TITLE: Mycobacteria Culture Maintenance	Revision Date: 07-April-2017	Issue Date: 07-April-2015
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Distribution: Mycobacteria Manual	Page: 1 of 7	
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PURPOSE:

To standardize the maintenance of Mycobacteria cultures from patients and quality control cultures.

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.

INOCULATED MGIT AND LJ’S:

MGIT & LJ cultures (and the test tube rack), should be wiped or sprayed with Accel TB before removal from the Mycobacteria BSC. The MGITs should be placed as soon as possible into the MGIT 960 analyzer. The LJ’s are taken out of the Mycobacteria room for placement inside the Mycobacteria incubator in the Bacteriology Lab. Keep PPE on while carrying LJ’s outside room.

MGIT’s:

- Follow MGIT 960 Analyzer for guidelines on MGIT insertion into analyzer.

LJ slants:

- Exit the Mycobacteria Room and place LJ slants into the current week rack on the top incubator. Return to Mycobacteria Room and place empty test tube rack under centrifuge counter.

ONGOING CULTURE MAINTENANCE PROCEDURES:

MGIT cultures:

- Refer to MGIT 960 Analyzer document for details how to remove positive & negative vials and print Unloaded Positives and Unloaded Negative reports

Positive Vials

- The machine detects positively and alerts accordingly. Positive cultures can be heard as a steady ongoing beep.
- When the alert is heard, remove positive MGIT tube(s) from machine.
- Request a print-out of the positive vial using the MGIT screen interface.
- After the print-out has printed, press the “OK” button to clear data from machine.
- Place MGIT tube in the **top** TB incubator in the rack labeled “Positives”. Place the MGIT print-out on the incubator by attaching the paper to the door with a magnet. Record results in LIS.

7 week negative MGIT cultures

- The machine will automatically flag negative MGIT tubes after full 7 weeks incubation.
- Alarm can be heard as a single beep that shuts off as soon as it sounds.
- Batch negative removal has been set-up. Remove all the negative tubes from MGIT in one go, and set aside in a test tube rack. Unnecessary to scan the vials before removing.

- Print the Unloaded Negative report. (Refer to MGIT 960 analyzer document for details).
- The Technologist must check the **Growth Units** on the Unloaded Negatives print-out.

7 week negative MGIT cultures with **Growth Unit >0**:

- If any MGIT tubes have a growth unit > 0, perform a Kinyoun smear terminal smear and evaluate for acid fast bacilli. If AFB seen, the culture is Positive, and must be referred to Prov Lab. *Rationale:* Very low numbers of Mycobacteria may not be detected by the instrument, causing false-negative results.

Note: It is acceptable to perform smears from culture when the analyzer reads the MGIT culture as negative but GU > 0; concentration of cultures are very low, and the majority of these situations are caused by non-MtB Mycobacteria such as M. gordonae.

7 week negative MGIT cultures with **Growth Unit = 0**:

- It is not necessary to visually examine the bottom of the tube for growth. A negative MGIT at the end-of-protocol with a GU of zero is considered negative for Mycobacteria after 7 weeks incubation.
- Using a pen, check off the MGIT cultures from the Unloaded Negative Report on the TB Worksheet, or attach the UN Report to the TB Worksheet. *Reason:* Recording when the MGIT portion of the CXAFB is complete helps keep track of which LJ's to pull out of the 7 week rack to terminally result and finalize. (LJ slants should only be considered negative after the MGIT protocol is complete).
- Discard negative MGIT tube in biohazard bag for autoclaving.

Note : MGIT product inset states to perform a visual check for growth however Provincial Lab in Edmonton has performed studies and it was determined this step is unnecessary.

LJ slants:

Weekly routine: Rotate the Rack and Paperwork during the 1st AFB run of the week.

Rotation of racks → Transfer previous week's LJ rack from the **top** incubator to the **bottom** incubator. Loosely tighten caps to prevent LJ media from drying out. Mycobacteria will survive on slants with caps tightened (caps are not O2/CO2 impermeable). The current week's culture rack will be placed in the **top** incubator.

Rotation of paperwork → Transfer previous week's printouts to the next week slot ie. move the paperwork in "6 week" file to the "7 week" slot, and move the "7 week" paperwork to the Mycobacteria Room and place on corkboard (check off negative 7 week MGIT's as they come off the machine). LJ's can only be resulted as negative when the MGIT cycle is complete.

Removal of 7 week cultures:

7 week negative LJ cultures

- When the corresponding MGIT culture completes its cycle and is negative after 49 days, the LJ slant is ready for its final inspection.
- Remove the 7 week LJ rack from the bottom incubator and locate the corresponding LJ culture.
- Observe LJ slant for growth. If negative, write "LJ neg" on the TB Worksheet.
- Discard LJ slant in Biohazard bucket for autoclaving.
- CXAFB cannot be considered negative until both MGIT and LJ are complete.

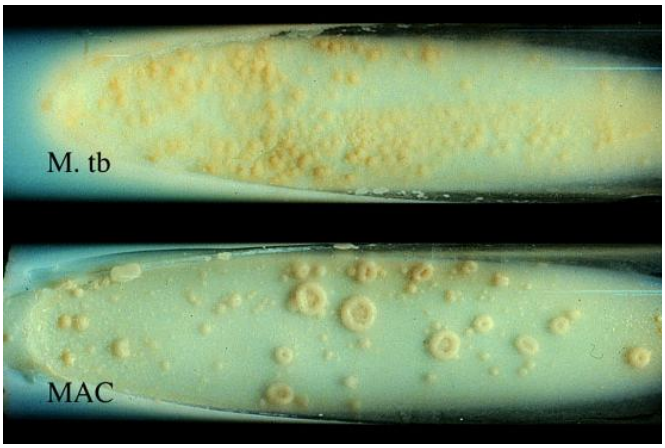
Note: The LJ culture cannot be considered negative until the MGIT has completed its length of protocol with either a GU = 0 or terminal smear is negative (if GU>0).

- Resulting: All negatives samples are resulted manually in LIS. (Refer to Mycobacteria Reporting document).

EXAMINING LJ SLANTS FOR GROWTH:

Check positivity:

"Check weeklies"→ Perform once a week at any time during the week. Observe 2-7 week old LJ slants for growth in the bottom incubator. Remove LJ slants that exhibit growth to the "Positives" rack. Check off "Checked Weeklies" in chart when done.



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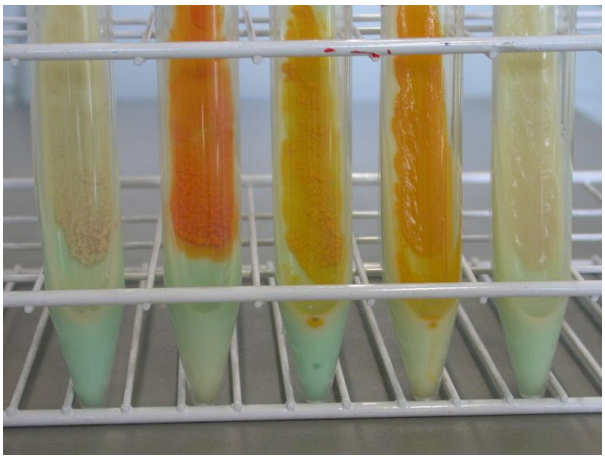

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- Follow Prov Lab's adage for the morphology of MtB: "**Rough, Tough, and Buff**". This describes typical Mycobacteria TB complex. Resembles dry **bread crumbs** or Oatmeal.

- M. avium/MAC is smooth and creamy (although still “buff” colour). Resembles **Cheerios**.
- Yellow/orange coloured growth indicates isolation of a Scotochromogen (*M. gordoni*)
- Buff colonies that become coloured if exposed to light are called Photochromogens *(M. kansasii*)
- Mycobacteria may growth rapidly (ie. *M. fortuitum)* or slowly, such as MtB.

Notes:

- LJ’s with positive growth and an accompanying positive MGIT should only have the MGIT referred to Provincial Lab.
- Keep the LJ stored at Stanton until Provincial Lab results are complete. Since opening up culture tubes is not advisable, **do not** plant a portion of the positive MGIT to a reference LJ (this is an old practice).
- Since nearly all positive MGITs have a corresponding positive LJ, the LJ slant will be our reference slant stored at Stanton for the referral in case a duplicate send-out is required.

QC Culture LJ Slants:

Every 2 weeks:

- Subculture QC isolates onto fresh LJ slants. Use pre-printed labels (located on Shared Drive). Write date of subculture on labels.
- Use a blue loop to transfer several old colonies to the fresh slant.
- Once growth is achieved on fresh slants, discard the older slants in the Biohazard bucket for autoclaving. Close caps tightly on older slants before removal from incubator and disposal.

Annually

- Aliquots of the Mycobacteria QC are frozen in glycerol beads in the -70° C freezer.

- Yearly subcultures onto LJ slants from glycerol are done to replace ageing stock.

RELATED DOCUMENTS:

- MGIT 960 Analyzer – for MGIT information.
- Mycobacteria Reporting

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	3-FEB-2015	Initial Release	L. Driedger
	03Feb2015	Review	S. Webber