TITLE: Quality Control of Mycobacteria Reagents & Culture	**Revision Date:** 07-April-2017	**Issue Date:** 07-April-2015
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Distribution: Mycobacteria Manual	**Page: 1 of 7**	
Approved by: Gloria Badari, Director, Corporate Services and Chief Financial Officer	**Signed by: (Original Signed Copy in Microbiology)**	

PURPOSE:

To standardize Quality Control methods in the Mycobacteria lab.

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.

QUALITY CONTROL:

The Mycobacteria Lab requires QC of the following items:

- Freshly prepared digestant (3% NaOH)
- Weekly TB’s
- Contamination rates

3% NAOH WORKING SOLUTION

*Note: See **MIC81800 Stock Reagent Preparation** for instructions.*

Frequency of preparation:

- AS NEEDED (dependent on # of samples processed) or annually.

Quality Control: Performed after the reagent is prepared to test for:

- Sterility of 3% NaOH

- Efficacy of decontamination of respiratory flora in sputum samples

Summary of QC procedure:

- Buffer is concentrated in a batch of two and inoculated to BA plates as a sterility check.
- Respiratory flora in two AFB negative sputum samples are quadrant streaked out to BA ("pre-test" results). Then they are spiked with a Mycobacteria ATCC strain or another lab strain and processed with the new batch of 3% NaOH.
- Preferred QC strain: *M. fortuitum* (fast-grower = faster QC results).
- Samples are re-planted to BA ("post-test" results), cultured onto MGITs & LJs, and examined for growth.

3% NaOH Quality Control Expected Results Record results on the 3% NaOH QC Worksheet		
Test	**Time**	**Results**
Sterility plates	24 hours	No growth If growth → autoclave buffer
Pre-test BA-C plate (un-spiked, untreated)	3 days	(1+) → (4+) growth of commensal respiratory flora
Post-test BA-C plate (spiked with MycoB strain, treated with 3% NaOH)	3 days	No growth (ideal) or scant growth of commensal respiratory flora
MGIT & **LJ**	Up to 3 weeks for positivity	Kinyoun smear (+) for AFB
If results are not as above: Do not use the new NaOH batch. Prepare a new batch and repeat QC.		

PROCEDURE TO QUALITY CONTROL NEW BATCHES OF 3% NAOH:

Supplies:

- 50 mL Falcon conical centrifuge tubes
- Yellow sterile 100 µL loops
- BA plates
- MGITs
- LJ slants
- Fresh batch 3% NaOH
- Sterile 1mL transfer pipette

Step	Actions
Sterility check:	
1	Label two 50mL Falcon conical centrifuge tubes. Label two Blood Agar (BA) plates.
2	Add 40 mL of freshly prepared 3% NaOH to each tube
3	Centrifuge tubes using the TB program setting.
4	Decant supernatant leaving 2.5 mL pellet in each tube.
5	Using a sterile yellow loop for each sample, inoculate each BA plate and quadrant streak.
6	Incubate plates in the Mycobacteria incubator at 37° Celsius for 24 hours.
7	Examine plates after 24 hours. Record results on the NaoH QC worksheet. See expected results chart above. If results are as expected, proceed to the steps below for decontamination testing.
Decontamination check:	
8	Obtain two AFB negative sputum samples from fridge. Label.
9	Obtain and label two 50mL conical centrifuge tubes, 4 BA plates (twe labeled “pre-test for Samples 1 & 2), and two labeled “post-test” for samples 1 & 2), two MGITs, two LJ’s.
10	**“Pre-test”.** Perform in either the Bacteriology or Mycobacteriology BSC: Using sterile yellow loops, inoculate the two pre-test BA plates for samples 1 & 2→ quadrant streak for isolated colonies → seal plates with parafilm → incubate plates in the Mycobacteria incubator at 37° Celsius for 24 hours. Save the sputum samples for the Post-test set-up.

11	**"Post-test"** Perform in the Mycobacteriology BSC: Spike both sputum samples with a specified standard of *M. fortuitum*. Vortex. Digest sputum samples as per **MIC80400 Mycobacteria Processing** using the new batch of 3% NaOH. After spiking the MGIT and LJ media, inoculate/streak/incubate the remaining two "post-test" BA plates using step 10 method.
12	Examine "post-test" plates after 24 hours. Record results on the NaoH QC worksheet. See expected results chart above.
13	MGIT should automatically flag the post test sample 1 & 2 as positive.
14	LJ slant should be checked weekly for growth.
15	*M. fortuitum* should growth enough for detection on the MGIT 960 in 2-5 days; however 3 weeks is the maximum allowable time to allow for variations in the Mycobacteria control standard. Record results on the NaoH QC worksheet. See expected results chart above.

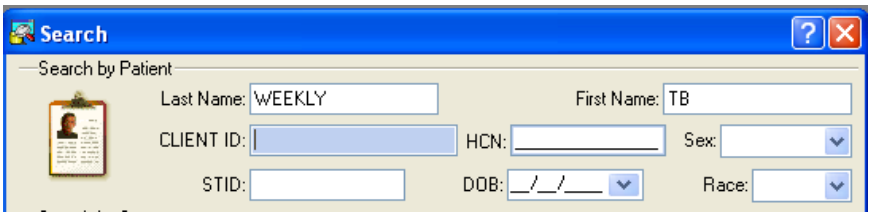
WEEKLY TB'S

Principle:

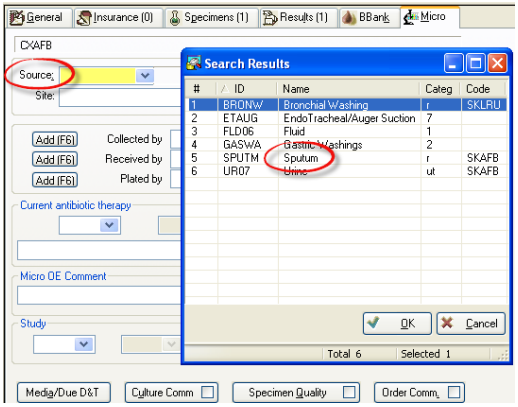
- To help evaluate the processing of samples for the week.
- Made with *M. gordonae* so its growth in culture indicates NaOH digestion is not too harsh.
- Monitoring Weekly TB's in conjugation with monitoring contamination rates helps to evaluate Mycobacteria processing methods.

Procedure:

- In Order Entry, accession the weekly control under name “AFB weekly”



- Test code “TB”. Source: “Sputum”. Site: type in “Made with M gordonae”.



- Collect, receive and plate all. Save. Labels will print.
- Label a 50 mL Falcon conical centrifuge tube with the NaOH label. Label MGIT and LJ slants. Prepare MGIT according to PANTA procedure with the patient samples.
- Add 2.5 mL 3% NaOH and either 2.5 mL AFB negative patient’s sputum sample OR 2.5 mL sterile water into the labeled centrifuge tube.
- Put on PPE and move over to the Mycobacteria BSC.
- Spike with a small blue loop-ful of *M gordonae*.
- Process (digest) with patient samples. See **MIC80400 Mycobacteria Processing**.
- Inoculate into MGIT and LJ media. Make a Direct Smear.
- Detection of QC isolate in the Direct Smear helps assure that enough of the isolate has been spiked into sample for culture to grow.

- Growth in MGIT and LJ ensures processing is gentle enough for M. gordonae detection.

CONTAMINATION RATES

Purpose:

- Monitor effectiveness of NaOH processing.

Frequency:

- Monthly or every 6 months, depending on how many Mycobacteria samples the lab is processing.

Method:

- Review TB worksheets. Manually calculate the rate of contaminates (non-AFB) vs total samples processed and cultured using the TB worksheets.

$$\frac{\text{\# of samples containing non-AFB}}{\text{total \#of samples processed}} \times 100\% = \text{Contamination rate}$$

Goal:

- 3-5% contamination rate.
- >5% = too mild processing
- <3% = too harsh processing.
- Adjust NaOH concentration by either altering the digestion timing in future AFB sample processing (maximum of 20 mins in 3% NaOH) or adjust the strength of the NaOH reagent.

REFERENCES:

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

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	12-Feb-15	Initial Release	L. Driedger