MOTILITY B MEDIUM w/ and w/o TTC

INTENDED USE
Remel Motility B Medium w/ and w/o TTC are semisolid media recommended for use in qualitative procedures to demonstrate motility, primarily in nonfermentative, gram-negative bacilli.

SUMMARY AND EXPLANATION
Nonfermentative gram-negative bacilli, such as pseudomonads, require oxygen for optimal growth.[1] Motility B Medium was formulated by Gilardi with a reduced agar concentration to facilitate the appearance of motility by such organisms.[2,3] The Gard plate technique consists of Motility B Medium dispensed into a petri dish and allowed to solidify.[4] It is used to select a motile strain from a population of cells which otherwise appears nonmotile. Kelly and Fulton recommend the addition of 2,3,5-triphenyltetrazolium chloride (TTC) to enhance the macroscopic appearance of motile organisms in motility media.[5]

PRINCIPLE
Casein peptone and yeast extract supply amino acids, peptides and vitamins to promote the growth of nonfermentative gram-negative bacilli. Sodium chloride provides essential electrolytes and maintains osmotic equilibrium. Agar provides a semisolid medium which makes motility interpretations macroscopic. TTC is incorporated in the medium to facilitate interpretation of results. Tetrazolium salt is colorless, but as the organism grows the dye is incorporated into the bacterial cells and is reduced to an insoluble red pigment, formazan. The red color forms only in the area where the bacteria are growing.

REAGENTS (CLASSICAL FORMULAE)*
Casein Peptone	10.0 g	Yeast Extract	3.0 g
Sodium Chloride	5.0 g	Agar	3.0 g
		Demineralized Water	1000.0 ml

pH 7.2 ± 0.2 @ 25°C

The following optional ingredient is available per liter of medium:
2,3,5-Triphenyltetrazolium Chloride (TTC) ..... 0.05 g

*Adjusted as required to meet performance standards.

PROCEDURE
Note: Due to their semisolid nature, the surface tension of these media may be broken during transport. If media is liquefied upon receipt or after storage, gently heat in a boiling water bath with caps loosened and allow media to cool and re-solidify in an upright position prior to inoculation.

1. Using a pure, 18-24 hour culture of the test isolate, inoculate Motility B Medium with an inoculating needle by stabbing down the center of the medium.
2. If using Motility B Medium w/ TTC, incubate an uninoculated tube of Motility B Medium w/ TTC simultaneously for use as a control. The control tube must remain clear and colorless for the test to be valid.
3. Incubate tubes aerobically at room temperature for 24-48 hours. Because some flagellar proteins are not synthesized at higher temperatures, the optimal incubation temperature for pseudomonads is 20-25°C.
4. If negative at 48 hours, continue incubation for 5 days.

Note: Because some organisms fail to grow deep in semisolid media, the use of pour plates (Gard technique) may provide more accurate results. Consult appropriate references for further instructions.[3,4]

INTERPRETATION OF THE TEST
Positive Test - Diffuse growth extending away from the stab line; cloudiness or turbidity may be observed throughout the medium
Negative Test - Growth confined to the stab line

Note: If TTC is present in the medium, a red color is present in the area of growth.

QUALITY CONTROL
All lot numbers of Motility B Medium w/ and w/o TTC have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Motility B Medium:		
Pseudomonas aeruginosa ATCC® 27853	Aerobic, 18-24 h @ 33-37°C	Growth with spreading away from the stab line
Acinetobacter baumannii ATCC® 19606	Aerobic, 18-24 h @ 33-37°C	Growth confined to the stab line
Motility B Medium w/ TTC:		
Pseudomonas aeruginosa ATCC® 27853	Aerobic, 18-24 h @ 33-37°C	Red color with growth spreading away from the stab line
Acinetobacter baumannii ATCC® 19606	Aerobic, 18-24 h @ 33-37°C	Red color with growth confined to the stab line

(Continued on back)

LIMITATIONS

1. Confirm weak or equivocal motility results by flagellum stain or wet mount microscopy (hanging drop).[6,7]
2. Motile organisms kept as stock cultures on artificial medium over a long period of time tend to lose their motility.[6]
3. When using Motility B Medium w/o TTC, an uninoculated tube of Motility B Medium w/o TTC can be used for comparison with the test isolate tube to facilitate interpretation of results.[6]
4. Nonenteric gram-negative bacilli may grow at 37°C; however, flagellar proteins are optimally synthesized at lower temperatures. Ideally, Motility B Medium should be incubated at room temperature.[3]
5. Tetrazolium salts may be inhibitory to some fastidious organisms.[7]
6. Heat exposure may render organisms nonmotile.[6]

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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