**PURPOSE:** The aerotolerance test is used to designate an organism as an anaerobe.

## SAMPLE INFORMATION:

| | |
|---|---|
| **Type** | Organisms growing on anaerobic culture media and not present on aerobic culture media. |

## REAGENTS and/or MEDIA:

- Chocolate agar
- Brucella agar

## SUPPLIES:

- Disposable inoculation needles
- Glass microscope slides
- Anaerobic trays or jars
- Anaerobic packs and indicators
- 35° ambient air and 37° $CO_2$ incubators

## SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

## QUALITY CONTROL:

- Refer to MIC60010 – Microbiology Quality Control for anaerobic tray and jar quality control.

## PROCEDURE INSTRUCTIONS:

| Step | Action |
|---|---|
| **Performing the aerotolerance test** | |
| 1 | Subculture a single, well-isolated colony of each morphological type suspected to be an anaerobe. Using one colony ensures that the same organism goes onto both agar plates and the slide. |
| 2 | Touch each colony with a loop or sterile stick and subculture to Chocolate agar, Brucella agar and a labelled, glass microscope slide. |
| 3 | The Chocolate agar should be inoculated first, so that if only the Brucella agar plate grows there is no question of not having enough organisms to initiate growth. |
| 4 | Incubate the Chocolate agar in $CO_2$ incubator for 24 hours. If no growth is seen, re-incubate for an additional 24 hours. |
| 5 | Incubate the Brucella agar in anaerobic jar or tray for 48 hours along with original anaerobic media. |

## INTERPRETATION OF RESULTS:

| IF | THEN |
|---|---|
| Isolate grows on Chocolate agar at 24 or 48 hours<br>**AND**<br>Isolate grows on Brucella agar at 24 or 48 hours. | Organism is not a strict anaerobe and can be identified using routine methods. |
| Isolate does not grow on Chocolate agar at 24 or 48 hours<br>**AND**<br>Isolate does grow on Brucella agar at 24 or 48 hours. | Organism is a strict anaerobe. |
| Isolate does not grow on Chocolate agar at 24 or 48 hours<br>**AND**<br>Isolate does not grow on Brucella agar at 24 or 48 hours. | Test is inconclusive:<br>• Reasons: organism died or media not inoculated.<br>• Repeat aerotolerance testing with colony on original anaerobic media. |

## LIMITATIONS:

1. Chocolate agar should be used for aerotolerance testing. *Haemophilus* spp. will grow anaerobically on Brucella agar and therefore will be mistaken for anaerobic Gram-negative rods if Chocolate agar is not used.
2. Some authorities have suggested performing aerotolerance testing in multiple environments (ambient air, $CO_2$, a microaerophilic environment) on problem isolates. This is not necessary for identification of the most commonly isolated anaerobes, but it may be considered for isolates when their exact atmospheric requirement is difficult to determine using the above procedure.
3. If anaerobic organism processing occurs on the open bench, all plates should be promptly incubated anaerobically as some clinical isolates may die after relatively short exposure to oxygen.

## REFERENCES:

- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016.
- Jorgensen J.H., Pfaller M.A., Carroll K.C., Funke G., Landry M.L., Richter S.S., Warnock D.W. 2015. Manual of Clinical Microbiology, 11th edition, ASM Press, Washington, D.C.

## REVISION HISTORY:

| REVISION | DATE | Description of Change | REQUESTED BY |
|---|---|---|---|
| 1.0 | 23 MAR 19 | Initial Release | L. Steven |
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