IFU

HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

MOTILITY TEST MEDIA

Cat. no. Q10	Motility Test Medium, 16x100mm Tube, 8ml Deep	20 tubes/box
Cat. no. Q11	Motility Test Medium with TTC, 16x100mm Tube, 8ml Deep	20 tubes/box

INTENDED USE

Motility Test Medium with and without Triphenyltetrazolium Chloride (TTC) is recommended for the detection of motility in gram-negative enteric bacilli. TTC is added to enhance visualization of bacterial growth.

SUMMARY

Hardy Diagnostics Motility Test Medium is a modification of the formula employed by Tittsler and Sandholzer.[6] Small amounts of agar and gelatin are added to provide a semi-solid medium which allows macroscopic examination of bacterial motility.

Organisms are stabbed into the medium which is then incubated for a 24 hour period. Motile organisms extend from the stab line and produce turbidity or cloudiness throughout the medium. Non-motile organisms grow only along the stab line and leave the surrounding medium clear.

Motility Test Medium with Triphenyltetrazolium Chloride (TTC) is used in the same manner, however, TTC adds visual enhancement of bacterial growth. TTC is a colorless dye that, when reduced by bacterial cells, produces formazan, an insoluble red pigment.[7] The red color forms only in the area of bacterial growth. Motile organisms produce a pink color that diffuses from the stab line. Organisms that are non-motile produce a pinkish-red pigment that is confined to the stab line.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Gelatin	10.0gm
Sodium Chloride	5.0gm
Beef Extract	3.0gm
Agar	4.0gm

In addition, Motility Test Medium with TTC contains:

Triphenyltetrazolium Chloride	0.5gm

Final pH 7.3 +/- 0.3 at 25ºC.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-30ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Specimen collection is not applicable since this medium is not intended for primary isolation from clinical specimens. As a general rule, infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation. Consult listed references for information on specimen collection.[1-5]

Method of Use:

1. Using isolated colonies from an 18-24 hour culture, inoculate the medium by stabbing the center of the medium to a depth of 1/2 inch.

2. Incubate the inoculated medium aerobically at 35ºC. for 18-24 hours.

3. If using Motility Test Medium, observe for a diffuse zone of growth flaring out from the line of inoculation. If using Motility Test Medium with TTC, observe for a pink color diffusing from the line of inoculation.
Note: When using Motility Test Medium with TTC, an uninoculated control must be incubated and must remain clear and colorless in order to consider the test valid.

INTERPRETATION OF RESULTS

Motility Test Medium
A positive motility test is indicated by a diffuse zone of growth flaring from the line of inoculation.
A negative motility test is indicated by growth confined to the stab line.

Motility Test Medium with TTC
A positive motility test is indicated by a pink color diffusing from the line of inoculation.
A negative motility test is indicated by a pinkish-red line that is confined to the stab line.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Media containing TTC may develop a pinkish-red diffusion within the medium when stored and/or incubated exposed to light. For best results store and incubate this medium in the dark.

False-negative reactions may occur if bacterial flagella are damaged due to heating, shaking, or other trauma. Such environmental shock will render the organism non-motile.

Organisms which are weakly motile may result in false-negative reactions.

Some microorganisms do not produce flagellar proteins at 35-37 degrees C. If such an organism is suspected, two tubes should be inoculated simultaneously. One tube should be incubated at 22-25 degrees C. for 5 days and the other at 35-37 degrees C. for 5 days.

When inoculating semi-solid media, it is important that the inoculating needle be removed along the exact same line used to inoculate the medium. A fanning motion may result in growth along the stab line that may result in false-positive interpretation.

TTC may be inhibitory to certain fastidious bacteria.

When using Motility Test Medium with TTC, an uninoculated control must be incubated and must remain clear and colorless in order to consider the test valid.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Motility Test Medium:					
Escherichia coli					Positive motility: growth

ATCC® 25922**	D	24-48hr	35°C	Aerobic	spreading from line of stab
Klebsiella pneumoniae ATCC® 13883**	D	24-48hr	35°C	Aerobic	Negative motility: growth confined to line of stab
Motility Test Medium with TTC:					
Escherichia coli ATCC® 25922**	D	24-48hr	35°C	Aerobic	Positive motility: pinkish-red pigment extends from line of stab
Klebsiella pneumoniae ATCC® 13883**	D	24-48hr	35°C	Aerobic	Negative motility: red pigment is confined to line of stab

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

USER QUALITY CONTROL

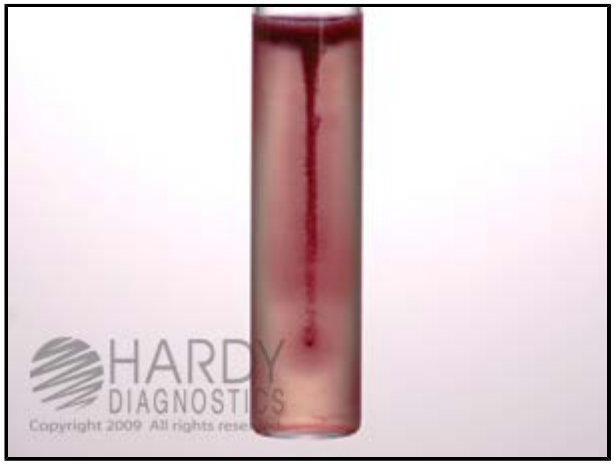
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

- Motility Test Medium is a semi-solid deep that should appear slightly opalescent, and light amber in color.
- Motility Test Medium with TTC is a semi-solid deep that should appear slightly opalescent, and whitish in color.

Escherichia coli (ATCC® 25922) growing in Motility Test Medium (Cat. no. Q10). Incubated aerobically for 24 hours at 35ºC. Showing positive motility.

Klebsiella pneumoniae (ATCC® 13883) growing in Motility Test Medium (Cat. no. Q10). Incubated aerobically for 24 hours at 35ºC. Showing negative motility.



Escherichia coli (ATCC® 25922) growing in Motility Test Medium with TTC (Cat. no. Q11). Incubated aerobically for 24 hours at 35ºC. Showing positive motility.

Klebsiella pneumoniae (ATCC® 13883) growing in Motility Test Medium with TTC (Cat. no. Q11). Incubated aerobically for 24 hours at 35ºC. Showing negative motility.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

5. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

6. Tittsler, R.P. and Sandholzer, J.A. 1936. *J. Bacteriol.*; 31:575.

7. Kelly, A.T. and Fulton, M. 1953. *Am. J. Clin. Pathol.*; 23:512.

ATCC is a registered trademark of the American Type Culture Collection.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

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