TITLE: Mycobacteria Sample Receipt & Accessioning	**Revision Date:** 07-April-2017	**Issue Date:** 07-April-2015
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Distribution: Mycobacteria Manual	**Page 1 of 9**	
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PURPOSE:

To standardize how to receive, accession and prepare patient samples for Mycobacteria Processing & Culture.

SPECIAL SAFETY PRECAUTIONS:

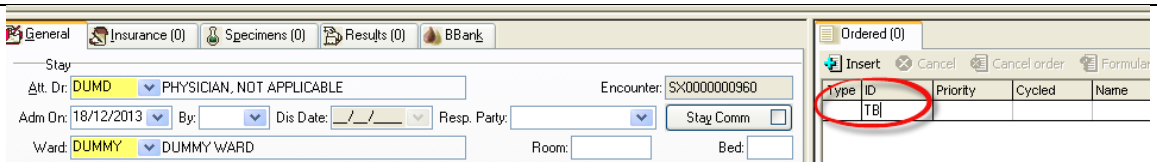
- Handle all patient samples and testing reagent using “Routine Practices”
- Please refer to the Northwest Territories Infection Prevention and Control Manual, March 2012
- Prior to testing all patient are to be identified as per I-0500 Use of Two Patient Identifiers.

SAMPLE RECEIPT& ACCESSIONING FOR MYCOBACTERIA CULTURE:

Provincial Lab has done a study and found that viable MtB has been recovered from the **OUTSIDE** of collection containers submitted for AFB testing. Therefore, before handling the collection container and labelling, it should be decontaminated with Accel TB after removal from the Biohazard bag inside the BSC.

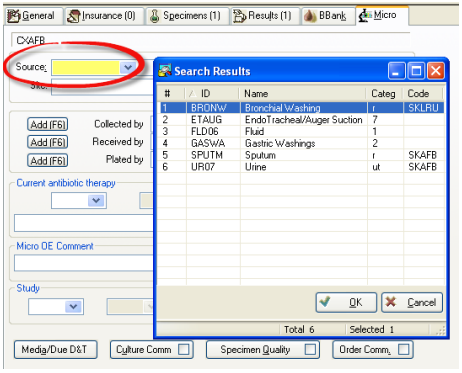
Procedure 1.0 - Sample receipt to Bacteriology Lab:	
1.1	Remove requisition from pouch. Place by accessioning computer.
1.2	Examine sample container for proper labeling through the Biohazard bag (keep sample in bag) or leaking samples.
1.3	Place sample into the fridge until ready to accession.
1.4	Follow steps below for sample decontamination (outer surface of container).

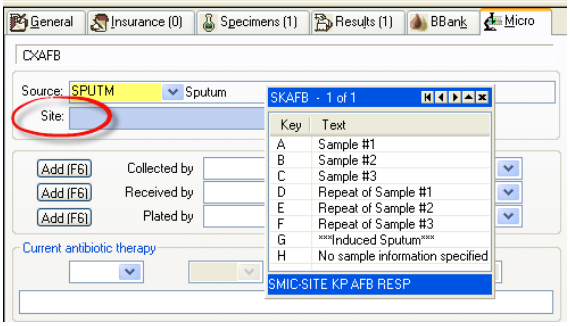
Procedure 2.0 - Sample decontamination steps (before labelling):	
2.1	Start up the Bacteriology BSC. Follow "Biological Safety Cabinet " LSM30200 and Mycobacteria CL-2 BSC document.
2.2	Place a metal tray in the BSC. Line the bottom with an Accel TB wipe or 4x4 gauze pads soaked in Accel TB.
2.3	Take bagged samples to BSC. Check sample integrity (ie. Leaking samples). Do not open leaking containers. Sample must be rejected and request a recollection. Remove acceptable specimens from Biohazard bags and place collection containers on metal tray. Discard Biohazard bag in waste bin beside BSC.
2.4	Wipe down or spray outside of sample container with Accel TB to decontaminate. Once sample is decontaminated and removed from BSC any sample deficiencies will be written on requisition and added as a Specimen Quality comment in the LIS.
Procedure 3.0 - Sample accessioning:	
3.1	Remove metal tray with decontaminated samples to the accessioning area.
3.2	Match up samples with requisitions and organize them (3 sputum samples from same patient should be organized together with the earliest sample date/time accessioned 1st).
3.3	Check for sample deficiencies (low volume, QNS) and check for extra testing such as C&S or cytology. Make a note on requisition.
3.4	Medipatient if required. Accession Mycobacteria samples through Order Entry. <u>Referred out samples</u> (to Provincial Lab in Edmonton) are the following: • Tissues, other biopsy material (ie. bone), blood cultures, stools for AFB. • *Test code: "**MRAFB**"* <u>All others are cultured at Stanton.</u> Test code: **"CXAFB"** for Mycobacteria culture. Quick code **i**s **"TB"** (see below).



Choose “source” as appropriate from the drop-down menu.

If multiple samples collected (Sputum samples 1, 2 and 3), key the sample information in the “Site” line to differentiate between them. The keypad gives options.



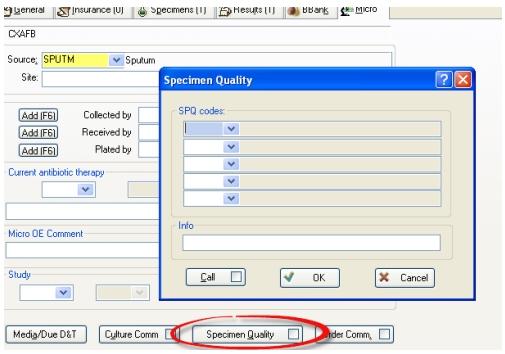


Fill out “collected” and “received” information.

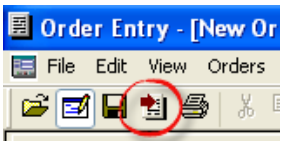
Fill out “Plated” information if processing ASAP.

Leave “Plated” empty if not processing immediately.

Key in any specimen quality comments in the SQ area or OE Comments area.



Click the “Add Next Order” button to accession additional tests on same patient if required ie. C&S, NGYNE cytology, etc.



ACCESSIONING NOTES:

Note 1 - centrifugation	If sample is a fluid or Bronch wash, it may require centrifugation before processing. Centrifugation should be done before holding the sample for AFB testing. • **Perform C&S 1st** (if requested) if concentration is not required for C&S, and then centrifuge for AFB processing using pre-programmed TB setting. Centrifugation guidelines (based on volume of sample received): • CSF & Body fluids **>2. 5 mL** (pleural, synovial, urines, bronch washes, bronch lavage and gastric washings). • See "Centrifugation notes" for Sterile Fluids if fluid is very thick. *In general, any sample that is LIQUID gets centrifuged if above 2.5 mL.* *Sputum is not concentrated.*
Note 2 – sample quality	Pay attention to the criteria in the charts below for sample handling, sample quality comments or additional ordering instructions. • Low volume samples may have minimal media inoculation requirements that require documenting in LIS.
Note 3 – single collection container; multiple tests	If a C&S culture is requested, accession both tests at the same time. • Perform C&S 1st and then keep the container in fridge for AFB culture the next possible day. If referral tests are ordered such as fungal or cytology, aliquot the sample into a separate container for referral. • If aliquotting causes low volume for any requested tests, phone ward for prioritizing. If ward cannot be reached, make the decision to reject if NSQ or process AFB culture with low volume SPQ codes.

ACTIONS BASED ON SAMPLE VOLUMES:

Sample volume and sample handling chart:		
Type	**Handling**	**Processing**
Respiratory (sputum, BW, etc) Optimal volume: 2.5-5 mL	Volume < 0.5 mL • Insufficient quantity – reject/cancel in Result Entry with canned text code "XNSQ" and "XUNS" Volume between 0.5 → 2.5 mL. • Document volume in OE, add SPQ code "AFBLO" Volume between > 2.5 mL. • Sputum: No centrifugation required. A 2.5 mL portion of sample (chose most purulent/mucous) will be used for culture. • If respiratory fluid, (BAL/BW, etc) centrifuge all of sample and use pellet for Mycobacteria culture.	3% NaOH digestion
Fluids Optimal volume: 1-5 mL CSF: 1-4 mL **Urines** (Suprapubic & Cystoscopy) ≥5 mL	Volume <0.5 ml – do not decontaminate, do not do a sterility plate. • Document volume in OE, add SPQ code "AFBFL" • Place ALL of sample directly into MGIT (no processing with NaOH). Do NOT do a direct smear or LJ culture. Cancel LJ slant in plate log & cancel Direct Smear test in Result Entry using canned text reject code "XTBS". Volume < 2.5mL • Document volume in OE; add SPQ code "AFBFL" however a smear and full culture can be done. • No centrifugation required, do not do Sterility plate. Volume > 2.5 mL • Centrifuge all of submitted sample • Perform decontamination as appropriate given sterility plate results. • Inoculate MGIT & LJ and perform Direct Smear	Sterility check for processing requirements (check C&S results) unless <0.5mL

	Centrifugation notes: Use pre-programmed setting on Allegra. (3800g for 20 mins). Decant supernatant into a sterile centrifuge tube, leaving 2.5 mL fluid and sediment behind. If a sterile fluid sample is very thick, **dilute with an equal amount or more of Sterile distilled water** (to reduce viscosity for processing) and then spin down. If a CSF sample is FROZEN – OK for processing however a SPQ code must be added "AFBFZ".	
Urines Optimal volume ≥10mL	Volume ≥ 10mL • Concentrate by centrifugation • Volume acceptable Volume < 10 mL • Insufficient quantity – reject/cancel in Result Entry with canned text code "XNSQ" and "XUNS" Note: Suprapubic/cystoscopy urines follow Sterile fluid guidelines.	3% NaOH digestion
Gastric washings Optimal volume ≥5mL	Volume ≥ 5mL • Concentrate by centrifugation • Volume acceptable Volume 2.5 → 5 mL • Low volume. Document volume in OE, add canned text in "AFBFL". • Concentrate by centrifugation Volume < 2.5 mL • Insufficient quantity – reject/cancel in Result Entry with canned text code "XNSQ" and "XUNS" Note: Acceptable without buffer within 4 hrs of collection and then place into buffer in the lab. Reject sample if received >4 hours without buffer.	3% NaOH digestion

SPECIMEN QUALITY (SPQ) CODES WITH RATIONALE:

- Add these in Order Entry

Code	Meaning	Comment & Rationale
Chart of SPQ codes with associated comments and reasoning.		
DELAY	Transport delay	***Comment:*** "Delayed transport may adversely affect pathogen recovery" ***Rationale:*** Add code if the sample is received > 7 days from collection. AFB is hardy and delays in transport to lab are somewhat more "forgiving" than typical bacteria testing.
AFBLO	AFB respiratory sample volume low	***Comment:*** Scant specimen received for AFB examination. Sensitivity of culture may be reduced due to inadequate volume of specimen received. Please submit at least 2.5 mL of specimen for Mycobacteria culture
AFBFL	AFB Fluid sample volume low	***Comment*:** Culture results may be compromised due to the suboptimal specimen volume received. Negative results should be interpreted with caution; re-collect if clinically indicated. ***Rationale:*** Sterile fluids are never rejected for culture even if very low volume received.
AFBFZ	AFB sample received frozen or from glycerol	***Comment:*** "Culture for AFB is compromised when performed from a frozen clinical specimen. Negative culture results should be interpreted with caution."

DIRECT SMEAR TEST CANCELLATION CANNED TEXT CODES WITH RATIONALE:

- Reject in Result Entry

Code	Meaning	Comment & Rationale
Chart of Cancellation canned text codes with associated comments and reasoning.		
XTBS	TB Direct Smear cancelled (MGIT culture is ongoing, LJ cancelled).	***Comment:*** "Sample will be cultured for Mycobacteria only. Direct smear cannot be performed due to very low volume of sample received." ***Rationale:*** <0.5 mL sample is only enough for MGIT. Due to increased sensitivity of culture vs. smear, priority is to inoculate the MGIT for best results.

CRITERIA FOR MYCOBACTERIA CULTURE REJECTION:

List of Rejection Criteria for Mycobacteria samples and follow-up actions.	
• Nasal or throat swabs. Swabs from other sources are acceptable as long as they are not in anaerobic transport media. Dry swabs are acceptable. • URICULTS • Coagulated blood • Blood in EDTA or aerobic/anaerobic blood culture bottles • Request for Urine TB Cx w/o significant Hx • Mismatched patient name on requisition vs. sample. • Any samples in fixatives or preservatives • Sample in viral transport media • Anaerobic transport media. Aerobic transport media IS ACCEPTABLE. • Pooled sputum samples & 24 hr urines (high rate of contamination) • Unlabeled samples **(XUNL) (XLBL)** • Leaking samples (Biohazard risk) **(XLIT)** • Insufficient quantity sample received **(XNSQ)**	***Follow-up action:*** Reject sample by cancelling the test in Result Entry if test is a "BacTe" test or in Order Entry if test is a "chemistry" test. Attach a comment when rejecting WHY the sample is being rejected and add the following comment below so the health care practitioner Is aware to recollect the sample: **(XUNS)** "Sample is unsuitable for culture. Please recollect sample if clinically indicated".

If samples will not be processed immediately after accessioning, replace labeled samples back in bacteriology fridge so they are kept cool and place media labels underneath samples.

If samples are to be processed right away, move samples and labels to the Mycobacteria room.

A maximum of 12 samples can be processed in one run.

Concentrate fluids, if necessary, before returning to fridge or to the Mycobacteria room so they are ready-to-go for the Mycobacteria processing technologist.

RELATED DOCUMENTS:

- MIC80410 - Mycobacteria Processing Job Aid Chart
- MIC80300 – Preparation of Fluids for Culture
- LSM30200 - Biological Safety Cabinet
- LSM30210 - BSC Monthly Maintenance Record

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	27-JAN-2015	Initial Release	L. Driedger