**Bacteri**

**PURPOSE:**

This document provides instructions for determining the motility of bacteria.

**SCOPE:**

This procedure applies to UPMC Hanover laboratory

**PRINCIPLE:**

The motility test is used to detect the presence of flagella of bacteria, allowing them to travel in and out of the microscopic field or beyond their initial inoculation in agar.

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**MATERIALS AND REAGENTS:**

TSB broth

Coverslips

Microscope slides

Sterile inoculating loop

Tube media (semisolid) such as CTA agar

**Wet Mount Preparation:**

1. Inoculate fresh growth from an agar plate to approximately 0.5 ml TSB. The inoculum should not be visibly turbid.

2. Place a drop of broth on the center of a microscope slide and coverslip. Allow organisms to settle for 1 minute.

3. Observe under the 40X objective.

**Tube Media:**

1.With a sterile inoculating wire, pick an isolated colony and stab the media straight down through the center of the agar to a depth of about 1/2 inch for small tubes and 1 " for large tubes.

2. Incubate as follows:

a. At 35ºC. for *Enterobacteriaceae*  for 24 hours.

b. At 30ºC. for non fermenting, Gram negative rods and enterococci for 24 hours.

c. If there is a question regarding a negative result, incubate at 25º C.

d. For Listeria and Yersinia, incubate 2 tubes, one at 35ºC and one at 25ºC.

**Interpretation:**

**Wet Mount:**

Positive

Directional purposeful motility. Motile organisms change position with respect to one another.

Negative

Random jiggling or shaking (Brownian movement)

**Tube Media:**

Positive

Diffuse outward growth away from the stab line.

Negative

A clear tube (the same as uninoculated media) with growth only along the line of inoculation.

**Limitations:**

Non motile Klebsiella strains may give a false positive motile reaction in tube media. This is due to mucoid strains spilling between the media and the tube giving a cloudy appearance and may be confused with motility.

Excessive heat on a microscope slide can affect the results. False negative reactions may occur to bacteria flagella damage.

**REFERENCE:**

Clinical Microbiology Procedures Handbook, 4th edition. Leber, Amy L. Editor in chief. Section 3.17.31 Motility Tests. 2016.

**REFERENCES:**

Site documents or publications, which are not maintained with the SOP. If the document is in response to a regulatory/accreditation requirement, the agency reference, standard, and/or number should be sited.