TITLE: Mycobacteria Media Inoculation & Direct Smear Preparation	**Revision Date:** 07-April-2017	**Issue Date:** 07-April-2015
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Distribution: Mycobacteria Manual	**Page: 1 of 5**	
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PURPOSE:

To standardize the inoculation of Mycobacteria culture media and the preparation of Direct Smears.

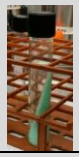
SPECIAL SAFETY PRECAUTIONS:

- 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.

SUPPLIES:

- 50 mL conical FALCON tubes
- 5 ml unsterile Pasteur pipettes
- 1 mL graduated Transfer pipettes, individually wrapped, sterile
- Yellow Waste bucket (1/3rd full with fresh Accel TB)
- Vortex mixer
- Alcohol wipes
- Clean frosted glass slides
- 5% phenol alcohol
- Pencil
- Paper towels
- 4x4 gauze
- 50 mL beaker

MEDIA & EQUIPMENT:

Criteria	Information
List of supplies, reagents, media and equipment required for Mycobacteria processing:	
LJ slants 	• Manufacturer: Remel • Vendor: Unipath/Oxoid • Storage: 2- 8 Celsius
MGIT 	• Storage: In-use box kept by Allegra with the lid closed to protect from light (light degrades the fluorescent compound inside tube). • Refer to PANTA document for additional informaion
MGIT 960 (analyzer) 	• Manufacturer: Becton Dickinson • Refer to Equipment document for use and maintenance
BSC (CL-2 protection) 	• Manufacturer: LABCONCO • Refer to Equipment document for use and maintenance

MEDIA INOCULATION & DIRECT SMEAR PROCEDURE:

Step	Action
Inoculate in the following order: MGIT tube → LJ → Slide **Inoculate samples and slide one sample at a time (only one sample is open).**	
1	Prepare work area: Bring labeled MGIT tube and LJ slants into BSC. Labeled slides should be sitting in the metal tray in the BSC (prepared before sample processing). Ensure the Accel TB work-surface is still moist with disinfectant from the processing steps. Place empty spare rack on work-surface and use to hold the tubes steady as they are being inoculated. Work on patient samples one-by-one on the moistened work-surface.
2	Place sample concentrate conical tube in 50mL beaker on the work surface.

	Obtain prepared MGIT and LJ and place into rack on the work-surface.  *Figure 1 (above): Work-surface area; specimen concentrate ready for culture*
3	Unwrap a sterile Transfer pipette. The same pipette can be used on MGIT and LJ cultures per one patient sample.
4	Unscrew MGIT tube. Using your pinkie, keep cap in hand so it doesn't touch the surface of the BSC.
5	Using the graduated marks on the pipette withdraw 0.5 mL of patient sample → inoculate MGIT tube. → Place pipette into patient sample tube → Close MGIT cap with free hand.
6	Invert several times to mix. Return MGIT to the plastic rack.
7	Unscrew LJ cap. Using your pinkie, keep cap in hand so it doesn't touch the surface of the BSC.
8	With the same pipette, inoculate LJ slant by slanting the tube and dispensing two drops of patient sample onto slant → close cap LOOSELY. Return LJ slant to the plastic rack.
9	Discard pipette in yellow discard bucket with Accel TB

10	Locate slide in metal tray (leave in metal tray but know its location). With a yellow 10 uL inoculating loop prepare a smear on the pre-labeled patient slide: Dip loop inside conical tube and touch the loop on the center of the labeled slide to dispense the drop on the slide. Thin the drop out with the loop to cover a **dime sized** area. *Note: A slide for direct smear does not require a drop of albumin since there is plenty of protein in the patient sample.*
11	Discard loop in yellow discard bucket with Accel TB. Close cap on the conical centrifuge tube. Move on to next patient sample.
12	Repeat steps 1 → 9 for each sample.
13	Allow smears to completely air dry before fixing. Leave BSC sash open while drying (and fixing) for proper protective airflow. Refer to Mycobacteria Smear Fixing.
14	Wipe or spray sample concentrate tube with Accel TB before removal from BSC. Wipe or spray down inoculated MGIT and LJ's before removal from BSC. • Refer to MGIT 960 procedure for insertion into MGIT analyzer • Refer to LJ Culture Procedure for LJ slant incubation Wipe or spray down the "TB Concentrates" bag before removal from BSC. Save the concentrates in the media fridge on bottom shelf in a Labeled Biohazard Bag "TB Concentrates" or similar label for 48 hours. Direct Concentrates may be sent for PCR if Direct Smear positive, at the request of the physician.

RELATED DOCUMENTS:

- MIC 80810 Mycobacteria Smear Fixing
- MIC 81500 Auroamine-Rhodamine Stain
- MIC 81600 Kinyoun Stain

REFERENCES:

- Central Public Health Laboratory. (2003). *Mycobacteriology Bench Manual.* Ottawa.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	31-Jan-15	Initial Release	L. Driedger
	03Feb2015	Review	S. Webber