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

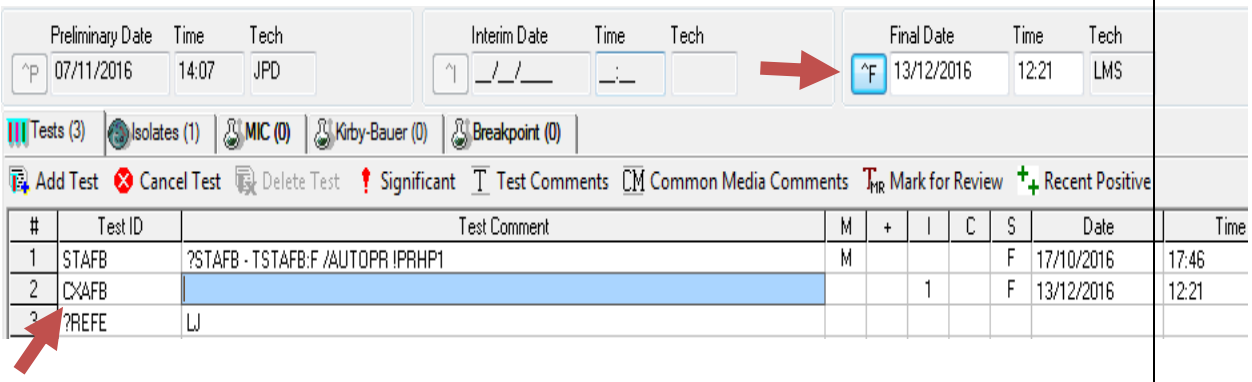
Positive Vials

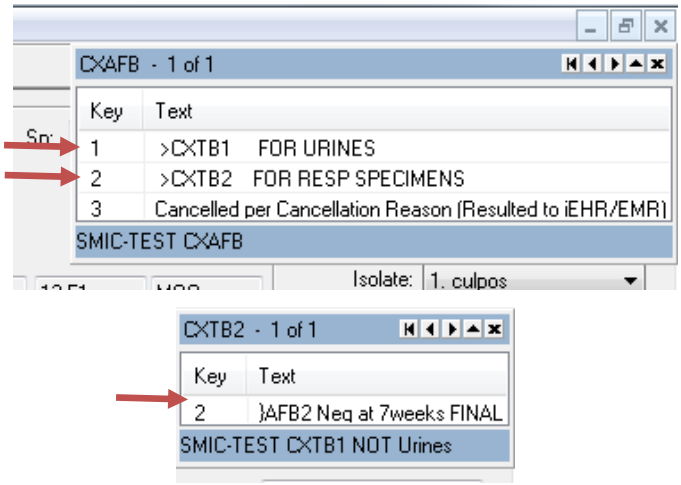
- The machine detects positively and alerts accordingly. Positive cultures can be heard as a steady ongoing beep.
- When the alert is heard, remove positive MGIT tube(s) from machine.
- Request a print-out of the positive vial using the MGIT screen interface.
- After the print-out has printed, press 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 discard.

LJ slants:

Maintaining LJ slants	
1	Rotate LJ racks in CO_2 incubator: 1. Week 7 → Week 8 2. Week 6 → Week 7 3. Week 5 → Week 6 4. Week 4 → Week 5 5. Week 3 → Week 4 6. Week 2 → Week 3 7. Week 1 → Week 2 8. Current week → Week 1 **Please Note:** If there is no Current Week LJ rack in the incubator (because TB was not performed previous week) still insert a rack into the Week One spot in order to easily rotate racks for the following weeks.
2	Result Week Eight rack: 1. Remove Week Eight LJ rack from the incubator 2. Scan tube 3. Check LJ tube to make sure no suspicious growth is present 4. If negative, no need to enter in any observations for tube 5. Ensure MGIT is negative and positive MGIT tube has not been sent out. 6. If MGIT was positive and sent out, finalize **CXAFB** (don't need to add any information, just finalize blank) 

	7. IF MGIT was negative (it should be finalized at this time), click on **>CXTB1** or **>CXTB2** (depending on specimen type) then select **{AFB2 Neg at 7weeks FINAL**  8. Finalize specimen.
3	<u>Record weekly observations for LJ tubes:</u> 1. Remove Week One rack from CO2 incubator and bring to computer. 2. Check all the tubes for any suspicious growth. Put these tubes aside. 3. Open up AFB/Fungus Worklist under Results tab in SoftMic. 4. Enter in results for tubes with suspicious growth as either Growth not typical of Mycobacterium species or as positive and proceed appropriately. 5. Refresh screen so these specimens are no longer marked as will be doing batch reporting next. 6. Scan all no growth tubes into list. 7. Once all no growth tubes are scanned, click on **Define MC** → Clear all → **CXAFB** → **LJ.** In Individual Media Comments box choose result from keypad. 8. For example, if you are resulting Week One LJ, choose "**No growth week one"** 9. Click on **Add Results** to add the observation to the specimen results. 10. A **GREEN !** will appear beside the specimens you just results. **Do not refresh** this list to keep the **!** there. 11. Continue this process with the remaining 6 racks.

12. When finished entering in results for all 7 racks look at list to see which specimens are missing the **!**. Go into each of these specimens and investigate why. Should either be because they were positive and sent out or had suspicious growth.

EXAMINING LJ SLANTS FOR GROWTH:

Check positivity:

Photo source;
http://www.uaz.edu.mx/histo/pathology/ed/ch_9b/c9b_mtb_mac.htm

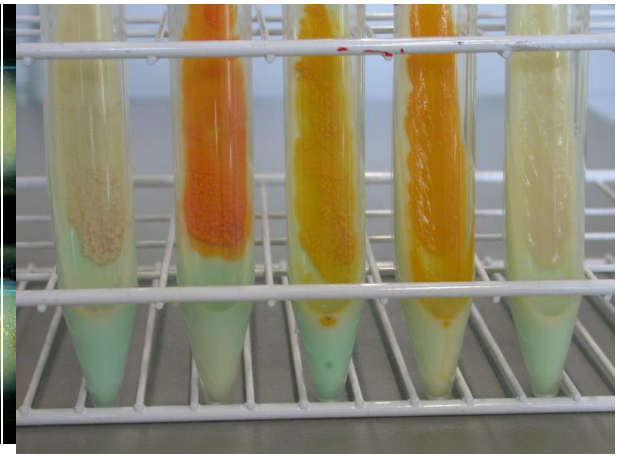


Photo source:
http://vdshahane.hpage.co.in/gallery243955_1.html

- 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 month:

- 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
3.0	13Mar2017	Update LJ culture maintenance	L. Steven