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Section: Technical Manual	Subject Title: Motility Test Medium	
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MOTILITY TEST MEDIUM

Principle

Motility Test Medium is a semi-solid agar designed to demonstrate motility by diffusion.

Motility Test Medium is a modification of the formula of Tittsler and Sandhoizer. The medium contains small amounts of agar and gelatin, as well as triphenyltetrazolium chloride (TTC). TTC is a soluble compound which is taken up by the bacterial cells. Once the substance has been absorbed by the bacteria, it is reduced, releasing the acid formazan, a highly pigmented red, insoluble compound.

Organisms are stabbed into the medium with an inoculating wire. If the organisms are motile, they will diffuse into the soft medium laterally from the line of inoculation, resulting in a diffuse, pink color throughout the medium. Nonmotile organisms grow along the line of inoculation only, producing a pinkish-red line with no diffusion.

Storage

Upon receipt store at 2-8⁰C away from direct light. Media should not be used if there are signs of contamination, deterioration (shrinking or discoloration), or if the expiration date has passed.

Limitations

Motility tests often show a false-negative reaction. The organism may be weakly motile, or the flagella may be damaged due to heating, shaking, or other trauma. A hanging drop motility may be performed from an inoculated tryptone broth incubated for 2-4 hours to confirm motility results. Consult appropriate microbiological texts for procedure.

TTC may be inhibitory to some fastidious bacteria.

Most motility of bacteria should be interpreted at 35⁰C: however, certain bacteria such as *Yersinia enterocolitica* demonstrate the best motility at 25⁰C.

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Organisms that require oxygen for growth, such as *Pseudomonas aeruginosa*, will produce a spreading film on the surface of the medium, and will not fan out from the inoculation line where oxygen is depleted.

Procedure

1. Tube Method

Prior to inoculation, the medium should be brought to room temperature. Inoculate selected colonies of a pure 18 to 24 hour culture, or from a turbid broth culture 4-8 hours old. Using a straight needle, stab the center of the medium about 1/4" from the top. Incubate the tubes with the caps loose at 35⁰C (see "Limitations") for 18-24 hours. Observe for motility.

2. Plate Method

If using a multipoint inoculation system, make a pour plate from the 18 ml tube by gently melting the agar in a boiling water bath and dispensing the liquid medium into a sterile petri dish. Prepare the inoculum by touching the top of one or two well isolated colonies and inoculating into a broth. Stab the inoculum into the medium using the modified pins of a replicator or by using a straight needle. Incubate aerobically at 35⁰C (see "Limitations") for 16-18 hours. Examine for the presence of a pink diffusion from the point of inoculation.

Interpretation

Positive: A diffuse pink color occurring throughout the medium.
 Negative: A pinkish red line at the stab site with no diffusion.

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Quality Control

Test the following positive and negative control organisms each time the test is performed:

Positive: *Escherichia coli* (ATCC 25922)
 Negative: *Klebsiella pneumoniae* (ATCC 13883)

References

1. Finegold, S.M., and E. J. Baron, Bailey and Scott's Diagnostic Microbiology, 7th ed., C.V. Mosby, St. Louis, 1986. Koneman, E.W., et al., Color Atlas and Textbook of Diagnostic Microbiology, J.B. Lippincott, Philadelphia, 1979. Lennette, E.H., et al., Manual of Clinical Microbiology, 4th ed., American Society for Microbiology, Washington, D.C., 1985.
2. MacFaddin, J.F., Biochemical Tests for Identification of Medical Bacteria, Williams and Wilkins, Baltimore, 1980. Tittsler R.P., and L.A. Sandhoizer, J. Bacteriol., 31:575, 1936.