Motility Test Medium Protocol

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Information

History

From the early days in the field of microbiology, the ability of bacteria to move has been used as a means of differentiation and classification (11, 12, 15). In the late 1800s, we find published reports describing the use of Koch's method of observing living organisms in a suspended drop of fluid (1, 2). While microscopic visualization of movement was often used in conjunction with media results (see below) to determine motility, the use of the hanging drop had many disadvantages (9, 18). Microscopic examination may lead to the possibility of overlooking a small number of actively motile cells in a sea of nonmotile ones. Motility may exhibit a cumulative time effect and if examination occurs at the wrong time, no motile cells will be seen (9). For these and other reasons, a macroscopic approach for determining motility was sought.

During the infancy of bacteriology, as scientists were experimenting with culture media composed of potatoes, gelatin, beef products, albumin, and agar, it was also discovered that the combinations of these ingredients had an impact on the visualization of motility (2, 7). In an effort to develop a better method to differentiate between the "typhoid bacillus" and other colon bacilli, in the early 1900s, Hiss developed a semisolid medium of 0.5% agar and 8% gelatin that allowed the typhus bacilli to demonstrate uniform turbidity within just 18 hours, while colon bacilli took much longer (6, 7). In 1934, motility GI medium (formulated with gelatin and heart infusion, hence the name) was developed as a means of demonstrating motility in microorganisms (9). In the study where motility GI medium was formulated, the researchers were able to demonstrate that the motility of microorganisms varies with the temperature of incubation. In the 1930s, Tittsler and Sandholzer developed a semisolid agar (17, 18) that gave over 99% concordance with hanging drop examinations. Motility was determined by the diffuse spread of growth beyond the stab line of inoculation. Realizing that the visualization of growth can sometimes be difficult, Kelly and Fulton modified Tittsler and Sandholzer's formula by the addition of triphenyltetrazolium chloride (TTC) (10). As organisms grow, they incorporate and reduce TTC, creating a diffuse red color, which is much easier to visualize.

Readers are directed to view the Flagella Stain protocol for further information on bacterial motility.

Purpose

Motility test medium is used to determine the motility of microorganisms. Although there is a single function test medium, motility tests are often part of multitest media used in the differentiation of the *Enterobacteriaceae*. These include motility-indole-lysine (3, 14), motility-indole-ornithine (3, 4), and sulfide-indole-motility (3, 5, 16) tests.

Theory

Motility has long been recognized as an important taxonomic tool and biological characteristic of microorganisms (9, 11, 12, 15). Motility in bacteria can be provided by a variety of mechanisms, but the most common involve flagella (8, 9). The presence of flagella occurs primarily in bacilli but there are a few flagellated cocci, thus motility is a very important means of identification in the family *Enterobacteriaceae* (9, 12). Motility test medium with triphenyltetrazolium chloride provides an easy method for determining motility. TTC in its oxidized form is colorless. As bacteria grow in the presence of TTC, the dye is absorbed into the bacterial cells where it is reduced to the insoluble red-colored pigment formazan (13). Growth is indicated by the presence of the red color, and as motility occurs, small to very large regions of color can be observed around the area of inoculation.

Recipes

Motility test medium (3)

Beef extract	3.0 g
Pancreatic digest of casein	10.0 g
Sodium chloride	5.0 g
Agar	4.0 g

Bring to 1 liter with distilled water and heat to boiling to melt agar. Add 5 ml of 1% TTC solution. Dispense in 5-ml aliquots into tubes and autoclave at 121°C under 15 psi pressure for 15 minutes. Cool upright in racks. After inoculation, incubate at 35°C for 18 hours or until growth is evident.

Motility test medium is commercially available in premixed forms and prepoured tubes from biological supply companies.

PROTOCOL

To test for motility, use a sterile needle to pick a well-isolated colony and stab the medium to within 1 cm of the bottom of the tube. Be sure to keep the needle in the same line it entered as it is removed from the medium. Incubate at 35°C for 18 hours or until growth is evident (Fig. 1). A positive motility test is indicated by a red turbid area extending away from the line of inoculation. A negative test is indicated by red

growth along the inoculation line but no further (Fig. 1).

FIG. 1. The tube on the left was inoculated with the motile bacterium *Proteus mirabilis* and shows a positive test for motility. The tube on the right was inoculated with the nonmotile bacterium *Staphylococcus aureus* and shows a negative test for motility.

Alternate methods of detecting motility using multitest media

Motility-indole-lysine (MIL) medium (3, 14)

Peptone	10.0 g
Tryptone	10.0 g
Yeast extract	3.0 g
L-lysine hydrochloride	10.0 g
Dextrose	1.0 g
Ferric ammonium citrate	0.5 g
Bromcresol purple	0.02 g

Agar	2.0 g

Bring to 1 liter with distilled water and heat to boiling to dissolve agar. Dispense in 5-ml aliquots in screw-top test tubes. Autoclave at 121°C under 15 psi pressure for 15 minutes.

To test for motility, use a sterile needle to pick a well-isolated colony and stab the medium to within 1 cm of the bottom of the tube. Be sure to keep the needle in the same line as it entered as it is removed from the medium. Incubate at 35°C for 18 hours or until growth is evident. A positive motility test is indicated by a diffuse cloud of growth away from the line of inoculation. The MIL medium is a multitest medium used to test for motility while simultaneously determining other metabolic characteristics. Please see the Comments and Tips section for more information.

Motility-indole-ornithine (MIO) medium (3,4)

Yeast extract	3.0 g
Peptone	10.0 g
Tryptone	10.0 g
L-ornithine HCl	5.0 g
Dextrose	1.0 g
Bromcresol purple	0.02 g
Agar	2.0 g

Bring to 1 liter with distilled water and heat to boiling to dissolve agar. Dispense in 5-ml aliquots in screw-top test tubes. Autoclave at 121°C under 15 psi pressure for 15 minutes.

To test for motility, use a sterile needle to pick a well-isolated colony and stab the medium to within 1 cm of the bottom of the tube. Be sure to keep the needle in the same line as it is inserted as it is removed from the medium. Incubate at 35°C for 18 hours or until growth is evident. A positive motility test is indicated by a diffuse cloud of growth away from the line of inoculation. The MIO medium is a multitest medium used to test for motility while simultaneously determining other metabolic characteristics. Please see the Comments and Tips section for more information.

Sulfide-indole-motility (SIM) medium (3, 5, 16)

Peptone	30.0 g
Beef extract	3.0 g
Ferrous	0.2

ammonium sulfate g

Sodium thiosulfate 0.025 g

Agar 3.0 g

Bring to 1 liter with distilled water and heat to boiling to dissolve agar. Dispense in 5-ml aliquots in screw-top test tubes. Autoclave at 121°C under 15 psi pressure for 15 minutes.
To test for motility, use a sterile needle to pick a well-isolated colony and stab the medium to within 1 cm of the bottom of the tube. Be sure to keep the needle in the same line as it is inserted and removed from the medium. Incubate at 37°C for 18 hours or until growth is evident. A positive motility test is indicated by a diffuse cloud of growth away from the line of inoculation. The SIM medium is a multitest medium used to test for motility while simultaneously determining other metabolic characteristics. Please see the Comments and Tips section for more information.

SAFETY

The ASM advocates that students must successfully demonstrate the ability to explain and practice safe laboratory techniques. For more information, read the laboratory safety section of the ASM Curriculum Recommendations: Introductory Course in Microbiology and the Guidelines for Biosafety in Teaching Laboratories.

COMMENTS AND TIPS

In addition to motility, MIL medium can be used to detect indole production, lysine decarboxylase, and lysine deaminase activities, and thus is helpful for the presumptive identification of enteric pathogens. After incubation as discussed above, lysine decarboxylation is indicated by a purple color throughout the medium. Lysine deaminase is indicated by a red or brown-red color in the top 1 cm of the medium. To test for the presence of indole, a by-product of tryptophan metabolism, add 3 to 4 drops of Kovács reagent to the medium. A positive indole test is indicated by a change in the color of the Kovács reagent to bright red within seconds of being added (3, 14).

In addition to motility, MIO medium can be used to detect indole production and ornithine decarboxylase activity. After incubation as discussed above, ornithine decarboxylation is indicated by a purple color throughout the medium. To test for the presence of indole, a by-product of tryptophan metabolism, add 3 to 4 drops of Kovács reagent to the medium. A positive indole test is indicated by a change in the color of the Kovács reagent to bright red within seconds of adding the reagent (3, 4).

In addition to motility, SIM medium can be used to detect indole and hydrogen sulfide production. To test for the presence of indole, a by-product of tryptophan metabolism, add 3 to 4 drops of Kovács reagent to the medium. A positive indole test is indicated by a change in the color

of the Kovács reagent to bright red within seconds of adding the reagent. Any blackening along the line of inoculation is considered to be a positive test for hydrogen sulfide, however, motility is thought to increase H_2S production (3, 16).

The success of this test depends upon proper stab technique and the quality of the inoculating needle. It is recommended that a straight inoculating needle be used. If this is not available, consider using a disposable inoculating needle.

It is critical to minimize the presence of water condensation in the test tubes as the presence of excess water may lead to false positives. The presence of condensation is usually apparent on the walls of the tube or on the surface of the agar. Problems can be minimized or avoided by preincubating the tubes at 37°C for 30 minutes to 1 hour with the cap loosened prior to inoculation.

Instructors have observed that known motile organisms occasionally give a negative motility result using this test. Therefore it is suggested that negative results are confirmed by using other methods such as direct observation of motility in a wet mount.

Motility in some organisms may be easier to observe in soft agar plates. Typically, the agar concentration ranges from between 0.1% to 0.4% in freshly poured plates. One advantage of this method is that it can give more quantitative information by measuring the diameter of turbidity. Another advantage is that one can assess other types of nonflagellar motility such as swarming, gliding, and twitching.

Another possible medium to consider using for motility is mannitol motility medium. This medium can detect both motility and mannitol fermentation and is used for the identification and differentiation of enteric pathogens.

As an alternative to the motility medium, nutrient broth (8.0 g/liter) can be substituted for the beef extract, pancreatic digest of casein, and sodium chloride.

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