

## DETECTION OF BACTERIAL MOTILITY

### I. OBJECTIVES

- To demonstrate bacterial motility by microscopic and macroscopic techniques.
- To observe flagella in prepared slides stained by specific flagellar stains.

### II. INTRODUCTION

Motility in bacteria may be studied by a variety of techniques. The movement of living bacteria may be examined in solid or semisolid agar media, microscopically in wet, unstained preparations or in stained preparations. These techniques are briefly described below:

#### Dark Field Microscopy

Some organisms would be difficult to see if it were not for the special dark field and phase contrast microscopes and techniques. Dark field illumination is achieved through the use of a special condenser that blocks the direct illumination of the specimen. The light that does reach the specimen comes from the light reflected across, not through, the field of vision. The field of vision is dark. The light is reflected past the lens. Any large particle in the preparation will scatter this reflected light through the lens of the microscope and the image of the specimen will appear light against a dark background. This scattering of light leads to an enlargement of the specimen, thus the dark field microscope is particularly useful for the examination of material that is beyond the resolution of a bright field microscope. The specimen used for dark field microscopy is not fixed or stained.

The dark field scope has several disadvantages: it is difficult to use by someone without extensive light microscope experience, the intense light utilized by the system will quickly dry out the specimen and it is too expensive to provide a separate scope for each student. However, this highly complicated, sensitive instrument is routinely utilized in research laboratories.

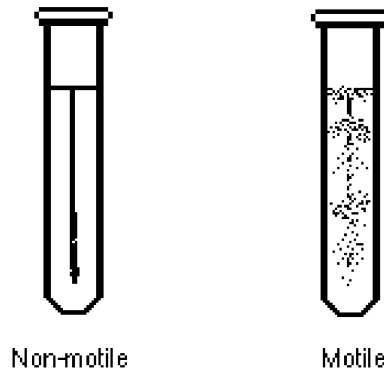
#### Bright Field Microscopy

The bright field microscope can be used to view motility in a wet mount by reducing the amount of light that passes through the specimen. This is the most common method of observing motility. It is quick and inexpensive.

## Motility Butts

Another method to detect motility involves the stab inoculation of a semi-solid motility agar in a tube (called butt) or in a Petri plate. Semi-solid medium differs from solid agar in that it contains less agar and thus allows motile bacteria to move through it. The medium also contains triphenyl tetrazolium chloride (TTC) which is a colorless, soluble substance. TTC acts as an artificial electron acceptor for the electron transport chain and gets reduced by either cytochrome b or c to turn into an insoluble, pink compound called formazan. So the presence of a pink color is an indication of presence of bacteria. If the bacteria are motile, they will spread from the stab line creating a pinkish feathering-effect (see drawing below). Often when the bacteria are highly motile, the whole medium may turn pink in color. Motile and highly aerobic organisms are seen to cover the top of the agar in the tube, so this is a good spot to keep under observation as well. Non-motile organisms will grow only along the stab line (see diagrams below).

Obviously, this procedure requires an incubation period of one to two days or longer, but the result of this procedure is easily noted with less eye-strain than the microscopic procedures. Also, butts may permit observation of motility that only occurs at one stage of the growth cycle in organisms that exhibit diphasic motility (i.e., are both motile and non-motile depending on the stage of growth). Many organisms are motile only during the early log to mid-log phase of growth.



## Swarming Bacteria

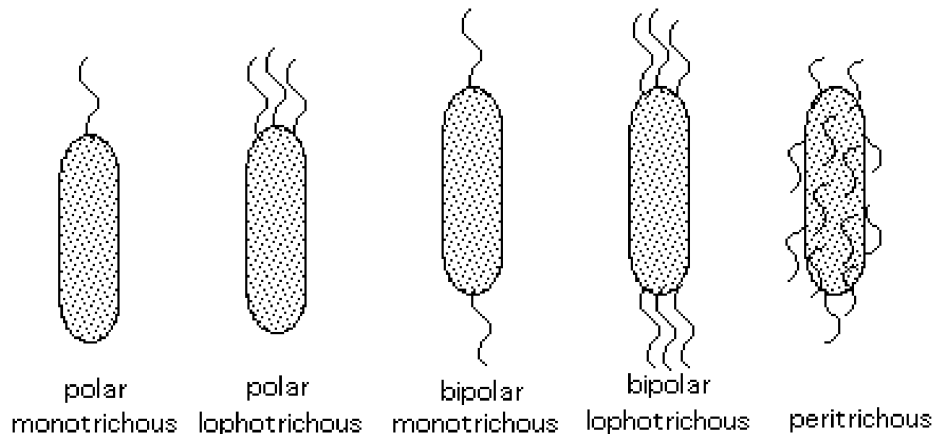
Some extremely motile bacteria are able to move through solid agar in chase of nutrients that they can metabolize for growth. This is a positive chemotactic behavior and is best observed in swarming *Proteus*. If a drop of a culture of this organism is placed on the center of an agar plate and the plate is incubated, the bacteria start to move out of the center towards the perimeter. During this movement, each bacterium absorbs nutrients and increases in size. At a certain distance from the center, they divide and the progeny continues to move out. This causes the formation of concentric rings (called swarms) on the agar plate, each ring showing the start of a new generation. Generation time for such bacteria can be estimated based on the number of rings formed during a measured time interval.

## Flagellar Stains

Finally, there are procedures available to stain flagella. One technique involves increasing the size of the flagella by precipitating alum along the length of the flagella and then staining this complex. This procedure is rather tedious and rarely used today.

The position and number of flagella on a bacterial body can be used as an aid in identification. If the flagellum is located at one end of the cell, the bacteria is said to possess a polar flagellum. Such flagella could be single (monotrichous) or in a tuft (lophotrichous). If both ends contain flagella, the bacterium is said to be bipolar. Bipolar bacteria can have monotrichous or lophotrichous flagella. If flagella are scattered all over the cell, the bacterium is called peritrichous.

Bacteria use their flagella to move towards nutrients (positive chemotaxis) or away from harmful chemicals (negative chemotaxis). They accomplish chemotaxis in series of swims and tumbles. Swims refer to the movement of bacteria while tumbles refer to the stop time after each swim. Swims may occur in random directions, except when nutrients or harmful chemicals are present in the environment for which chemotaxis occurs.



## III. LABORATORY SUPPLIES

Microscope slides	1 box/table
Coverslips	1 box/table
Motility butts (to be used for cultures A & B only)	2/student
Motility Plate (optional)	1/4 students
BHI plate	1/student
Vaspar (vaseline-paraffin 1:1 mixture)	2/table
Prepared slides	1 box/table
Cultures	
A	2 ml/table
B	2 ml/table
Swarming <i>Proteus</i>	2 ml/table

**IV. PROCEDURE** (Each student will work independently for this exercise.)**Wet Mounts**

(Keep all your specimens at 30-37°C water bath. Some motile bacteria become non-motile at lower temperatures. Also prepare your specimens at the time you intend to view them and not too far in advance as they may dry out or become immobile.)

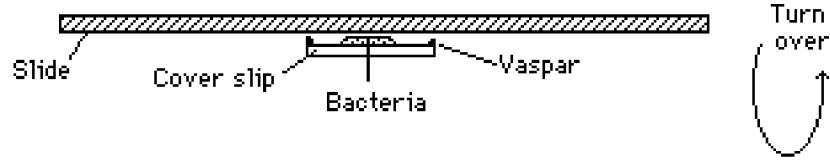
1. Remove a small amount of vaspar from the jar and spread it over a small area (~ 2 x 4 cm) at the edge of your palm.
2. Hold a coverslip between the thumb and index finger of your other hand. Drag the coverslip into the vaspar on your palm to transfer a narrow band onto the edge of the coverslip.
3. Carefully rotate the coverslip and repeat step 2 until there is a narrow band of vaspar on all sides of the coverslip.



4. Place the prepared coverslip on the tabletop, vaspar side up. Continue with step 5a or 5b depending on the source of your sample
- 5a. Transfer from a broth culture: Use a sterile loop to place a single small droplet of culture in the center of the coverslip. Continue with step #6.



- 5b. Transfer from a solid culture: Use a sterile transfer pipette to place 1 small drop of water in the center of the coverslip. Use a sterile loop to remove growth from the solidified medium and suspend it gently in the fluid on the coverslip.
6. Place a microscope slide over the coverslip to tightly seal all 4 edges. You do not need to press on the slide or you may make your preparation very messy.
7. Carefully turn over the slide so the coverslip is on top of the slide.



8. Place the slide with the suspension on the center of your microscope stage. Lower the condenser to the lowest position to reduce the amount of light passing through the preparation.
9. Using the 4X objective, focus on the edge of the drop.
10. Position the 40X objective and refocus on the edge. Now slowly move the specimen away from the edge and look for the presence of motility. You may find it necessary to further decrease the amount of light passing through the specimen to increase the contrast. This is accomplished by slightly closing the iris diaphragm.
11. Interpretation hints:
  - a. A truly motile cell will have movement across the microscope field of vision. Brownian movement is dancing or bouncing of the organisms caused by random bombardment by surrounding water molecules. Make sure specimen moves a distance before interpreting motion as true motility.
  - b. An incomplete vaspar seal results in a rapid flow of water through leakage and the consequent movement of the organisms. However, the movement will be in one direction; truly motile organisms will move in all directions!
  - c. Prepare your specimens at the time you intend to view them and not too far in advance as they may dry out or become immobile.
  - d. Unfavorable environmental conditions play a role in motility. For example if the temperature of the room is too low, many bacteria stop moving. It is therefore a good idea to warm up your preparation to at least 30°C before observing.

### Motility Butts

1. Obtain a tube of semi-solid agar (motility butt, 0.4% agar).
2. Flame-sterilize the inoculating needle and let it cool down. Dip it into culture A and stab the agar butt in the center and down the tube.

Note: It is important to give enough time for the needle to get cool enough not to kill the organisms before taking samples from either a broth or plate culture.

3. Withdraw the needle as vertically as possible to avoid spreading the inoculum beyond the original stab line. Label the tube and incubate it at 30°C for 2 days.
4. Repeat steps 1 to 4 with culture B.

Note: If a motility agar plate is provided, divide it into quadrants and stab each quadrant with a different sample. Give the unused portion of plate to another student to use/

5. Interpretation: examine the pattern of turbidity:
  - Non-motile: growth occurs only along the line of inoculation.
  - Motile: growth occurs throughout the agar. You may observe a feathering effect of growth away from the line of inoculation.

Note: Triphenyl Tetrazolium Chloride (TTC) is added to the motility butts to show where growth has occurred. The bacteria will reduce the TTC which is red in color.

6. All negative motility reactions are confirmed by the wet mount test on a fresh broth culture when doing diagnostic tests on an unknown sample. Cultures should not be more than 24-48 hours old.

### Swarming Bacteria

1. Place a loopful of the *Proteus* culture exactly in the center of a BHI plate.
2. Label your plate and incubate inverted at 30°C for 24 hours. After incubation, draw your observations.

### Flagellar Stains

1. Look at the prepared slides under the microscope. Increase magnification until you can observe flagella well.
2. Record the magnification and draw your observations.

*Use of any section of this Lab Manual without the written consent of Dr. Eby Bassiri, Dept. of Biology, University of Pennsylvania is strictly prohibited.*

**Results of the Bacterial Motility Exercises**

NAME \_\_\_\_\_ DATE \_\_\_\_\_ GROUP NAME \_\_\_\_\_

PARTNER(S) \_\_\_\_\_

1. Fill in the following table:

Organism	Magnification	Motility in	
		Wet Mount	Agar Butt or Plate
Culture A			
Culture B			

2. Draw what you observed on the plate for the swarming *Proteus*: How many generations do you see on your plate? If the incubation period was 24 hours, what is the generation time?



3. Fill in the following table for commercially stained slides of flagella:

<u>Organisms</u>	<u>Magnification</u>	<u>Drawing</u>	<u>Flagellar Type</u>
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