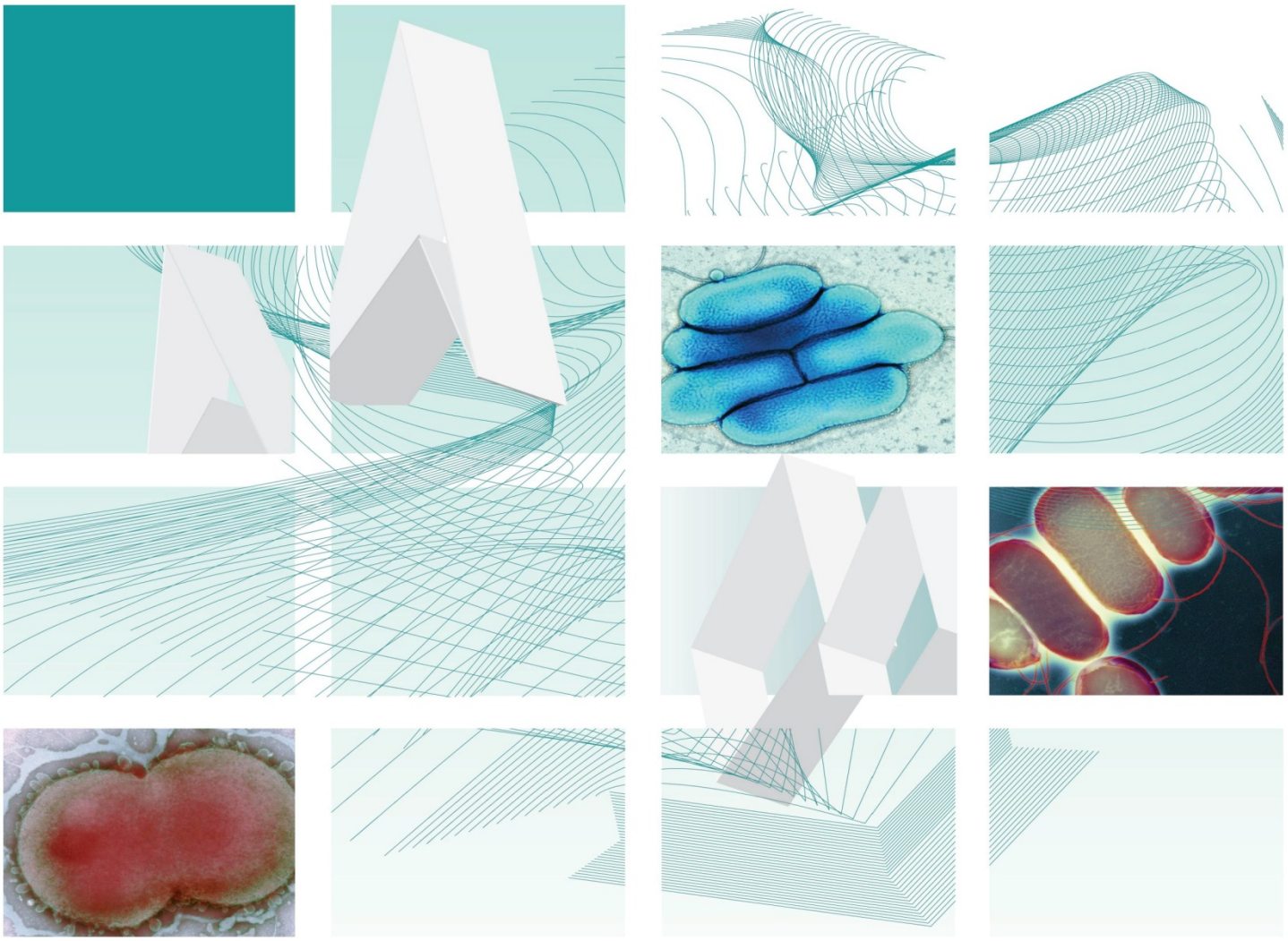
Public Health England

NHS

# UK Standards for Microbiology Investigations

## Motility Test



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Issued by the Standards Unit, Microbiology Services, PHE

# Acknowledgments

UK Standards for Microbiology Investigations (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. 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).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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UK Standards for Microbiology Investigations are produced in association with:


Logos correct at time of publishing.

# Contents

# Amendment Table

Each 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 No/Date. | 7/22.11.16 |
| Issue no. discarded. | 3 |
| Insert Issue no. | 3.1 |
| **Section(s) involved** | **Amendment** |
| Whole document. | Correction made to the spelling of *Acinetobacter lwoffii* in section 3 of the document. |

| | |
|---|---|
| Amendment No/Date. | 6/24.11.14 |
| Issue no. discarded. | 2.3 |
| Insert Issue no. | 3 |
| **Section(s) involved** | **Amendment** |
| Whole document. | Hyperlinks updated to gov.uk. |
| Page 2. | Updated logos added. |
| Introduction. | Information updated with references. |
| Technical information/Limitations. | This section has been updated and references added. |
| Safety Considerations. | Section updated. |
| Reagents/Equipment. | Updated with references. |
| Procedures and Results. | This has been updated with references. |
| Flowchart. | This has been amended for easy guidance. |
| References. | Some references updated. |

# UK Standards for Microbiology Investigations[#]: Scope and Purpose

## Users of SMIs

- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK.
- SMIs 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.
- SMIs 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 SMIs

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. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of 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

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 SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop 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.

SMIs are developed, reviewed and updated through a wide consultation process.

[#]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.

## Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development.

The performance of 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 SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting 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 SMIs are subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity. The 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

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, 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 SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time 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.

SMIs are Crown copyright which should be acknowledged where appropriate.

## Suggested Citation for this Document

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# Scope of Document

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

This SMI should be used in conjunction with other SMIs.

# Introduction

This test is used to determine if organisms are motile by means of flagella. The location of the flagella varies with 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 eg *Yersinia enterocolitica* is motile at 25°C but not at 37°C[1].

Some bacteria such as *Capnocytophaga* species, although non-motile, exhibit a gliding motility[2].

Occasionally bacteria such as *Campylobacter* species produce non-motile variants; these rarely revert to motile forms[2].

# 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 although direct examination of the blood culture broth can be useful as motile organisms are usually very motile as in liquid culture.

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

Some bacteria become less motile in old cultures. Repeat motility testing on a fresh subculture.

Environmental conditions affect motility in some strains. A strain actively motile when grown at 22°C may be practically non-motile when grown at 37°C; the motility of other strains remains apparently uninfluenced by changes in temperature[3].

The disadvantage of using the wet mount and the hanging drop methods is that there are significant risks associated with it, especially with highly pathogenic organisms eg salmonellae.

The semi-solid agar method permits the isolation of motile and non-motile strains from some cultures which were non-motile with the hanging drop technique.

The semi-solid agar method is particularly advantageous to use with testing of highly pathogenic organisms and routine testing, because the results are cumulative and macroscopic.

# 1 Safety Considerations[4-20]

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

All work likely to generate aerosols must be performed in a microbiological safety cabinet.

It is good practice that gloves should be worn when handling wet mounts or hanging drop suspensions.

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[21]

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

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

Coverslips

Inoculating loop

## Wet Mount Method[2]

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

Normal microscope slide without central depression

Inoculating loop

Coverslips

## Semi-solid Agar Method[22,23]

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

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

Test tube Motility medium

# 3 Quality Control Organisms

## Positive Control

*Proteus mirabilis* NCTC 10975

## Negative Control

*Acinetobacter lwoffii* NCTC 5866

**Note:** The reference strains have been validated by NCTC for the test shown.

# 4 Procedure and Results

## 4.1 Hanging Drop Method[21]

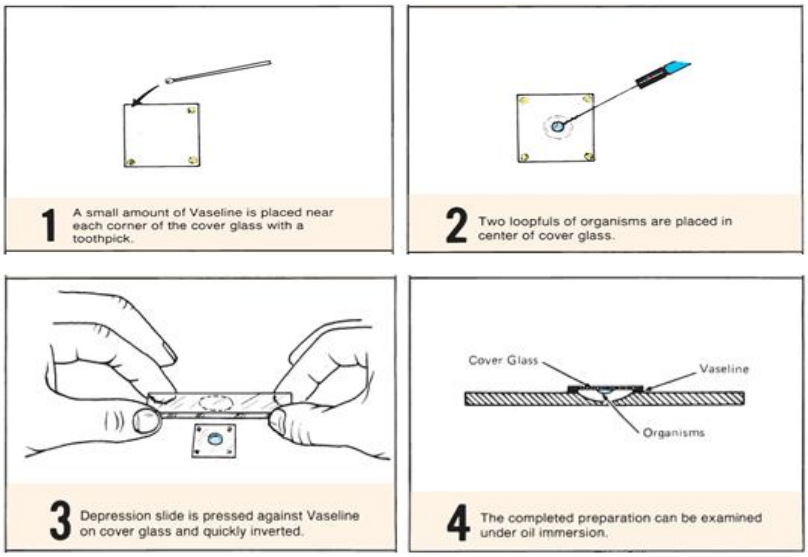



Fig. 1: Hanging drop method

(Adapted from the weblink: http://amrita.vlab.co.in/?sub=3&brch=73&sim=697&cnt=2 (copyright under the NME ICT initiative of MHRD))[24].

- Place a small drop of liquid bacterial culture in the centre of a coverslip
- Moisten the four edges of the coverslip with water to keep the coverslip firmly in place
- 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. The Vaseline-sealed depression or sometimes using plasticine also slows down the drying-out process, so the organisms can be observed for longer periods.

If too much Vaseline is used, it will be squeezed toward the centre and mix with the drop or squeeze out the edges and get on the objective lens of the microscope.

Alternative hanging drop methods are available.

**Positive Result**

A darting, zigzag, tumbling or other organised movement.

**Negative Result**

No movement or Brownian motion only.

## 4.2 Semi-solid Agar Method[2,22,23]

- Inoculate the liquid bacterial culture to the test tube motility slant medium using the stab technique. Inoculate the positive and negative controls as well as the control medium (uninoculated) at the same time
- Incubate at the relevant temperature for 24-48hr
- Examine the test tube slant for the presence or absence of growth along the line of the stab inoculation

  **Note:** Inoculation is with a straight wire/needle that is stabbed two-thirds of the way into the media.

**Positive Result**

Visible stab line, with cloudiness of the agar.

**OR**

Organisms migrate from the stab line and diffuse into the medium, causing turbidity.

**Negative Result**

Visible stab line and clear agar media.

**OR**

Growth accentuated along the stab line but no further and surrounding medium remains clear.

**Control Result (uninoculated)**

No growth, medium remains colourless and clear.

## 4.3 Wet Mount Method[2]

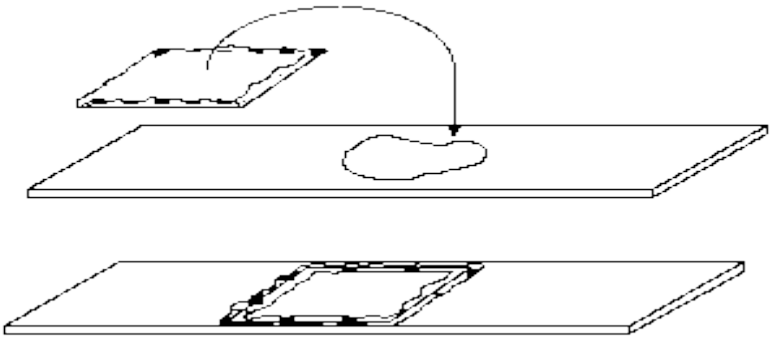


Fig.2: Wet Mount Method

(Adapted from the web link: http://www.ruf.rice.edu/~bioslabs/methods/microscopy/wetmount.html developed by David Caprette)[25].

- Set microscope slide according to Figure 2 below
- Place a small drop of bacterial culture in centre of the microscope slide
- Invert the coverslip gently over the prepared microscope slide to avoid bubbles. The coverslip should stick to the slide
- Examine microscopically (x400) for motile organism

**Note:** Examine a wet mount immediately, once it has been prepared, because motility decreases with time after preparation

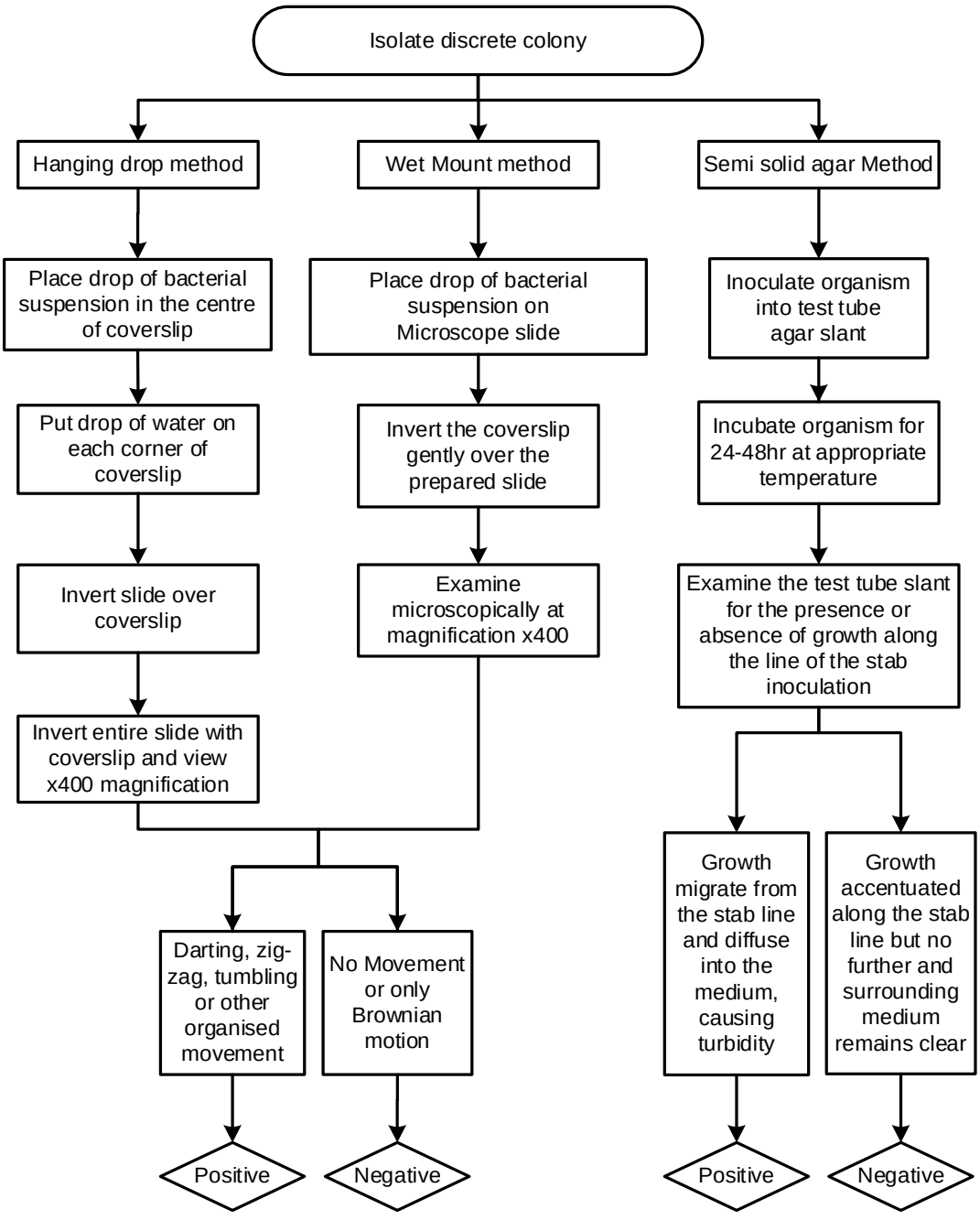
**Positive Result**

A darting, zigzag, tumbling or other organised movement.

**Negative Result**

No movement or Brownian motion only.

# Appendix: Motility Test




**Note:**
**Positive control:** *Proteus mirabilis* NCTC 10975
**Negative control**: *Acinetobacter lwoffii* NCTC 5866

The flowchart is for guidance only.

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