TITLE: Preparation of Fluid Samples for Mycobacteria Culture	**Revision Date:** 07-April-2017	**Issue Date:** 07-April-2015
Document Number: MIC80300	**Status: Approved**	
Distribution: Mycobacteria Manual	**Page: 1 of 5**	
Approved by: Gloria Badari, Director, Corporate Services and Chief Financial Officer	**Signed by: (Original Signed Copy in Microbiology)**	

PURPOSE:

To standardize the pre-processing steps required for processing and culture of Fluids for Mycobacteria.

INTRODUCTION:

All fluids for Mycobacteria culture and direct smear prep require centrifugation if greater than a certain volume.

Centrifugation should be done before sample processing (decontamination) to speed up processing times. Ideally centrifugation should be done immediately after sample receipt and accessioning. Pre-processed samples (centrifuged samples) should be held in the bacteriology sample fridge for the next available Mycobacteria run.

Fluids from a body cavity such as pleural, CSF, synovial for example, are considered “sterile” and should have a sterility plate streaked upon sample receipt and accessioning. Since NaOH can be harmful to Mycobacteria, its use can be minimized or eliminated in samples that do not normally have superficial contamination. Checking C&S results or performing a sterility check limits unnecessary NaOH exposure, therefore increasing recovery of potential Mycobacteria in the sample.

SPECIAL SAFETY PRECAUTIONS:

- Handle all patient samples and testing reagent using “Routine Practices”

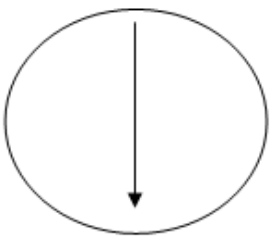
- Please refer to the Northwest Territories Infection Prevention and Control Manual, March 2012
- Prior to testing all patient are to be identified as per I-0500 Use of Two Patient Identifiers.

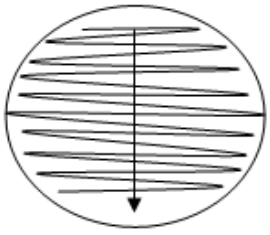
PRE-PROCESSING OF STERILE FLUIDS

The sterility plate is streaked out like a Urine culture and evaluated for growth the next day. If any bacterial growth is seen, the fluid receives digestion in NaOH equivalent to the degree of contamination seen in culture. If a C&S was ordered then a sterility plate is not required. The fluid C&S culture results should be checked before Mycobacteria processing. Sterile fluids should be concentrated by centrifugation before the sterility plate or C&S culture. The decision to centrifuge is based on the volume of sample received. Since centrifugation produces heat, and mild heat can kill Mycobacteria, use the **"TB" pre-programmed setting #1 on the Allegra to centrifuge any fluids for Mycobacteria culture (spun at 4° Celsius).**

STERILITY PLATE:

- In the Bacteriology BSC, on a labeled blood agar plate, streak out sample like a urine with a blue 0.1 uL loop.
- Streak downwards and then across in a zigzag pattern with the same loop. Discard loop in the Yellow Biohazard bucket.





- Incubate plate in CO2 for 16-24 hours. Check plate for growth. Interpret growth and adjust processing times according to the chart below.

Notes about specimen processing for Sterile Fluids:

- *Typically a C&S will be done the day before; examine for growth on plate.*
- *If a culture was not ordered, and if > 2.5 mL fluid received, a BA plate should have been inoculated for a sterility check to test for contamination.*
- *If >2.5 mL fluid received → pour into a labeled FALCON conical centrifuge tube and concentrate by centrifugation.*
- *Decant supernatant into a separate labeled conical tube or the original specimen container, leaving 2.5 mL fluid behind. Vortex the pellet in the remaining supernatant. Fluid is ready for processing.*
- *Volumes of sterile fluids < 2.5 mL should NOT be centrifuged or decontaminated with 3% NaOH and a sterility plate is not done.*

Follow the steps below to process sterile fluids for AFB smear and culture:

Action

If:	Then:
No Growth on C&S or sterility plate **<2.5 mL fluid**	**DO:** • Directly inoculate into MGIT and LJ • Prepare a direct smear. **DON'T**: • Do not decontaminate with 3% NaOH
<0.5 mL fluid	**DO:** • Directly inoculate MGIT tube. **DON'T**: • Do not inoculate LJ slant. • Do not make a direct smear. • Do not prepare a sterility plate (if a C&S was not done).
Growth	Note the quantity of bacterial contamination. In a conical FALCON centrifuge tube, digest the fluid. Adjust specimen processing time according to growth amounts below:

Sterility plate or C&S results: Processing adjustment chart

	Growth amount:	Digest for:
	1 + (1/3rd growth) C&S = scant/light growth	**5 minutes.** Process the fluid sample 10 minutes after the start of digestion of the other sputum samples.
	2+ (½ growth) C&S = mod growth	**10 minutes.** Process the fluid sample 5 minutes after the start of digestion of the other sputum samples.
	3+ or 4+ (¾ →full plate) C&S = heavy growth	**15 minutes.** Process the fluid sample at the same time as the other sputum samples.

PROCESSING OF RESPIRATORY SAMPLES, URINES & GASTRIC WASHINGS:

Samples from these sources typically contain contaminating bacteria and a sterility check is not required; it is assumed they contain superficial contamination. However these samples should be concentrated by centrifugation. The decision to centrifuge is based on the volume of sample received.

Notes about specimen processing unsterile fluids:
• *If volume > 2.5 mL and > 10mL for urines, transfer into a labeled conical FALCON centrifuge and concentrate by centrifugation.* • *Decant supernatant into a separate conical tube or the original specimen container, leaving 2.5 mL fluid behind. Vortex the pellet in the remaining supernatant. Fluid is ready for processing.* • *Do not centrifuge if volume < 2.5 mL*

- *Respiratory samples between 0.5 → 2.5mL volume: Add sterile water to samples n to make a total volume of 2.5 mL (equals the volume of 3% NaOH digestant aliquot).*
- *Urines <10 mL and Gastric washings < 2.5 mL and Respiratory samples < 0.5 mL are NSQ and are rejected at these low volumes (except aseptically collected urines).*

RELATED DOCUMENTS:

- MIC80200 Mycobacteria Sample Receipt & Accessioning
- MIC80400 Mycobacteria Processing
- MIC80100 Mycobacteria Sample Collection Guide

REFERENCES:

- Prov Lab Mycobacteria Manual. Job Shadow December 2014
- Central Public Health Laboratory. (2003). *Mycobacteriology Bench Manual.* Ottawa.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	19-Jan- 2015	Initial Release	L. Driedger
	03Feb2015	Review	S. Webber