATIONS ................................................................................................................ 5
1 SAFETY CONSIDERATIONS .................................................................................................................................. 6
2 REAGENTS AND EQUIPMENT ................................................................................................................................ 6
3 QUALITY CONTROL ORGANISMS ........................................................................................................................ 6
4 PROCEDURE AND RESULTS .................................................................................................................................. 7
   4.1 HANGING DROP METHOD .................................................................................................................................. 7
   4.2 SEMI SOLID AGAR (CRAIGIE) ............................................................................................................................. 7
   4.3 THREE COVER SLIP METHOD ............................................................................................................................ 7
5 ACKNOWLEDGEMENTS AND CONTACTS ............................................................................................................. 8
APPENDIX: MOTILITY TEST FLOWCHART ................................................................................................................ 9
REFERENCES ......................................................................................................................................................... 10
AMENDMENT PROCEDURE

<table>
<thead>
<tr>
<th>Amendment Number/ Date</th>
<th>Issue no.</th>
<th>Insert Issue no.</th>
<th>Page</th>
<th>Section(s) involved</th>
<th>Amendment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 23.04.10</td>
<td>1.1</td>
<td>2</td>
<td>All</td>
<td>Whole document</td>
<td>Document reviewed, no updates required</td>
</tr>
</tbody>
</table>

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.
MOTILITY TEST

SCOPE OF DOCUMENT

This test is used to determine if an organism is motile or non-motile. Motile organisms are generally bacilli although a few motile cocci do exist.

INTRODUCTION

This test is used to determine if organisms are motile by means of flagella. The location of the flagella is determined by the bacterial species. Non-motile bacteria do not possess flagella. The production of flagella is also subject to culture conditions; for example, some bacteria are motile at different temperatures from those at which they are normally incubated e.g. Yersinia enterocolitica is motile at 20-25°C but not at 30°C. Some bacteria such as Capnocytophaga species exhibit a gliding motility.

Occasionally bacteria such as Campylobacter species produce non-motile variants; these rarely revert to motile forms.

TECHNICAL INFORMATION/LIMITATIONS

Bacterial motility must be distinguished from Brownian motion. Weakly motile bacteria may require prolonged observation of individual cells.

Some bacteria on first isolation from blood cultures do not appear to be motile.

Motility results are difficult to determine for anaerobic bacteria. Only a positive result is significant.

Some bacteria are motile at one temperature and non motile when incubated at another.

Some bacteria become less motile in old cultures. Repeat motility testing on a fresh sub culture.
1 SAFETY CONSIDERATIONS

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

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

Hanging drop method

Liquid bacterial culture (incubation times and temperatures may vary depending on the species). Refer to the appropriate identification NSM.

Microscope slide with a central depression (or a ring of petroleum jelly or plasticine may be made on an ordinary microscope slide).

Coverslip

Semi-solid agar method (Craigie)

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

Three coverslip method

Normal microscope slide without central depression

Coverslips

3 QUALITY CONTROL ORGANISMS

Positive control: *Serratia marcescens* NCTC 10975

Negative control: *Acinetobacter Iwoffii* NCTC 5866
4 PROCEDURE AND RESULTS

4.1 HANGING DROP METHOD

- Place a small drop of liquid bacterial culture in the centre of a coverslip
- Place a small drop of water at each corner of the coverslip
- Invert a slide with a central depression over the coverslip
- The coverslip will stick to the slide and when the slide is inverted the drop of bacterial culture will be suspended in the well
- Examine microscopically (x400) for motile organisms

Note: If well slides are not available, a ring of Vaseline or plasticine may instead be made on an ordinary microscope slide

Positive result: A darting, zigzag, tumbling or other organised movement

Negative result: No movement or Brownian motion only

4.2 SEMI SOLID AGAR (CRAIGIE)

- Inoculate the test organism - the central glass tube
- Incubate at the relevant temperature for 18-24 hours
- Subculture from the outer section of the medium

Positive result: Organism can be recovered from the outer section of the medium

Negative result: Organism remains in the inner tube

4.3 THREE COVERSLLIP METHOD

- Set microscope slide according to Figure 1 below.

- Place a small drop of bacterial culture in centre of third coverslip.
- Place a small drop of water at each corner of the coverslip.
- Invert the prepared microscope slide over the third coverslip.
• The coverslip should stick to the other coverslips on the slide and when inverted the drop of bacterial culture should be suspended in the gap between the microscope slide and third coverslip.

• Examine microscopically (x400) for motile organism.

Positive result: A darting, zigzag, tumbling or other organised movement

Negative result: No movement or only Brownian motion

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
APPENDIX: MOTILITY TEST FLOWCHART

Isolate discrete colony

- Hanging drop method
  - Place drop of bacterial suspension on coverslip
  - Put drop of water on each corner of coverslip
  - Invert slide over coverslip
  - Invert entire side with coverslip and view at 400x magnification
    - Darts zig-zag, tumbling or other organised movement: Positive
    - No Movement or only Brownian motion: Negative

- Semi solid agar (Craigie)
  - Inoculate organism into glass tube
  - Incubate organism for 18-24h at appropriate temperature
  - Subculture from outer section of medium
    - Organism can be recovered from outer section of medium: Positive
    - Organism remains in the inner tube: Negative

- Three coverslip method
  - Set microscope slide with two coverslips as Fig 1
  - Place drop of bacterial suspension on third coverslip
  - Put drop of water on each corner of third coverslip
  - Invert prepared slide over third coverslip
  - Invert entire side and view at magnification 400x
    - Darts zig-zag, tumbling or other organised movement: Positive
    - No Movement or only Brownian motion: Negative

Notes:
- Positive control: Serratia marcescens NCTC 10973
- Negative control: Acinetobacter iwoff NCTC 5866

MOTILITY TEST
Issue no: 2  Issue date: 26.04.10  Issued by: Standards Unit, Department for Evaluations, Standards and Training  Page no: 9 of 10
BSOP TP 212
This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency
www.evaluations-standards.org.uk
Email: standards@hpa.org.uk
REFERENCES


7. Safety in Health Service Laboratories. Safe working and the prevention of infection in clinical laboratories and similar facilities. 2 ed. HSE Books; 2003.


