Public Health England

NHS

# UK Standards for Microbiology Investigations

## Motility test

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011.**"



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# Acknowledgments

UK Standards for Microbiology Investigations (UK 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. UK 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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Logos correct at time of publishing.

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# Amendment table

Each UK 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 number/date | 8/03.12.18 |
|---|---|
| Issue number discarded | 3.1 |
| Insert issue number | 4 |
| Anticipated next review date* | 03.12.18 |
| **Section(s) involved** | **Amendment** |
| Whole document. | Document and flowchart updated.<br>Technical limitations updated with subheadings.<br>References updated with grades. |
| Procedures and results. | This has been updated with a picture showing results for the semi-solid agar method. |

*Reviews can be extended up to five years subject to resources available.

# UK SMI#: scope and purpose

## Users of UK SMIs

Primarily, UK SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. UK SMIs also 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. The documents also 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 UK SMIs

UK 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. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of UK 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

UK 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 UK SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing UK SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop UK 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. UK SMIs are developed, reviewed and updated through a wide consultation process.

## Quality assurance

NICE has accredited the process used by the UK SMI working groups to produce UK SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of UK SMIs is certified to ISO 9001:2008. UK SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. UK SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level

# 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.

of complex laboratory investigation possible. In using UK SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. UK SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. UK SMIs also provide a reference point for method development. The performance of UK 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 UK SMI working groups are committed to patient and public involvement in the development of UK SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting UK 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 UK SMIs is subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The UK 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

While every care has been taken in the preparation of UK SMIs, PHE and the partner organisations, 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 UK SMI or any information contained therein. If alterations are made by an end user to an UK SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as UK SMIs have been developed for application within the UK, any application outside the UK shall be at the user’s risk.

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

UK SMIs are Crown copyright which should be acknowledged where appropriate.

## Suggested citation for this document

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# Scope of document

This document covers the procedure for motility test. The motility 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. It is also used to aid in differentiation between genera and species.

This UK SMI should be used in conjunction with other UK 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; some bacteria are motile at different temperatures from those at which they are normally incubated, for example, *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

**Brownian motion**

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 in liquid culture.

**Difficulty in interpretation of results**

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.

**False positive results**

Environmental conditions such as heating, shaking, or other trauma can damage bacteria flagella, rendering the organism non-motile and giving a false-negative reaction.

**Growth temperatures**

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

### Semi - solid agar method

The semi-solid agar method is useful for detecting bacterial motility. It permits the isolation of motile and non-motile strains from some cultures which were non-motile with the hanging drop technique. It is particularly advantageous to use with testing of pathogenic organisms and routine testing, because the results are cumulative and macroscopic. This method has excellent sensitivity as it picks up low levels of motility.

Staff should exercise caution when interpreting results using this method as it could be complex to interpret at times. Both the positive and negative control agar slopes should be included. Manufacturer's instructions should be followed.

### Wet mount Vs hanging drop methods

The disadvantage of using the wet mount method and the hanging drop method is that there are significant risks associated with them, especially with pathogenic organisms for example salmonellae.

# 1 Safety considerations[4-21]

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.

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[22]

Liquid bacterial culture (incubation times and temperatures may vary with different species). Refer to the appropriate identification UK 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 with different species). Refer to the appropriate identification UK SMI.

Normal microscope slide without central depression

Inoculating loop

Coverslips

## Semi-solid agar method[23,24]

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

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

Test tube Motility medium. There are different varieties of the motility media that are available. Laboratories should ensure that whatever media is used should be validated prior to use.

# 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[22]

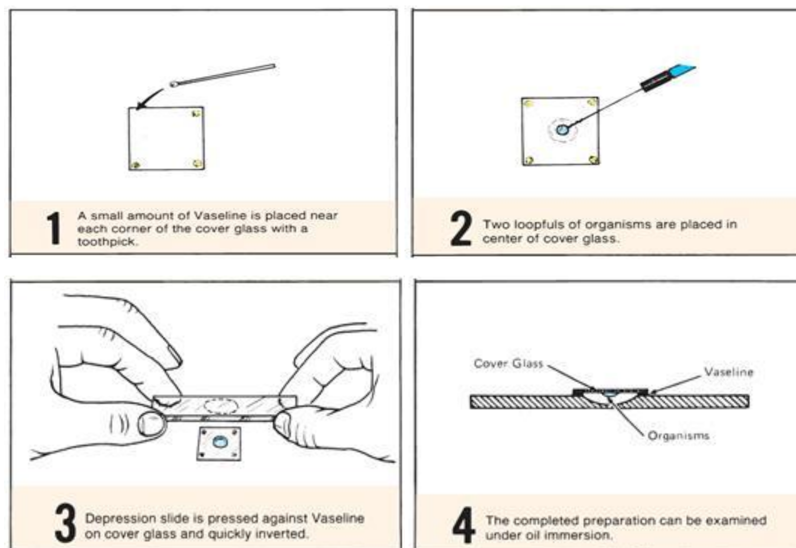



Fig. 1: Hanging drop method

(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))[25].

- moisten the four edges of the coverslip with water to keep the coverslip firmly in place

  A ring of vaseline or plasticine may be used in place of water to keep the coverslip firmly in place on a microscope slide if microscope slides with central depression are not available. The Vaseline-sealed depression or sometimes using plasticine also slows down the drying-out process, so the organisms can be observed for longer periods.

- place a small drop of liquid bacterial culture in the centre of a 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 immediately as the organisms become less motile with time

**Note:**

1. 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.
2. 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[2,23,24]

- 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 adding 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. Care should be taken to ensure that the wire/needle is in the exact same line when removed from the medium as it was when it was initially inserted for inoculation.

**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.

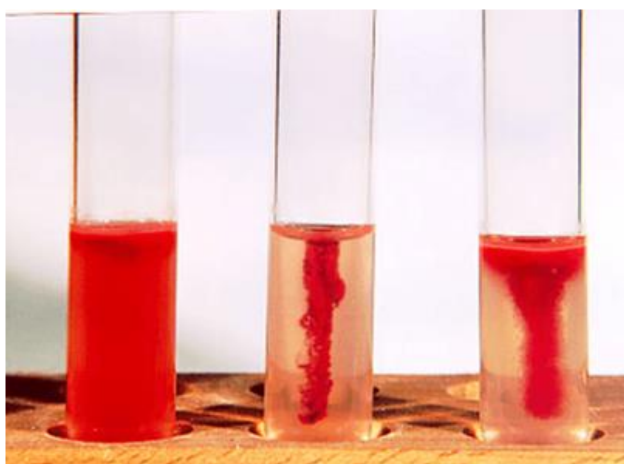


Fig.2: Semi-Solid Agar Method

*The first and last test tubes are positive as the organisms extend from the stab line while the middle test tube is negative and organism grows along the stab line.*

Adapted from the web link: https://microbeonline.com/tests-bacterial-motility-procedure-results/.

## 4.3 Wet mount method[2]

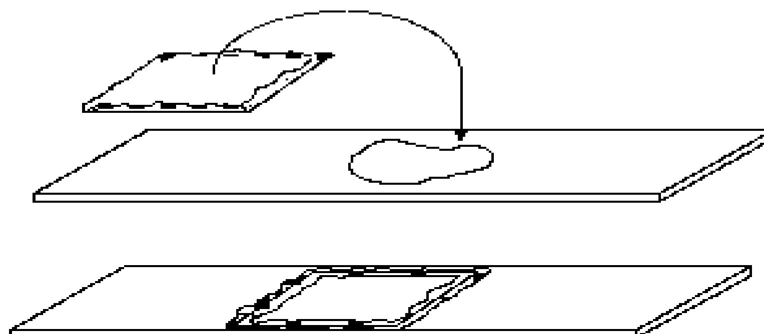


Fig.3: Wet Mount Method

(Adapted from the web link: http://www.ruf.rice.edu/~bioslabs/methods/microscopy/wetmount.html developed by David Caprette)[26].

- set microscope slide according to Figure 3 above
- 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

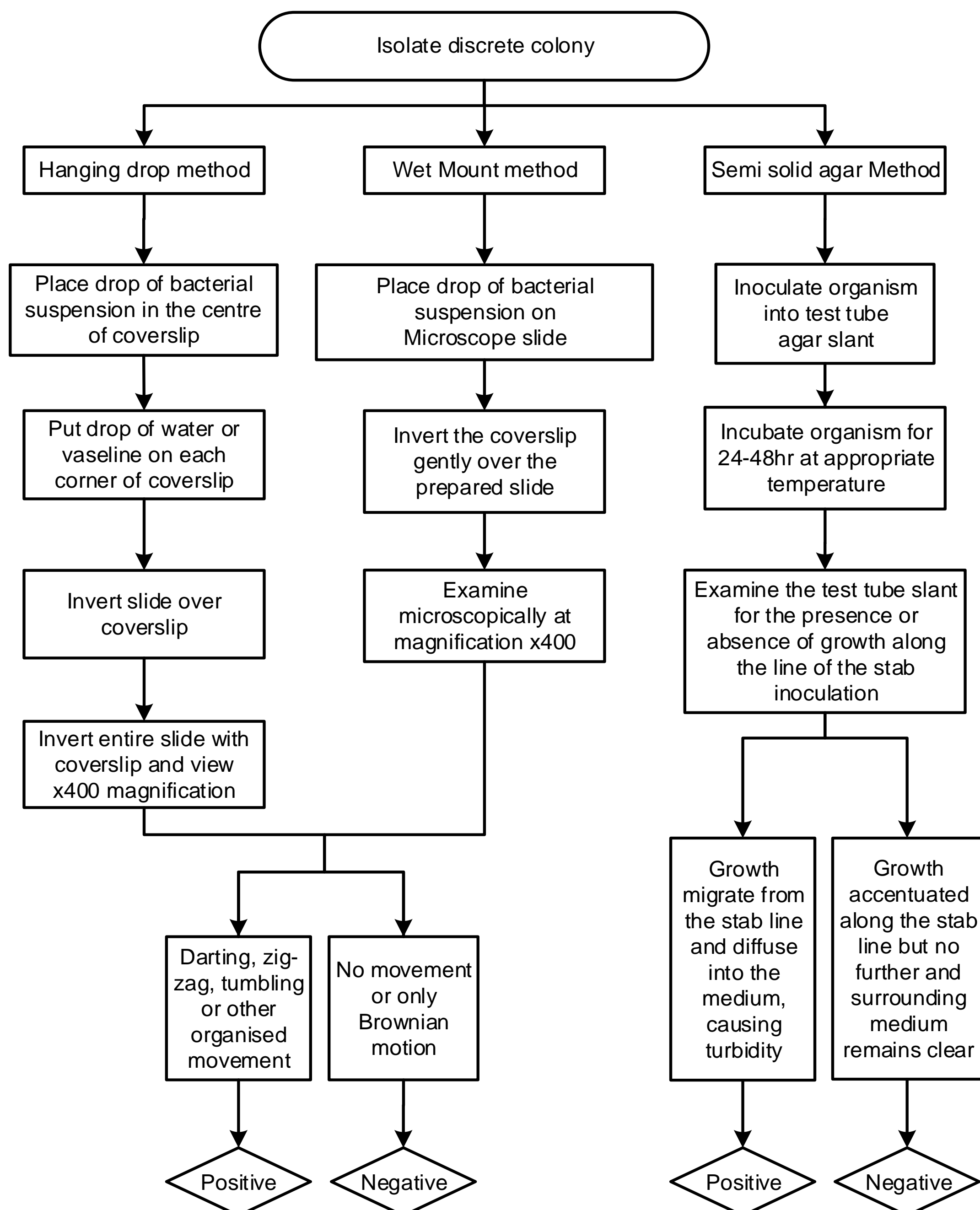
**Positive result**

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.

# References

### Modified GRADE table used by UK SMIs when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMIs for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VIII). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

| Quality/certainty of evidence | Types of evidence |
|---|---|
| A Strongly recommended | I Evidence from randomised controlled trials, meta-analysis and systematic reviews |
| B* Recommended but other alternatives may be acceptable | II Evidence from non-randomised studies |
| | III Evidence from documents describing techniques, methods or protocols |
| C* Weakly recommended: seek alternatives | IV Non-analytical studies, eg case reports, reviews, case series |
| D Never recommended | V Expert opinion and wide acceptance as good practice but with no study evidence |
| | VI Required by legislation, code of practice or national standard/ guideline |
| | VII Letter /short communication /editorials /conference communication |
| | VIII Electronic citation |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