



## MOTILITY AGAR (TTC)

- For in vitro use only -

Catalogue No. TM80

Our Motility Agar (TTC) is used for the detection of bacterial motility. This characteristic is useful for differentiating among *Enterobacteriaceae* and between *Yersinia* species.

Flagella are the complex whip-like structures responsible for bacterial motility. Bacterial motility can play a key component in bacterial survival as well as their ability to cause disease. Motility Agar is a semi-solid medium that elicits growth, and allows motile organisms to move about the medium.

Kelly and Fulton reformulated motility agar to include the colorless dye, 2,3,5-triphenyltetrazolium chloride (TTC). TTC can be reduced by bacteria to form formazan, an insoluble red pigment. Therefore bacterial growth in the medium appears pink or red and serves as a visual aid in the detection of bacterial motility.

### Formula per Litre of Medium

Pancreatic Digest of Gelatin.....	10.0 g
Sodium Chloride.....	5.0 g
Beef Extract .....	3.0 g
Agar.....	3.5 g
TTC.....	0.05 g

pH 7.3 ± 0.2

### Recommended Procedure

1. Obtain a fresh, overnight, pure culture of the test organism.
2. Inoculate the tube using a sterile inoculating needle and stab the centre of the medium approximately half way down the length of the tube. **Do not stab through to the bottom of the tube.**
3. Incubate aerobically at 35°C with the cap loose. Incubate an uninoculated tube as a negative control.

4. Check after 24 and 48 hours.
5. If negative, incubate at room temperature for up to 5 days.

### Interpretation of Results

Motile organisms are able to move about the semi-solid medium resulting in a pink or red diffuse growth throughout the medium.

Non-motile organisms will remain isolated at the inoculation point and will appear as pink or red growth along the stab line. Weakly motile organisms, such as *Enterococcus gallinarum*, will show only a small diffusion of growth from the stab line making interpretation difficult.

The uninoculated control tube is used to assess the stability of TTC as well as the sterility of the completed medium. After the incubation period the control tube should remain colorless and clear.

- *Strict aerobes, such as Pseudomonas aeruginosa, may only grow on the surface of the medium and not at deeper levels along the stab*
- *TTC may be inhibitory to some fastidious organisms*
- *Weakly motile organisms may give false negative results. All weak or equivocal motility results should be confirmed by a flagellum stain or by direct wet microscopy (hanging drop)*
- *Flagella may be destroyed or damaged if a bacterial culture tube is shaken violently and will result in false-negative results*
- *Some filamentous organisms may spread through the medium but are non-motile*

- Some flagellar proteins are not synthesized at higher temperatures. Also, some bacteria are motile at one temperature and non-motile when cultivated at another. *Yersinia enterocolitica* develops flagellar proteins at room temperature but not at 35°C
- *Listeria*, *Yersinia*, and *Corynebacterium* species require room temperature incubation. If it is an unknown pure culture, incubate as instructed since the 5 day RT incubation period should recover all motile species

### Quality Control

After checking the medium for correct pH, colour, depth, and sterility, the following organisms are used to determine the performance of the completed medium.

<u>Organism</u>	<u>Expected results</u>
<i>Escherichia coli</i> ATCC 25922	+ve Pink / red color throughout the medium
<i>Klebsiella pneumoniae</i> ATCC 33495	-ve Pink / red growth along the stab line with no diffusion of colour

### Storage and Shelf Life

Our Motility Agar (TTC) should be stored in an upright position at 4°C to 8°C. Under these conditions the medium has a shelf life of 26 weeks from the date of manufacture.

### References

1. Tittsler RP, Sandholzer JA. The use of semi-solid agar for the detection of bacterial motility. J Bacteriol 1936; 31:575-80.
2. Kelly AT, Fulton M. Use of triphenyl tetrazolium chloride in motility test medium. Am J Clin Path 1953; 23:512.
3. D'Amato RF, Tomfohrde KM. Influence of media on temperature-dependent motility test for *Yersinia enterocolitica*. J Clin Micro 1981; 14:347-8.
4. MacFaddin JF. Media for isolation-cultivation-maintenance of medical bacteria, Vol I. Baltimore: Williams & Wilkins, 1985.
5. Ewing WH. Edward's and Ewing's identification of *Enterobacteriaceae*. 4<sup>th</sup> ed. New York: Elsevier, 1986.
6. MacFaddin, JF. Biochemical tests for the identification of medical bacteria. 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2000.

Original: Dec 2001

Revised / Reviewed: January 2014