
MOTILITY TEST MEDIUM w/ and w/o TTC

INTENDED USE

Remel Motility Test Medium w/ and w/o TTC are semisolid media recommended for use in qualitative procedures to demonstrate motility in microorganisms.

SUMMARY AND EXPLANATION

This medium is a modification of the formulation of Tittsler and Sandholzer for detection of motility in enteric gram-negative bacilli.¹ They compared the results of motility determinations in hanging-drop preparations and semi-solid agar and obtained perfect correlation.² Kelly and Fulton recommended the addition of a tetrazolium salt to enhance the macroscopic appearance of motile organisms in motility media.³

PRINCIPLE

Peptones and beef extract supply vitamins and nitrogenous compounds essential for bacterial growth. Sodium chloride provides essential electrolytes and maintains osmotic equilibrium. Agar is added to the medium making it semisolid which enables macroscopic motility interpretation. TTC (2,3,5,-triphenyltetrazolium chloride) is incorporated in the medium to add visual enhancement of bacterial growth. Tetrazolium salt is colorless, but as the organism grows the dye is incorporated into the bacterial cells and reduced to an insoluble red pigment, formazan. The red color forms only in the area where the bacteria are growing.

REAGENTS (CLASSICAL FORMULAE)*

Gelatin Peptone	10.0 g	Beef Extract	3.0 g
Sodium Chloride.....	5.0 g	Agar.....	4.0 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, gently heat in a boiling water bath with caps loosened. 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 Test Medium with an inoculating needle by stabbing down the center of the medium to a depth of one-half inch.
2. If using Motility Test Medium w/ TTC, incubate an uninoculated tube of Motility Test Medium w/ TTC simultaneously as a control. The control tube must remain colorless and clear for the test to be valid.
3. Incubate aerobically at room temperature or at 33-37°C for 24-48 hours. If an organism is suspected of demonstrating motility at a lower temperature, inoculate two tubes simultaneously and incubate at 33-37°C and room temperature for 5 days. (The flagellar proteins of some bacteria are not synthesized at higher temperatures and require incubation at or near room temperature to demonstrate motility.³)

INTERPRETATION OF THE TEST

Positive Test - Diffuse growth extending away from the stab line of inoculation; cloudiness, turbidity, or feathery protuberances may be observed extending laterally

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 Test 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

Motility Test Medium:

Listeria monocytogenes ATCC® 7646
Enterobacter aerogenes ATCC® 13048
Klebsiella pneumoniae ATCC® 27736

INCUBATION

Aerobic, up to 48 h @ 33-37°C
Aerobic, up to 48 h @ 33-37°C
Aerobic, up to 48 h @ 33-37°C

RESULTS

Growth in the shape of an umbrella
Growth diffused throughout the media
Growth confined to the stab line

Motility Test Medium w/ TTC:

Enterobacter aerogenes ATCC® 13048
Klebsiella pneumoniae ATCC® 27736

Aerobic, 18-24 h @ 33-37°C
Aerobic, 18-24 h @ 33-37°C

Red color with growth spreading away from the stab line
Red color with growth confined to the stab line

LIMITATIONS

1. Confirm weak or equivocal motility results by flagellum stain or by wet mount microscopy (hanging drop).^{4,5}
2. Motile organisms kept as stock cultures on artificial media over long periods of time tend to lose their motility.⁴
3. When using Motility Test Medium w/o TTC, an uninoculated tube of media can be used for comparison with the test isolate tube to facilitate interpretation of equivocal results.⁴
4. Tetrazolium salts may be inhibitory to some fastidious organisms.⁵
5. Heat exposure may render organisms nonmotile.⁴

BIBLIOGRAPHY

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5. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.
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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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IFU 61408, Revised June 2, 2008

Printed in U.S.A.

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