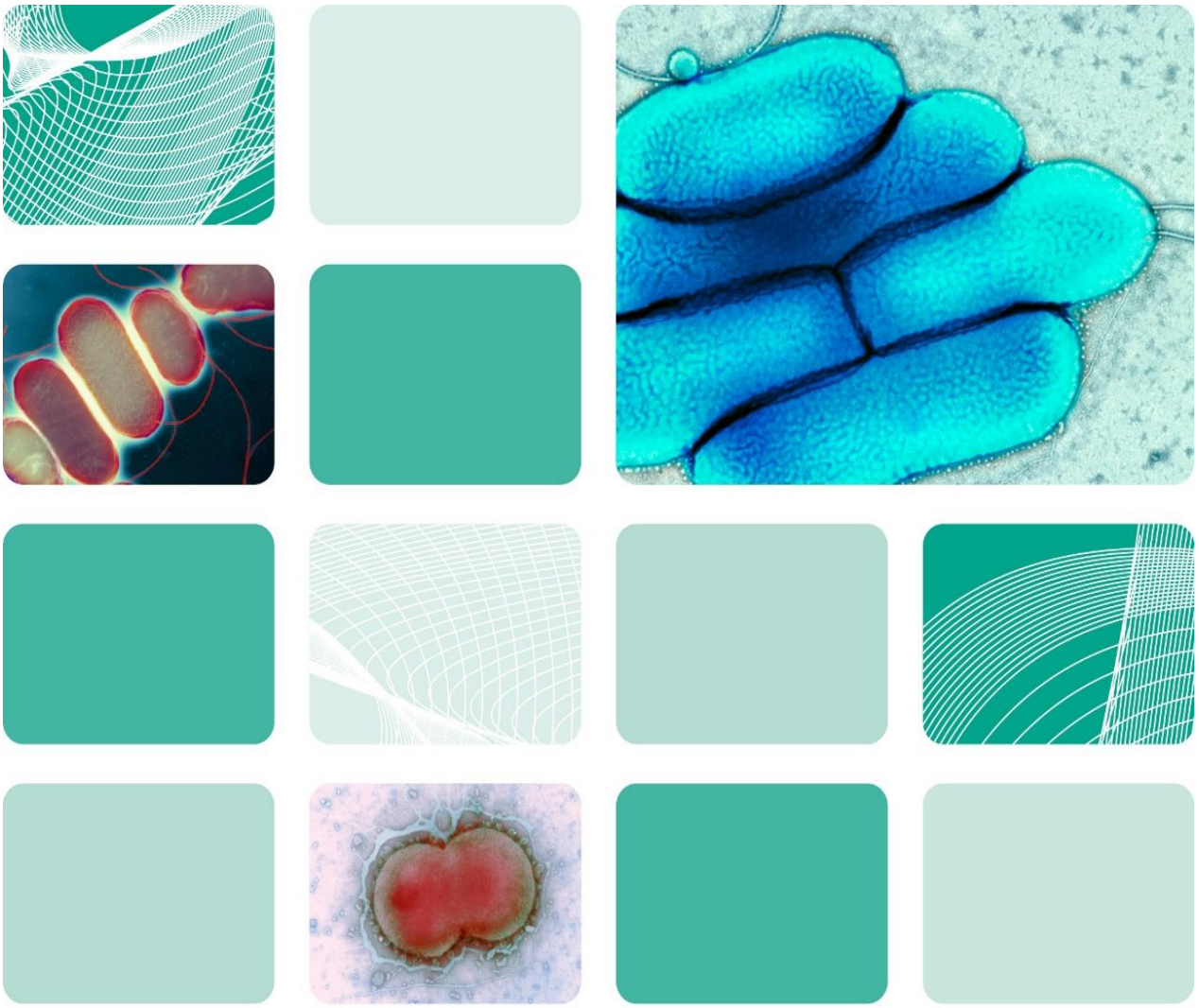
UK Health Security Agency

UK Standards for Microbiology Investigations

Motility test



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Test Procedures | TP 21 | Issue number: 4.1 | Issue date: 28.02.25 | Page: 1 of 14

Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on the UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a 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.

UK SMIs are produced in association with:

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Contents

Amendment table

Each UK SMI document has an individual record of amendments. The amendments are listed on this page. The amendment history is available from standards@ukhsa.gov.uk.

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	9/28.02.25
Issue number discarded	4
Insert issue number	4.1
Section(s) involved	**Amendment**
Whole document.	**This is an administrative point change.** **The content of this UK SMI document has not changed.** **The last scientific and clinical review was conducted on 03/12/2018.** Hyperlinks throughout document updated to Royal College of Pathologists website. Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms Partner organisation logos updated. Broken links to devolved administrations replaced. References to NICE accreditation removed. Scope and Purpose replaced with General and Scientific information to align with current UK SMI template.

Amendment number/date	8/03.12.18
Issue number discarded	3.1
Insert issue number	4
Anticipated next review date*	03.12.21
Section(s) involved	**Amendment**

Whole document.	Document and flowchart updated. Technical limitations updated with subheadings. 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 5 years where appropriate

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 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.

4 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].

5 Technical information/limitations

5.1 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.

5.2 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.

5.3 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.

5.4 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].

5.5 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.

5.6 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.

6 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.

7 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.

8 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.

9 Procedure and results

9.1 Hanging drop method[22]

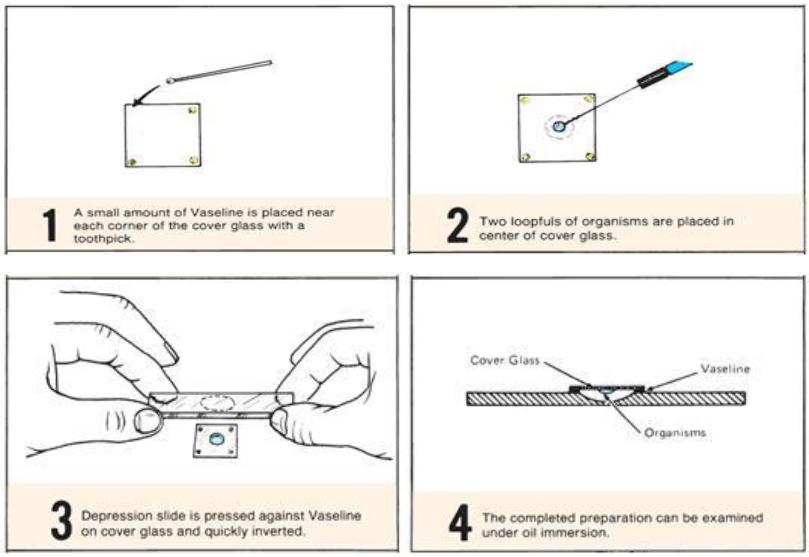


Fig. 1: Hanging drop method

(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.

9.2 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.

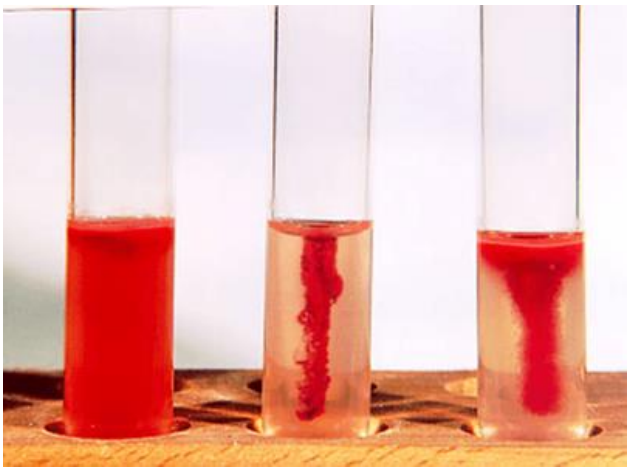


Fig.2: Semi-Solid Agar Method

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/.

9.3 Wet mount method[2]

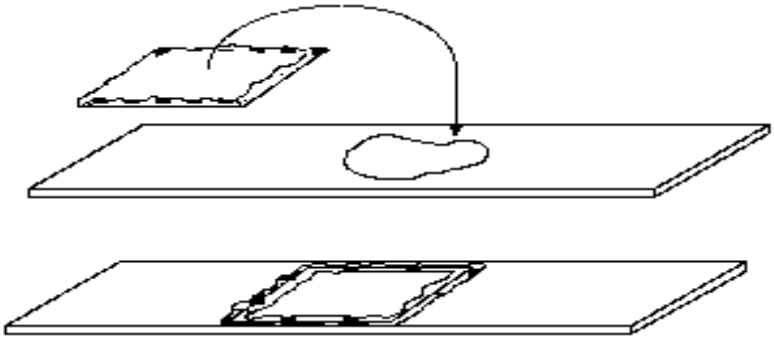


Fig.3: Wet Mount Method

(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

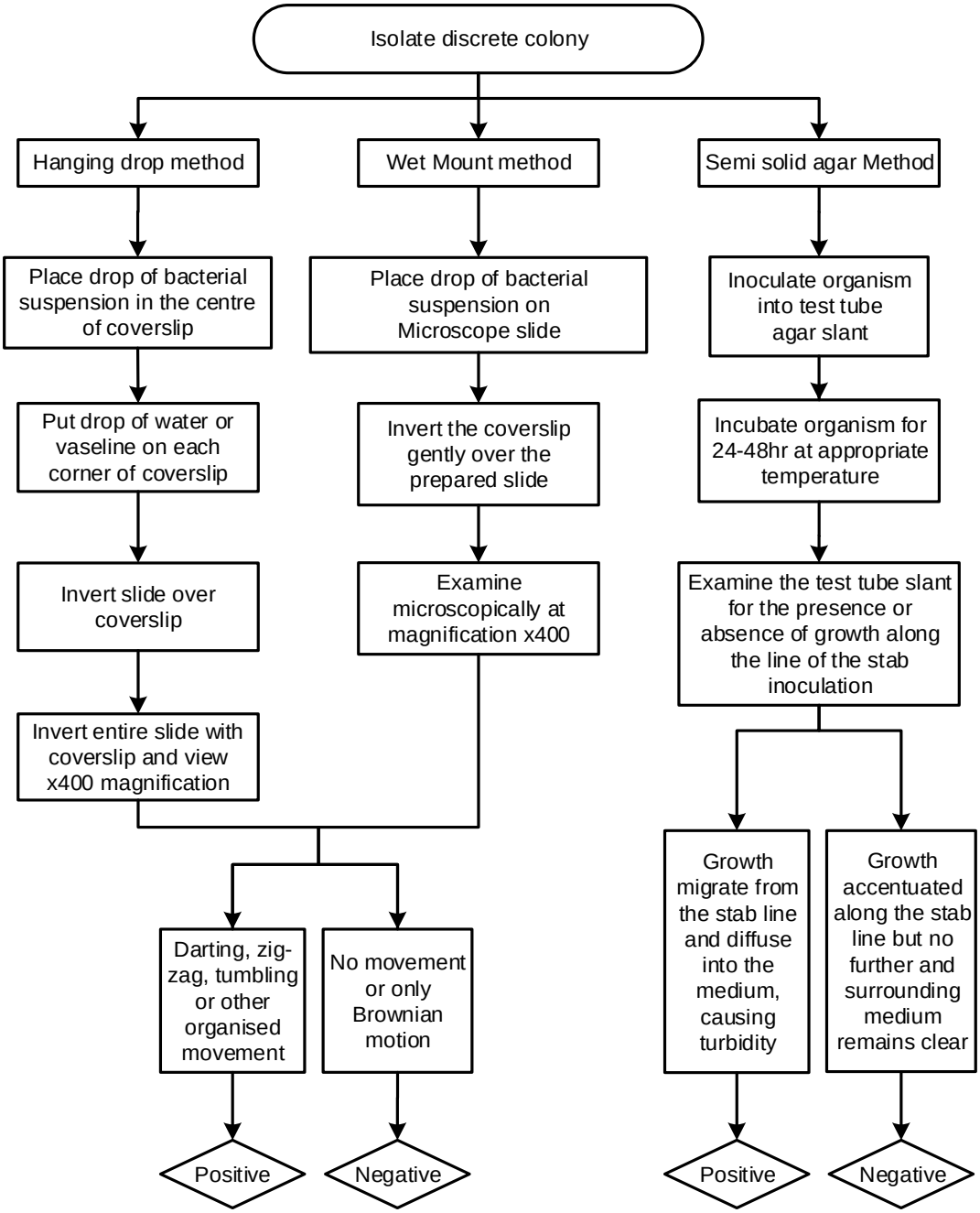
Positive result

A darting, zigzag, tumbling or other organised movement.

Negative result

No movement or Brownian motion only.

Algorithm: Motility test



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

The flowchart is for guidance only.

References

An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.

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the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes". 1998. **A, VI**

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