PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC53700 – Aerotolerance Test	Policy Number:
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s):	
Effective Date:	Effective Date:
Issuing Authority: Director, Health Services	Date Approved:
Accreditation Canada Applicable Standard: N/A	

GUIDING PRINCIPLE:
The aerotolerance test is used to designate an organism as an anaerobe.

PURPOSE/RATIONALE:
This standard operating procedure describes how to perform the aerotolerance test.

SCOPE/APPLICABILITY:
This procedure applies to Medical Laboratory Technologists (MLTs) performing the aerotolerance test.

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

EQUIPMENT
- 35° ambient air and 37° CO_2 incubators

SPECIAL SAFETY PRECAUTIONS:

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

- Ensure that appropriate hand hygiene practices be used.
- 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 of 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. Routine Practices 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 **AND** 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 **AND** 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 **AND** Isolate does not grow on Brucella agar at 24 or 48 hours	Test is inconclusive: • Reasons: organism did not survive, or media not inoculated • 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.

CROSS-REFERENCES:

- MIC60010-Microbiology Quality Control

REFERENCES:

1. Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016

APPROVAL:

_____
Date

_____

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	23 Mar 17	Initial Release	L. Steven
2.0	30 Jun 21	Procedure reviewed and added to NTHSSA policy template	L. Steven