Motility Wet Mount Test

Purpose

This procedure provides instructions for performing the Motility Wet Mount Test.

Principal and Clinical Significance

The Motility is used to detect the presence of flagella on bacteria, allowing them to travel in and out of the microscopic field. For the wet mount, a light inoculum if an organism in a drop of broth is suspended on a clean glass slide, a coverslip is added and he culture is observed microscopically for motility. Occasionally, the organism is incubated in the broth prior to examination.

Policy Statements

This procedure applies to Microbiologists who perform culture plate reading.

Test Code

MOT

Materials

	Reagents	Supplies
	• TSB (trypticase soy broth) for Bacillus spp. • BHI (brain hear infusion) • Saline for Gram negative organisms	• Sterile Loop or applicator stick • Disposable sterile pipette • Glass slide • KOVA slide • Coverslip

Specimen

Acceptable specimens:

- Bacterial growth less than 24 hours old from SB agar.

Special Safety Precautions

Microbiologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Microbiology Procedure Manual***.**

- *Biohazard Containment*
- *Biohazardous Spills*
- *Safety in the Microbiology Laboratory*

Quality Control

- **Positive Control:** *Escherichia coli ATCC 25922*
- **Negative Control:** *Klebsiella pneumoniae ATCC 700603*
- Perform QC with each day of testing. Record results on Desk 2 QC chart.
- If there is a QC failure, document observation and corrective action. Report QC problems that cannot be resolved to the microbiology technical specialist.

Procedure

1. With a loop or applicator stick, carefully remove a fresh colony from the agar and transfer to labeled TSB or BHI broth tube. Use a light inoculum.
2. Using the sterile pipette, remove a small portion and place a single drop on the glass slide. Place coverslip over the drop. Allow to settle for a minute.
3. Or, transfer a drop to the KOVA slide.
4. Observe slide using 40X or 100X oil objective on the microscope and observe motility of the organism. Close the diaphragm to decrease the light.
5. Directional motility is recorded as a positive result indicating a motile organism.

6. Organisms that do not change position in respect to each other is a negative result and indicate a non-motile organism.
7. If the test is negative, incubate the broth at 35ºC or room temperature depending on the organism for 18-24 hours and repeat the test.
8. Discard slide into sharps container.

Interpretation

- **Positive**: Directional movement of the organism
  - Examples: *Bacillus* species, *Escherichia coli, Pseudomonas aeruginosa*
  .
- **Negative**: No Directional movement of the organism
  - Examples: *B. anthracis, Acinetobacter species, Klebsiella species, Streptococcus species*

Limitations

1. Bacillus species are best tested directly from a fresh plate. If a fresh plate is not available, inoculate a plate and incubate for 4 hours. Then perform the test.
2. Excessive heat on the slide can affect results
3. False-negative reactions can occur if bacterial flagella are damaged due to heating, shaking, or other trauma.
4. Some organisms do not produce flagellar proteins at 35-37ºC but do so at 22ºC.

Result Reporting

1. Record results in Sunquest MRE in the Culture Entry tab and Workup section. An example is as follows:

Workups: Workup # 1
Med : SB
Desc: ;GROUND GLASS
Id: BACI

Workup Components
GMS:GPR, LARGE, BOXY
CAT :POS
MOT: POS
SC : SB

References

1. Leber, Amy. *Clinical Microbiology Procedures Handbook*, 4th edition. Vol. 1-3 (16.15.5). 2016. American Society for Microbiology, Washington D.C., 20036.
2. CDC Center for Disease Control and Prevention Basic Diagnostic Testing for the presumptive identification of Bacillus anthracis. 3/18/2022

Training Plan/ Competency Assessment

Training Plan	Initial Competency Assessment
1. Employee must read the procedure. 2. Employee will observe trainer performing the procedure. 3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.	1. Direct observation.

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1.0	Susan DeMeyere	6/1/2024	Initial Version