



Core Microbiology Skills

How to Perform a Motility Test

In this short video, you will learn how to perform the motility test. This test is a useful tool to differentiate bacteria by detecting if a bacterium is motile due to the presence of flagella. After this program, you will be able to: discuss the steps to perform both the wet mount and the tube motility tests and identify a positive, or motile, and negative, or non-motile, reaction in motility media and on a wet mount.

There are two primary methods for performing a motility test — the Wet Mount Method (or direct test) and the Motility Media Method. There are several species of bacteria that are motile by their attached flagella. The wet mount involves observing this motility in a drop of saline or broth. Bacteria can usually be seen moving at different angles of direction in the solution as compared to bacteria moving as a result of Brownian motion (which is the normal drifting of particles in a solution).

Motility media is a semi-solid media which is the result of a low concentration of agar. Because the media is semi-solid, there is an easy visual determination of motility which appears as diffuse growth outward from the line of inoculation or turbidity of the media. If using media with TTC (triphenyl tetrazolium chloride, a colorless salt that turns a red color when it becomes reduced as a result of bacterial metabolism), you will see a red color in the line of inoculation and a pink color that diffuses out from it if the organism is motile. Please note that some organisms are inhibited by the TTC.

For this procedure, you will need the following: personal protective equipment (PPE) such as gloves, laboratory coat, and safety glasses, sterile Pasteur pipettes, clean large 2x3 glass slides, a cover slip, sterile loops and/or needles, and your reagent. For this presentation, we will be using two types of motility media: motility media with and without TTC. Motility media with TTC (triphenyl tetrazolium chloride) is a colorless salt that turns a red color when it becomes reduced as a result of bacterial metabolism. For our samples, we have two agar plates containing two isolated colony types that are 18 to 24 hours old.

Wet mount procedure

Before you can perform the wet mount to determine motility, it is best if the organism is placed in an enrichment broth such as BHI (brain heart infusion) or TSB (trypticase soy broth). Using a plastic loop, isolated colonies are picked from a blood agar plate and transferred to a labeled tube of BHI broth. The loop is then discarded into the sharps container. Using a sterile Pasteur pipette, remove a small amount of broth and place a single drop on the center of a large glass slide.

Place a glass cover slip over the sample and allow the organisms to settle for a minute. Next, observe the sample under high power 40x on a microscope. Directional motility is recorded as a positive test indicating a motile organism. If the organisms do not change position with respect to one another, that is Brownian motion, or a negative test, or a non-motile organism. If the initial wet mount (direct test) is negative, either repeat it with a tube test or incubate the broth at 35 degrees Celsius or the temperature appropriate for that organism for 18-24 hours. After 18 to 24 hours, remove the broth from the incubator and place it on your bench. Mix the broth gently but do not invert the tube. Non-

motile or aero-tolerant organisms may have settled to the bottom. Repeat the steps above for observing motility.

With a sterile inoculating needle, do not use a loop because it is too wide and will make it difficult to interpret. Instead, using a sterile inoculating needle, pick an isolated colony and stab the labeled tube medium straight down through the center to a depth of one-half inch from the bottom of the tube. Do not go all the way to the bottom of the tube. This prevents the organism from growing up the sides of the tube. It is important that you remove the needle from the media along the same path used to enter the media. Discard the needle in the sharps container. Incubate at 35 degrees Celsius or the temperature appropriate for that organism for 18 to 24 hours.

A positive test in motility media without TTC is diffuse growth away from the site where the media was inoculated or turbidity of the media. A negative test is indicated where there is only growth along the side of the inoculum and the media is clear around it. Please note: some organisms will grow better at the top of the tube where there is more oxygen.

For TTC media, a positive test for a motile organism is indicated by a red turbid area that extends away from the line that the medium was inoculated, and the media is turbid. If there is red growth only along the line of inoculum and the media is clear around it, then the test is negative, for a non-motile organism.

And now, a review of the steps: for the Wet Mount, place a drop of the broth containing the isolated organism on a slide and cover it with a cover slip. Look for directional movement of the bacteria. If negative, repeat after incubation or with a tube test. For motility media with or without TTC, pick a colony with a needle and stab the motility straight down a half-inch from the bottom of the tube. After 18 to 24 hours, look for turbidity or a red turbid area extending away from the stab in the medium.

Link to video job aid: <https://reach.cdc.gov/jobaid/how-perform-motility-test>.