MOTILITY TEST

Principle and Purpose

Many bacterial species possess flagella. These structures, which come in various arrangements, enable a bacterium to move from one location to another. Flagella can be visualized with a microscope using a special staining procedure which is tedious to perform. Motility can also be visualized in a wet mount of bacterial cells. However, sometimes Brownian motion causes bacterial cells to appear motile when they are not.

One manner to readily test for bacterial motility is to use a medium having a semi-solid consistency. When this medium is inoculated, the diffusion of the bacterial species causes the medium to become cloudy, thereby indicating motility. Sulfide-Indole-Motility (SIM) medium takes away some of the subjectivity if the microbe also generates hydrogen sulfide which combines with iron in the medium to produce a black precipitate. If the precipitate diffuses away from the inoculation stab line, then motility is readily discerned. A simpler, yet similar method is to incorporate the compound triphenyltetrazolium chloride (TTC) into the semi-solid medium. TTC, which is a colorless dye, enhances visualization of bacterial growth. When reduced by bacterial cells, an insoluble red pigment (formazan) is produced. Hence, a red color shall form only in the area of bacterial growth. Therefore, motile organisms will produce a pinkish-red color that diffuses from the stab line, whereas non-motile microbes exhibit a pinkish-red pigment confined to the stab line (Fig. 1).

In this exercise, students will use Motility Test Medium containing TTC to assess motility among selected bacterial species.

Figure 1. Motility Test Medium Using TTC. *Escherichia coli* (left image) and *Klebsiella pneumoniae* (right image) were stab inoculated into Motility Test Medium containing TTC. The media were then incubated at 37°C for 24 hours. The images show that *E. coli* readily moves away from the stab line, whereas *K. pneumoniae* does not. Hence, *E. coli* is motile and *K. pneumoniae* is not.

Learning Objectives

Upon completion of this exercise, a student should be able to:

- Understand the biochemical basis of the motility test;
- Properly perform the motility test; and
- Discern how this information can be used to differentiate and identify microbial species.

Materials Required

The following materials are necessary to successfully conduct this exercise:

Organisms - The following organisms should be provided as 18-24 hour TSA slant cultures:

- *Enterobacter aerogenes* (ATCC 13048) [abbreviated as *Ent. aerogenes*]
- *Klebsiella pneumoniae* (ATCC 13883) [abbreviated as *K. pneumoniae*]

Materials

- Motility Test Medium with TTC (Cat. No. Q11; Hardy Diagnostics, Santa Maria, CA; https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/MotilityTestMedia.htm)

Procedures

Students shall review and use the BIOL 3702L Standard Practices regarding the labeling, incubation, and disposal of materials.

1) Obtain two tubes of Motility Test Medium with TTC. Label each tube with the microbe to be used and other relevant information.
2) Inoculate each tube with the appropriate microbe (preferably from an 18-24-hour culture) by stabbing the center of the medium to a depth of 1/2 inch.
3) Incubate both tubes at 37°C for 18-24 hours. Be sure to loosen the caps, but not to the degree at which they would fall off.
4) Remove the tubes from the incubator and observe each for a reddish-pink color diffusing from the stab line.

Interpretation of Results: The development of a reddish-pink color that diffuses away from the stab line into the medium indicates that the test organism is motile (Fig. 1). If a reddish-pink color remains along the stab line, then the microbe used to inoculate the medium is non-motile.

Record your observations on the report sheet attached to this exercise.

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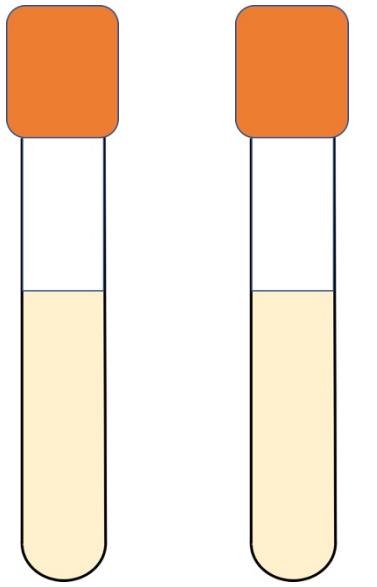
Student Name: _____ **Group Designation** _____

COMPLETE THE FOLLOWING TABLE BASED UPON YOUR OBSERVATIONS, THEN SKETCH THE RESULTS ON THE FIGURES BELOW EACH ORGANISM LISTED IN THE TABLE.

Observations of Inoculated Motility Test Media with TTC (yes or no)	**Bacteria Tested**	
	Enterobacter aerogenes	*Klebsiella pneumoniae*
Diffuse Red Pigment?		
Red Pigment Along Stab Line Only?		
Motile or Non-Motile?		



E. aerogenes *K. pneumoniae*

Discussion Question:

What are the possible reasons that a tube of Motility Test Medium containing TTC shows no red pigment production at all after incubation at 37°C for 48 hours.