14th National Conference on
Laboratory Aspects of Tuberculosis

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Back to Basics: Don’t Forget to Pack the Essentials

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Table of Contents

Conflict of Interest Statement

No Potential Conflicts of Interest

Speaker: Toby Bennett, Rhode Island State Health Laboratory

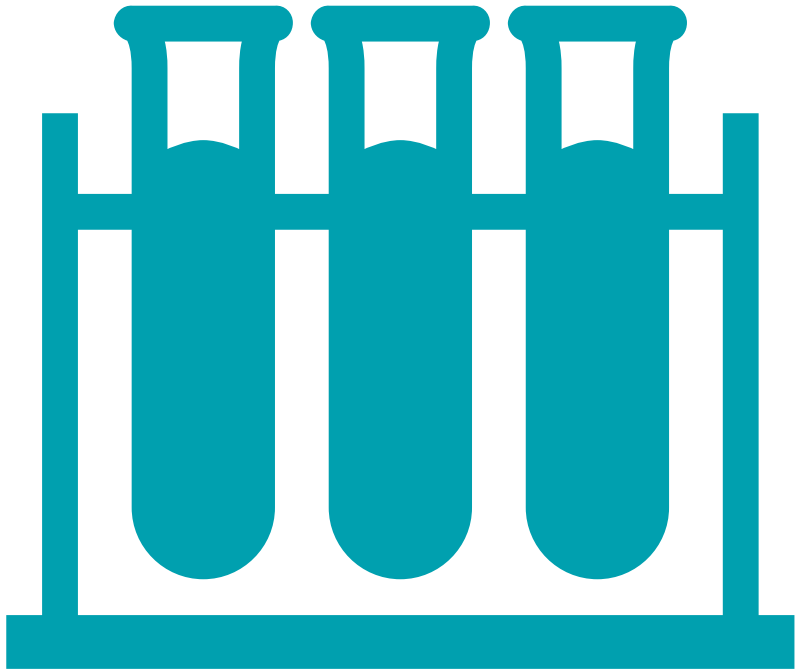
I have NO conflicts of interest related to this presentation to disclose.

Speaker: Calvin Lung, Contra Costa Public Health Laboratory

I have NO conflicts of interest related to this presentation to disclose.

Speaker: Angie Schooley, Michigan Department of Health and Human Services

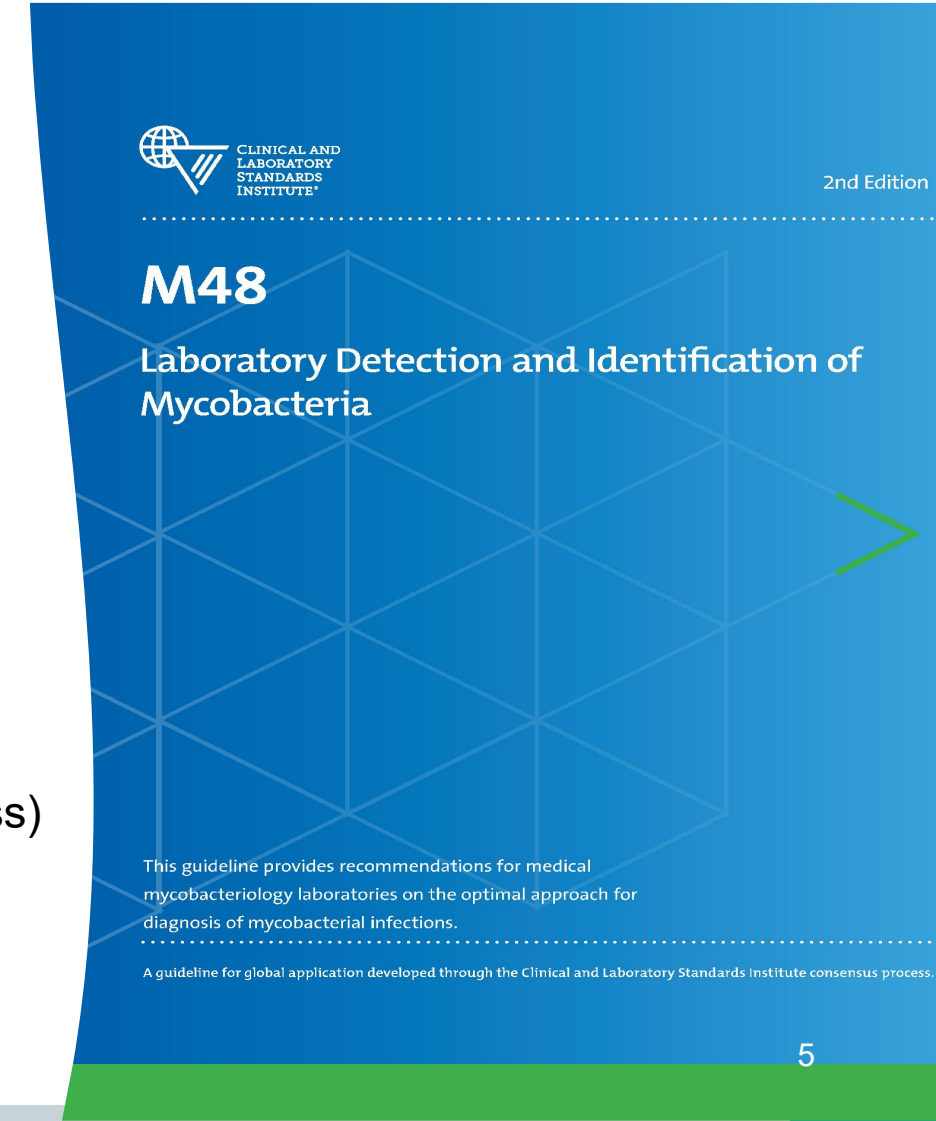
I have NO conflicts of interest related to this presentation to disclose.



AFB Processing

Specimens (Types and Volumes) - RISHL

- Acceptable sources:
  - Abscess
    - aspirate fluid preferred, tissue if possible, swab in saline (if only specimen available)
  - Body fluids
    - Any sterile fluid, recommend 15 mL, minimum of 2 mL requested (disclaimer if less)
  - Bone marrow aspirate
    - In SPS tubes or sterile container
  - CSF
    - Recommend 10 mL, minimum of 2 mL requested (disclaimer if less)



Specimens (Types and Volumes) - RISHL

- Gastric lavage/wash
  - Must contact RISHL for instructions
- Respiratory (lower)
  - BAL, brushing, washing, endotracheal aspirate, transtracheal aspirate
  - Minimum volume of 3 mL (disclaimer if less)
  - No pooling of specimens
- Sputum
  - Recommend 5-10 mL, minimum volume of 3 mL (disclaimer or cancel if less)
  - Pooling of specimens not accepted

Specimens (Types and Volumes) - RISHL

- Stool
  - Minimum amount of 1 g, collected in sterile container
  - No preservatives and no swabs
- Tissue or Lymph Node
  - As much tissue as possible; swabs in saline (disclaimer or rejected)
  - May be pre-ground by the submitting client
- Urine
  - Preferred 40 mL first morning
  - Minimum of 15 mL (disclaimer if less)
  - Pooling of specimens not accepted

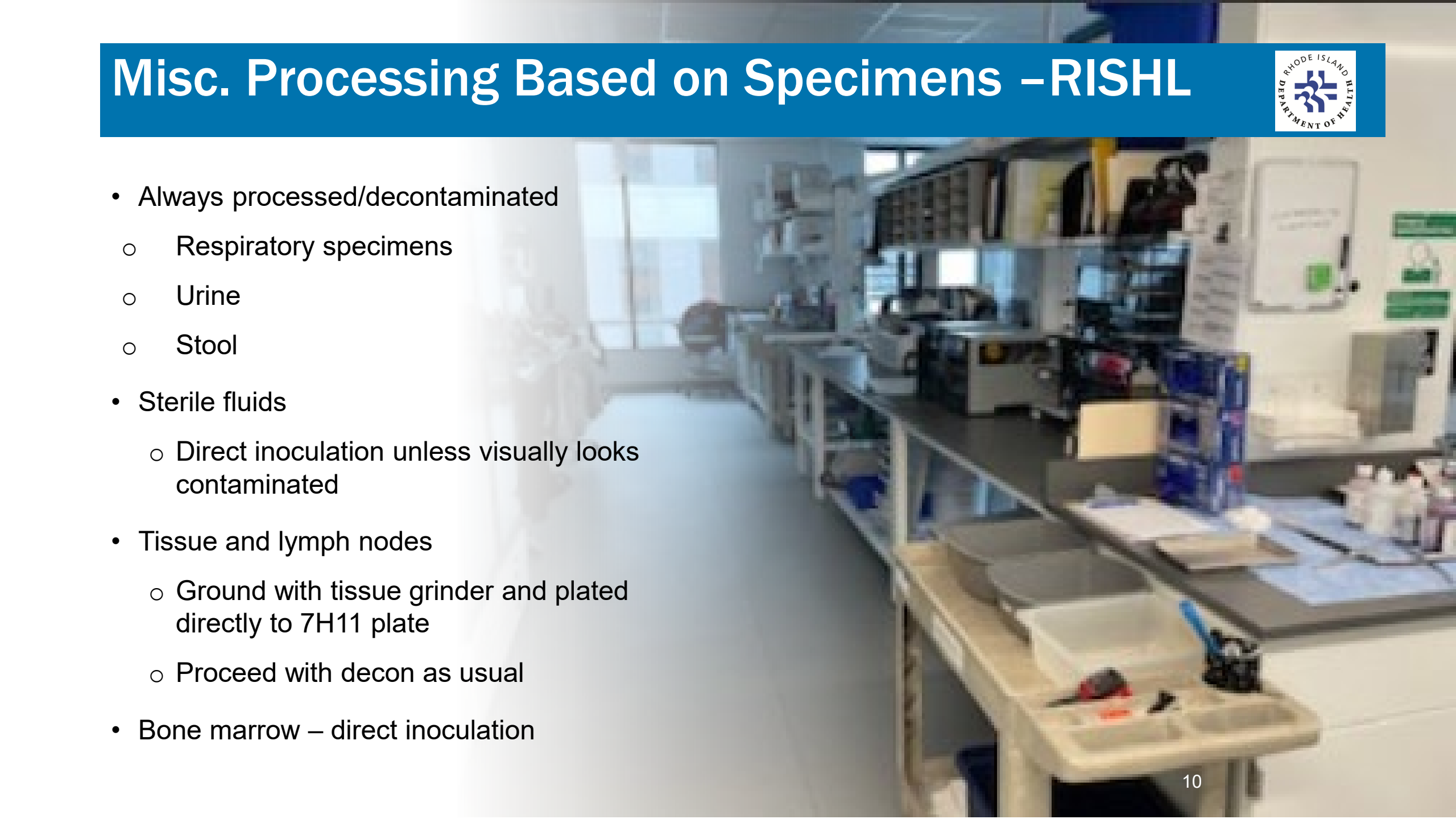
Specimens (Types and Volumes) - Michigan

- 3 mL recommended for respiratory specimens but will process any volume w/disclaimer
- Gastric Lavage
  - Must be neutralized to a pH 6.0-8.0 using sodium bicarbonate within 4 hours of collection
  - Must be received within 72 hours of collection
  - Specimens are unacceptable and will not be tested if both criteria not met
- Blood and Bone Marrow
  - MDHHS will accept SPS (yellow) and Heparin (green)
  - Store at room temperature before delivery

Specimens (Types and Volumes) - CCPHL

- Blood is only accepted in blood culture vial
- Vast majority of specimens received are respiratory specimens
  - Require 5 mL for respiratory specimens
  - Volume minimum of 2 mL
- CSF volume minimum 1 mL
- Urine volume minimum 40 mL

Misc. Processing Based on Specimens –RISHL



- Always processed/decontaminated
  - Respiratory specimens
  - Urine
  - Stool
- Sterile fluids
  - Direct inoculation unless visually looks contaminated
- Tissue and lymph nodes
  - Ground with tissue grinder and plated directly to 7H11 plate
  - Proceed with decon as usual
- Bone marrow – direct inoculation

Misc. Processing Based on Specimens - Michigan

- Decontamination times according to table
- Tissues ground first then decontaminated
  - Use disposable tissue grinders for most tissue grinding, larger tissues are ground in mortar and pestle with sterile sand
- Sterile sites >10ml are concentrated before decontamination
- Swab specimens are soaked in sterile water for 5 minutes and vortexed before decontamination

Source	Decontamination Time	Description
Feces	30 minutes	Hard-formed, soft, liquid Color, volume of specimen
Urine	15 minutes If >10 ml, concentration prior to decontamination required	Clear, cloudy, bloody Color, volume of specimen
Gastric	15 minutes Check pH: must be 6-8 and <72 hours old	Mucoid, serous, purulent, bloody Color, volume of specimen, pH
Sputum Bronchial washing Tracheal aspirates Wounds Abscesses (external) Feet - bone and tissues (also other sources that are mucoid or purulent)	15 minutes If >10 ml, concentration prior to decontamination required (NOT SPUTUM)	Mucoid, serous, purulent, bloody Color, volume of specimen
Body fluids Tissues	5 minutes If >10 ml, concentration prior to decontamination required	Clear, cloudy, purulent, bloody Color, volume of specimen
CSF	Direct If >1 1/2 ml, concentration required	Clear, bloody Color, volume of specimen
Blood Bone marrow	Direct lyse If >7 ml, split into multiple tubes	Volume of specimen
Animal Tissue	20 minutes	Volume of specimen
Animal Feces	45 minutes	Volume of specimen

Misc. Processing Based on Specimens - CCPHL

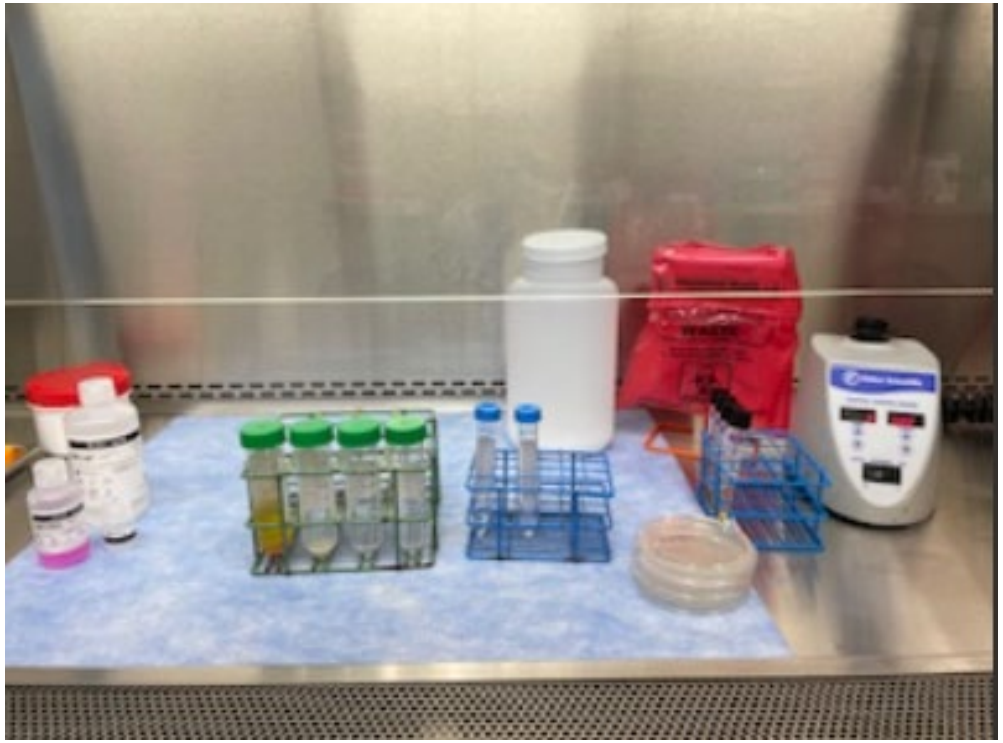
- Blood specimens must be collected in a BD Myco/F Lytic culture vial
  - Will notify provider if fungus is present but they will have to add on an order if they want it worked up
- Tissue/wound specimens ground down and decontaminated

Decontamination Reagents – RISHL

- AlphaTec Systems NAC-PAC RED (3.0%)
  - NALC crystals
  - NaOH solution with indicator
  - NPC-67 Neutralizing Buffer
  - Pellet Resuspension Buffer (PRB)

Decontamination Processing –RISHL

- 1:1 volume of specimen with NAC-PAC RED
- Vortex 30 seconds initially and at least 4x during 18 minute decon process
- Add neutralizing buffer until clear
- Centrifuge at 3000 x g for 15 minutes in refrigerated centrifuge at 4°C
- Decant supernatant, leaving 1 mL of pellet
- Add 1.5 mL of PRB and vortex

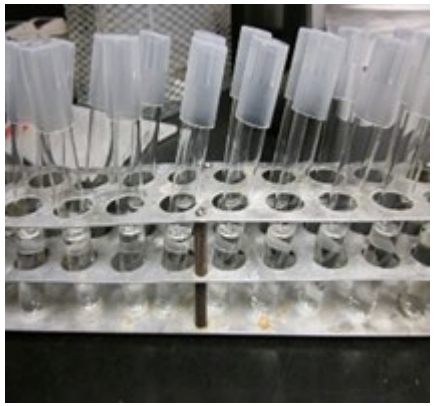


Decontamination Reagents - Michigan

- All reagents are made in house
  - 2.9% Sodium citrate stock solution: store 2-8° for up to 3 months
  - Sodium Hydroxide (NaOH) stock solution: store 2-8° for up to 3 months
  - N-acetyl-L-cysteine-sodium hydroxide solution (NALC-NaOH) made based on the following chart

Total volume	100 ml	200 ml	400 ml	500 ml
NaOH	50 ml	100 ml	200 ml	250 ml
Sodium citrate	50 ml	100 ml	200 ml	250 ml
N-acetyl-L-cysteine	0.5 g	1.0 g	2.0 g	2.5 g

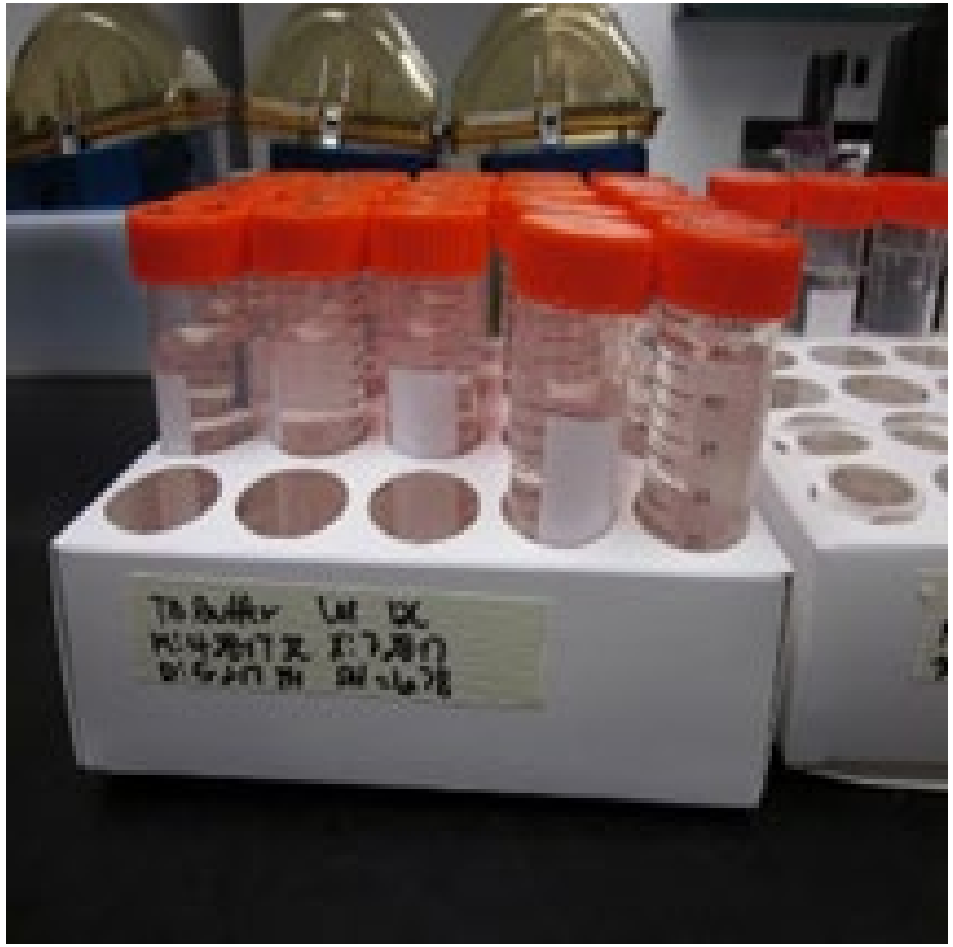
  - Combine the amounts from the chart above into a sterile 1L flask to reach the volume needed for the number of specimens based on 10 ml per specimen. Cover with aluminum foil and allow digestant to self-decontaminate for 30 minutes before using.



- Individual tube with digestant sodium hydroxide (NaOH) and N-acetyl-L-cysteine (NALC)

Decontamination Reagents - Michigan

- 0.067 M Phosphate Buffer
  - Made in house 4-6 L per batch
  - Aliquot in 50 ml centrifuge tubes for single use
  - Store 2-8° for up to 3 months



Decontamination Processing - Michigan

- Transfer total volume of specimen a new 50 ml centrifuge tube
- Add a volume of digestant equal to the volume of specimen
- Vortex to liquify the specimen (2x for respiratory specimens)
- Decontaminate for designated time
- Add buffer to within ½ in from the top of the tube
- Centrifuge >4000 RPM at 9°C for 18 minutes
- Decant the supernatant into a plastic splash-proof flask filled with 50 ml of disinfectant, leaving approximately 0.5 ml in the tube

Decontamination Reagents - CCPHL

- Immy MycoDDR Trident Kit (3.0%)
  - NALC
  - NaOH (3.0%)
  - Neutralization Buffer B
  - Resuspension Buffer C

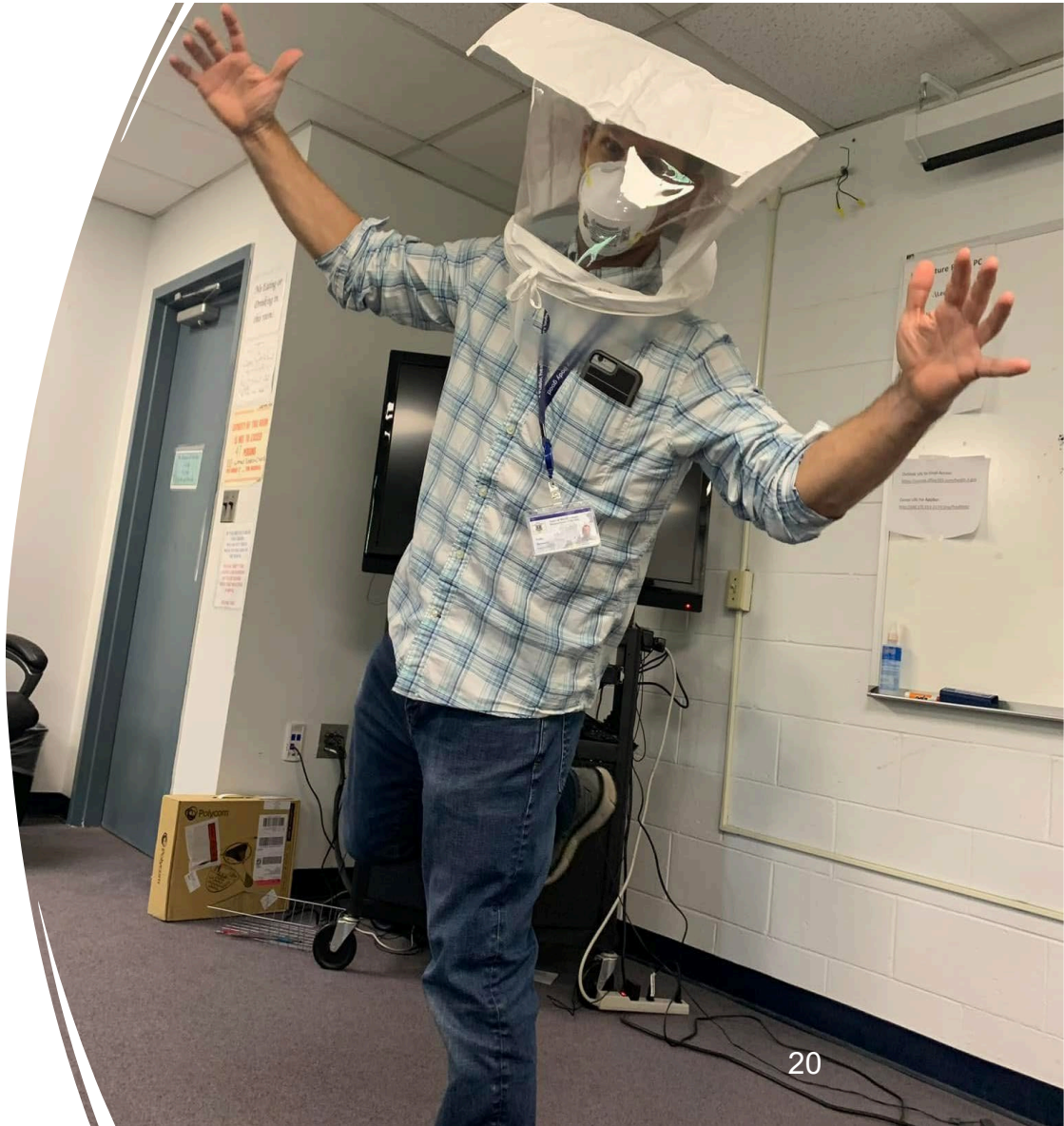
Decontamination Processing - CCPHL

- 1:1 specimen with NALC/NaOH.
- Vortex for ~15 seconds. Specimen should be digested.
- Let sit for 15 min.
- Neutralize with neutralization buffer.
- Mix by inverting several times.
- Solution should be clear. If solution is not clear, add addition buffer until clear.
- Centrifuge for 20 minutes at 3300 RPM at 10°C
- Pour off supernatant

Processing Practices – RISHL

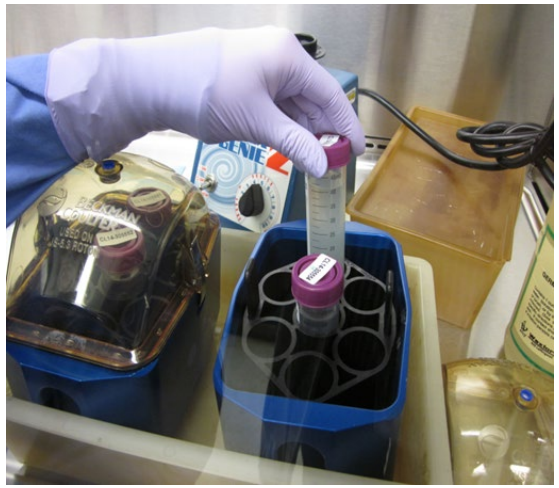
- All AFB processing performed in a BSC within BSL-3 lab

- TB PCR processing runs done separately
- Known MTB positive patients processed at end of run
- PPE
  - N95 respirator (fit-tested) or PAPR
  - Tyvek gown (front facing)
  - Double gloves
  - Goggles/face shield (optional)



Processing practices - Michigan

- Watch for wet caps during every step of processing!
  - If we notice a wet cap, the lip of tube is wiped with disinfectant soaked gauze then wiped with dry gauze and new cap placed on tube.
- Centrifuge bucket carriers are loaded and unloaded within the BSC.
  - When unloading, tubes are wiped with disinfectant soaked towel and inspected for cracked or leaking tubes.
- During decanting of supernatant:
  - Do not touch the lip of tube to the funnel on the splash proof flask
  - Watch pouring speed to ensure no aerosol is created



Processing Practices - CCPHL

- Processing performed in BSL-2 with BSL-3 practices
  - We have a separate BSL-3 for TB work
- Entry into room allowed only when staff is not working in BSC
- Patient specimens are processed separately from competency specimens and patient specimens to decrease risk of contamination
- PPE: Double lab coats, with one front facing, N95, and gloves

Smear and Media

Slides (Type and Fixing) - CCPHL

- Each specimen gets one slide
- Made from pellet prior to adding resuspension buffer
- A smear roughly the size of a nickel, or a 2 cm X 2 cm square is made with 2 sterile wooden sticks
- Slide is heat-fixed at 85°C for 15-30 min in BSC
  - Temperature is measured with a slide thermometer

Staining procedure - CCPHL

- After fixing, the slide is stained with AlphaTec (Now Calibre Scientific) Auramine-Rhodamine
  - If slide is positive, slide is overstained with AlphaTec Kinyoun
- We include one BD AFB slide in each AR and KY run.
- Our specimen volume is low enough that we would only need one slide for each stain a day.
  - We normally only perform one KY run a week for cultures

Slides (Type and Fixing) and Staining Procedure - RISHL

- Single pipet drop onto each of two 10mm circles on FA glass slide
- Air dry then heat fix at >80°C on slide warmer for 15 minute minimum
  - All within BSC
  - time tracked/logged
- Stain with AlphaTec Auramine O, TB decolorizer, potassium permanganate
- Smear made after resuspension of pellet

Slides (Type and Fixing) and Staining Procedure - Michigan

- Use 2 sterile wooden applicator sticks, mix the sediment PRIOR to suspension of the pellet
- Transfer the material between the sterile wooden sticks to a numbered TB slide
- Place on a slide warmer for ~15-20 minutes at 70°C
- Transfer the slides to a passive capture hood and heat fix with a Bunsen burner
- Stain using in-house Auramine-O prepared stains
  - Positive and negative control slides are stained with each batch
  - MDHHS prepares our own QC slides
- If AO is positive, a second slide is made for Ziehl-Neelsen

Reporting Guidelines - CCPHL

- QC slides must be valid prior to reading patient specimens
- Report AR and KY results based off the MCM guidelines

TABLE 2 Acid-fast smear evaluation and reporting[a]

Report	No. of AFB seen by staining method and magnification		
	Fuchsin stain, ×1,000	Fluorochrome stain	
		×250	×450
No AFB seen	0	0	0
Doubtful; repeat	1–2/300 F[b] (3 sweeps)[c]	1–2/30 F (1 sweep)	1–2/70 F (1.5 sweeps)
1+	1–9/100 F (1 sweep)	1–9/10 F	2–18/50 F (1 sweep)
2+	1–9/10 F	1–9/F	4–36/10 F
3+	1–9/F	10–90/F	4–36/F
4+	>9/F	>90/F	>36/F

[a]Adapted from reference 98.
[b]F, microscope fields.
[c]In all cases, one full sweep refers to scanning the full length (2 cm) of a smear 1 cm wide by 2 cm long.

- AR: Read at 400X (Bacilli, beaded, orange)
- KY: Read at 1000X (Bacilli, pink)
- Both results (if AR positive) are reported

Reporting Guidelines - RISHL

- Scan slides at 400x and report using CLSI M48 ED2:2018 guidelines for 1000x
  - Report 1-4+ and report number seen if one or two AFB are seen on entire smear
  - All positive primary smears are co-read by another scientist prior to reporting

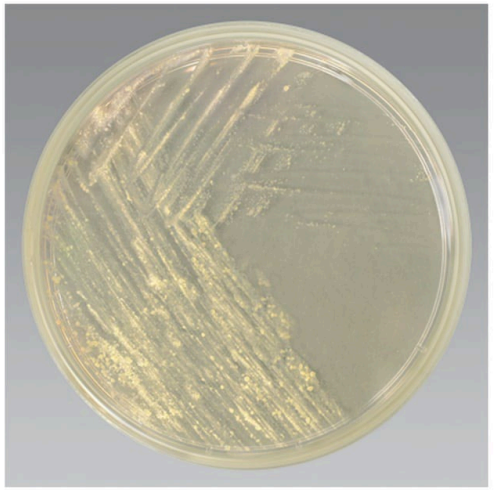
Fluorescence Microscopy		Ziehl-Neelsen	
250×*	450×*	1000×	Report as:
0/smear	0/smear	0/smear	No AFB seen
1–2/30 fields†	1–2/70 fields†	1–2/300 fields†	Report exact count Recommend submission of repeat specimen†
1–9/10 fields	2–18/50 fields	1–9/100 fields	1+
1–9/field	4–36/10 fields	1–9/10 fields	2+
10–90/field	4–36/field	1–9/field	3+
> 90/field	> 36/field	> 9/field	4+

Reporting Guidelines - Michigan

- Read the entire AO smear under 20X
- When fluorescent AFB are seen, a second slide is stained by ZN for confirmation
- Results are based on the ZN result and reported as:
  - ≥10 AFB seen on ZN = Acid Fast Bacilli - Found
  - 3-10 AFB seen on ZN = Acid Fast Bacilli - Few Found
  - <3 AFB seen on ZN = Acid Fast Bacilli - Not Found

Media Inoculated, Volume, and Incubation - CCPHL

- Sediment is resuspended with 1.2 mL of resuspension buffer (CSF specimens are directly inoculated)
- Resuspended sediment is aliquoted into:
  - 0.5 mL into MGIT tube with 0.8 mL PANTA/OADC
  - 0.25 mL LJ slant
  - 0.25 mL 7H10 plate
  - If it's a tissue/wound specimen additional media are also inoculated and incubated at room temperature:
  - 0.25 mL LJ slant
  - 0.25 mL 7H10 plate
  - 0.25 mL chocolate plate



Media inoculated, volume, and incubation - RISHL

- MGIT tube w/PANTA: 0.5 mL
- Middlebrook 7H11 agar plate
  - 4-5 drops from sterile pipet incubated at 37°C w/CO_2
  - Second 7H11 agar plate inoculated if wound, ulcer, skin source incubated at 30°C (non-CO_2)
  - Solid agar currently held 8 weeks
- Chocolate agar plate if suspected species is indicated on requisition form

Media Inoculated, Volume, and Incubation - Michigan

- 0.5 mL MGIT tube
- 0.5 mL LJ incubated @ 36 ± 1°C with 5-10% CO_2
- 0.5 mL 7H11 incubated @ 36 ± 1°C with 5-10% CO_2
  - only on sputum specimens
- 0.5 mL per LJ and 0.2 mL per chocolate agar slant for superficial wounds and ulcers of the limbs incubated @ 30± 1°C non-CO_2

Contaminated MGIT - CCPHL

- Positive MGITs will be examined via KY stain at 100X and 1000X
  - 1 mL aliquot centrifuged
  - Supernatant is pour off
  - Smear is made with pellet
  - If positive, will be resulted as "AFB present. Identification to follow". MGIT tube is stored.
  - If negative (non-AFB), the MGIT tube is stored at room temperature. (This is considered contaminated.)
  - If no bacteria is seen at all, the smear is re-made.
  - If bacteria is still not seen, incubated until growth is visible to the naked eye and smear is remade. (Very rare)

Contaminated Solid Media - CCPHL

- Cultures are read weekly
- Any growth, whether AFB, bacteria, or fungus is documented on our weekly log sheets.
- The media is only considered "contaminated" if 4+ growth is present.
- All media (liquid and solid) must be contaminated for the specimen to be unsatisfactory and cancelled.

Contaminated MGIT - RISHL

- 1 mL aliquot centrifuged – FA stain and Gram stain
- If AFB negative and bacteria seen on Gram stain will re-decontaminate a 1 mL aliquot if within 7 days of initial processing
  - If >7 days then MGIT is logged as contaminated on worksheet
- If AFB neg and no bacteria seen on Gram stain will reincubate and hold 7 days and repeat 1 mL aliquot smear

Contaminated Solid Media - RISHL

- Solid agar read once per week
  - Currently plates are held 8 weeks
- Marked as contaminated if:
  - overgrown (2/3 of plate)
  - unable to see through agar (agar turns opaque or discolors)
  - If contaminated with mold then marked as such on log for tracking
- Reported out as contaminated if both solid and liquid medium are contaminated

Contaminated MGIT - Michigan

- Make ZN smear from bottom of the tube
- If the original specimen was negative for AFB – discard
- If contamination is from a sterile site, we call the submitter to ask of they recovered anything on routine culture, consider sending to reference bacteriology or mycology for further identification
- If the original specimen was positive for AFB – decontaminate MGIT broth for 20 minutes and inoculate a new MGIT tube

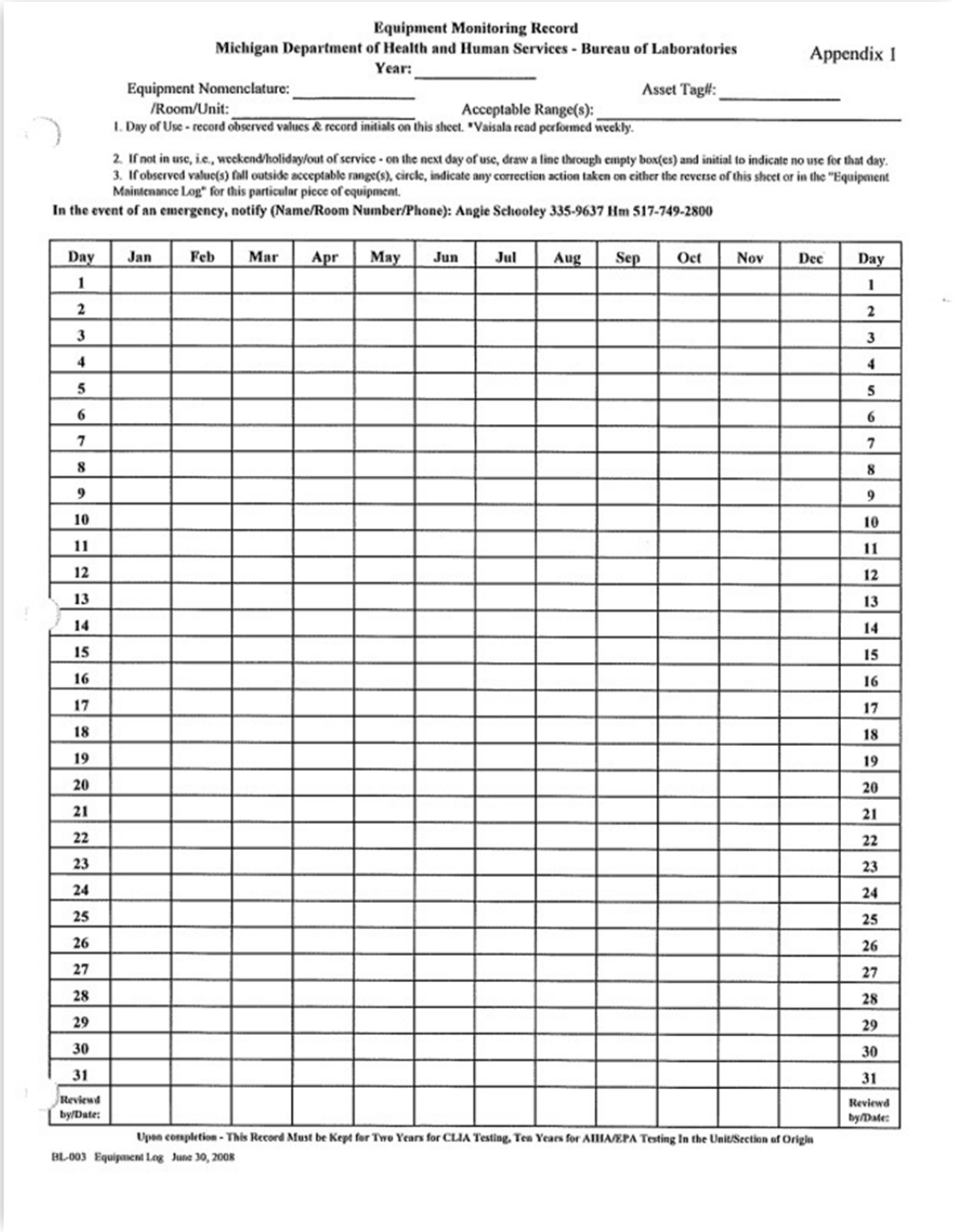
Contaminated Solid Media - Michigan

- Discard the slant if greater than 2/3 of the tube is contaminated with non-AFB
- If contamination is from a sterile site, we call the submitter to ask of they recovered anything on routine culture, consider sending to reference bacteriology or mycology for further identification
- Solid media is discarded after 6 weeks

Quality assurance and quality control

Temperature and CO_2 Monitoring – Michigan

- The morning routine:
  - CO_2 is recorded daily by reading the digital readout on the incubator and once a week by using a Vaisala hand-held Carbon Dioxide reader. This system confirms the accuracy of the digital reading.
  - Temperatures of incubators, refrigerators, and freezers are continuously monitored with probes linked to the Vaisala continuous monitoring system. Managers receive a text or email when temperatures are out of range.
  - Air flows (Phoenix valves) are checked to make sure airflows are correct in the BSL3.
  - Biosafety cabinets are turned on and the magnahelic (Baker hoods) or the filter % (Labconco) are recorded on daily monitoring sheets.
  - Eyewashes are turned on for 10 minutes once a week.
  - Other pieces of equipment such as water baths, heat blocks, oven are recorded each day of use.



Equipment Monitoring Record
Michigan Department of Health and Human Services - Bureau of Laboratories Appendix I
Year: _____

Equipment Nomenclature: _____ Asset Tag#: _____
/Room/Unit: _____ Acceptable Range(s): _____

1. Day of Use - record observed values & record initials on this sheet. *Vaisala read performed weekly.

2. If not in use, i.e., weekend/holiday/out of service - on the next day of use, draw a line through empty box(es) and initial to indicate no use for that day.
3. If observed value(s) fall outside acceptable range(s), circle, indicate any correction action taken on either the reverse of this sheet or in the "Equipment Maintenance Log" for this particular piece of equipment.

In the event of an emergency, notify (Name/Room Number/Phone): Angie Schooley 335-9637 Hm 517-749-2800

Day	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Day
1													1
2													2
3													3
4													4
5													5
6													6
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28													28
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30													30
31													31
Reviewd by/Date:													Reviewd by/Date:

Upon completion - This Record Must be Kept for Two Years for CLIA Testing, Ten Years for AIHA/EPA Testing In the Unit/Section of Origin

BL-003 Equipment Log June 30, 2008

Temperature and CO_2 Monitoring - CCPHL

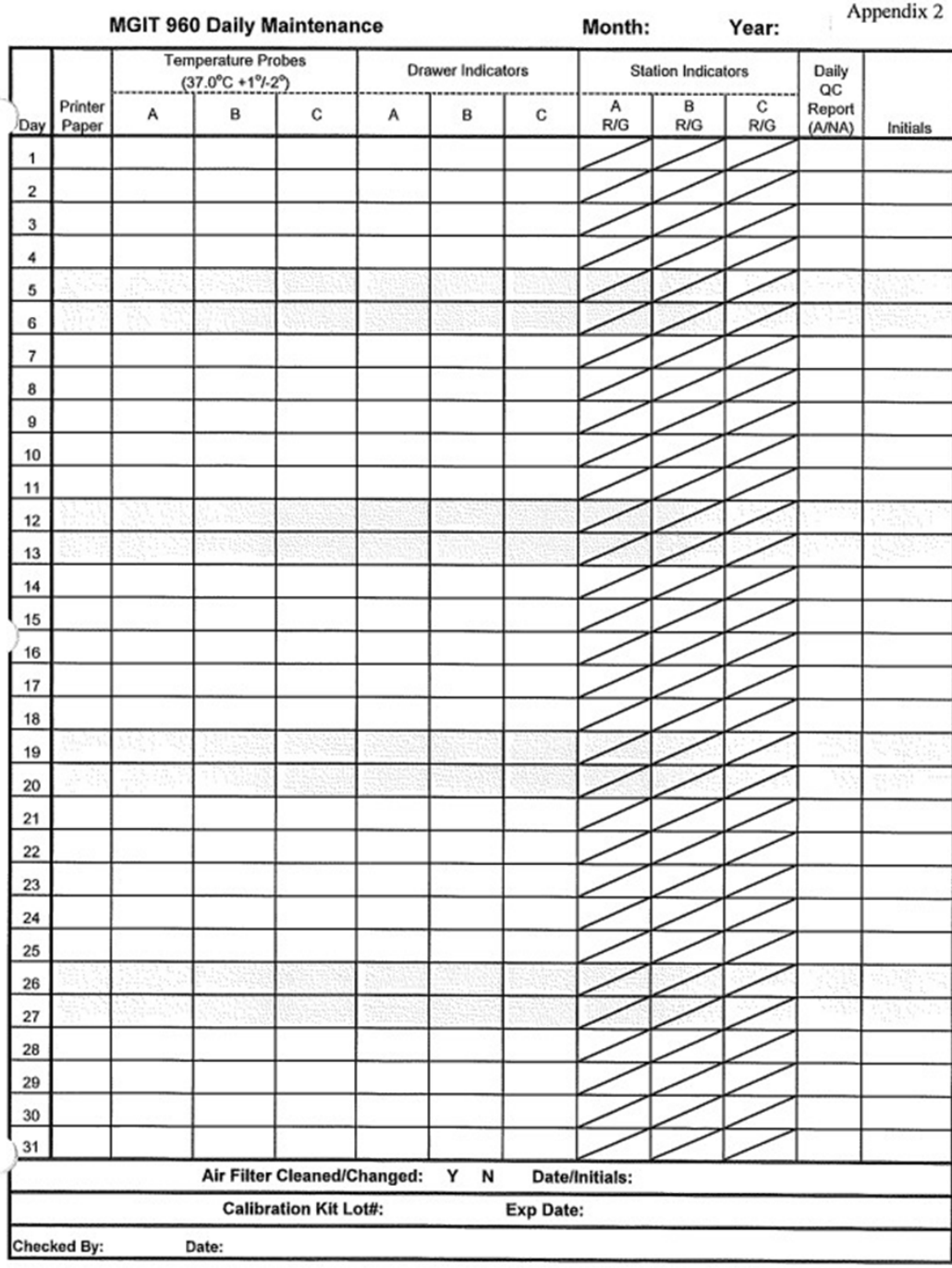
- Manual daily temp monitoring once a day
  - Room temperature and negative pressure is also measured
  - CO2 incubators are checked once a week with Fyrite. Note: Fyrite is discontinued so we will eventually need to move to a digital method
- Monthly review of temp/CO_2 logs by SPHM
- Digital logging system is also logging but we haven't completely moved to digital yet

Temperature and CO_2 Monitoring – RISHL

- Daily temperature/CO_2 reading 2x/day of all equipment (am/pm)
  - Includes room temperature and room relative humidity
  - Room temp/humidity range adjusted for instrumentation within lab space
- Fyrite checked monthly for CO_2 incubators
- Charts reviewed monthly by supervisor

MGIT Daily Maintenance – Michigan

- MGIT 960 and 320
  - Perform daily checks and print QC report
  - Unload negatives and print report, any negative with a GU, make a ZN
  - Calibrator tube expiration dates are monitored monthly
  - Clean and change filters monthly



Appendix 2

MGIT 960 Daily Maintenance **Month:** **Year:**

Day	Printer Paper	Temperature Probes (37.0°C +1°/-2°) A	B	C	Drawer Indicators A	B	C	Station Indicators A R/G	B R/G	C R/G	Daily QC Report (A/NA)	Initials
1												
2												
3												
4												
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29												
30												
31												

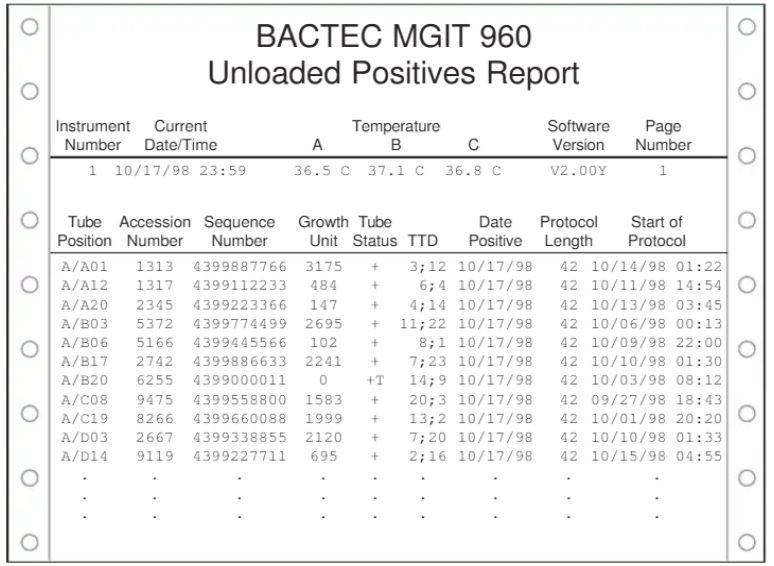
Air Filter Cleaned/Changed: Y N Date/Initials:

Calibration Kit Lot#: Exp Date:

Checked By: Date:

MGIT Maintenance - CCPHL

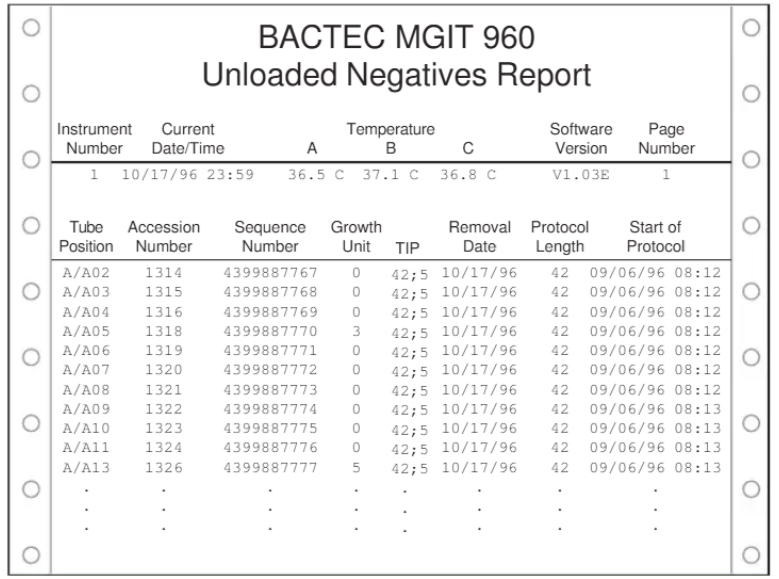
- Daily:
  - Positive and negative MGITs are removed
  - Temperatures, and all lights are checked daily
- Monthly:
  - Calibrator tubes
  - Filters are cleaned monthly
  - Maintenance log printed monthly



BACTEC MGIT 960
Unloaded Positives Report

Instrument Number	Current Date/Time	Temperature A	Temperature B	Temperature C	Software Version	Page Number
1	10/17/98 23:59	36.5 C	37.1 C	36.8 C	V2.00Y	1

Tube Position	Accession Number	Sequence Number	Growth Unit	Tube Status	TTD	Date Positive	Protocol Length	Start of Protocol
A/A01	1313	4399887766	3175	+	3;12	10/17/98	42	10/14/98 01:22
A/A12	1317	4399112233	484	+	6;4	10/17/98	42	10/11/98 14:54
A/A20	2345	4399223366	147	+	4;14	10/17/98	42	10/13/98 03:45
A/B03	5372	4399774499	2695	+	11;22	10/17/98	42	10/06/98 00:13
A/B06	5166	4399445566	102	+	8;1	10/17/98	42	10/09/98 22:00
A/B17	2742	4399886633	2241	+	7;23	10/17/98	42	10/10/98 01:30
A/B20	6255	4399000011	0	+T	14;9	10/17/98	42	10/03/98 08:12
A/C08	9475	4399558800	1583	+	20;3	10/17/98	42	09/27/98 18:43
A/C19	8266	4399660088	1999	+	13;2	10/17/98	42	10/01/98 20:20
A/D03	2667	4399338855	2120	+	7;20	10/17/98	42	10/10/98 01:33
A/D14	9119	4399227711	695	+	2;16	10/17/98	42	10/15/98 04:55



BACTEC MGIT 960
Unloaded Negatives Report

Instrument Number	Current Date/Time	Temperature A	Temperature B	Temperature C	Software Version	Page Number
1	10/17/96 23:59	36.5 C	37.1 C	36.8 C	V1.03E	1

Tube Position	Accession Number	Sequence Number	Growth Unit	TIP	Removal Date	Protocol Length	Start of Protocol
A/A02	1314	4399887767	0	42;5	10/17/96	42	09/06/96 08:12
A/A03	1315	4399887768	0	42;5	10/17/96	42	09/06/96 08:12
A/A04	1316	4399887769	0	42;5	10/17/96	42	09/06/96 08:12
A/A05	1318	4399887770	3	42;5	10/17/96	42	09/06/96 08:12
A/A06	1319	4399887771	0	42;5	10/17/96	42	09/06/96 08:12
A/A07	1320	4399887772	0	42;5	10/17/96	42	09/06/96 08:12
A/A08	1321	4399887773	0	42;5	10/17/96	42	09/06/96 08:12
A/A09	1322	4399887774	0	42;5	10/17/96	42	09/06/96 08:13
A/A10	1323	4399887775	0	42;5	10/17/96	42	09/06/96 08:13
A/A11	1324	4399887776	0	42;5	10/17/96	42	09/06/96 08:13
A/A13	1326	4399887777	5	42;5	10/17/96	42	09/06/96 08:13

MGIT Daily Maintenance – RISHL

- Morning daily check, print QC report, unload neg/pos and print both reports
  - Any GU for negative tubes is reviewed
- Complete BACTEC MGIT 960 Maintenance Log
- Calibrator tube check on PM schedule for expiration date
- Filters cleaned and changed monthly

Media Quality Control – Michigan

- MDHHS maintains working stock cultures for QC of media and test methods. Aliquots of working stock cultures are stored at –70° to preserve biological characteristics of each organism. Michigan has a procedure dedicated to maintaining stock cultures.
- Commercial LJ and 7H11
  - 5 tubes from each new lot, inoculate 1 slant for each organism with 0.1 ml of a 1:100 dilution of a 7-day broth culture of the following mycobacterium:
    - *M. tuberculosis, M. kansasii, M. scrofulaceum, M. intracellulare, M. fortuitum*
    - Incubate the slants at 36 ± 1°C in 5-10% CO_2 for up to 3 weeks.
    - Record all results on the Lowenstein Jenson Quality Control F-787 form.
    - Expected results: Mature growth after 3 weeks of incubation
    - Inhibition testing is also performed by inoculating a 7H11 slant and TSA slant with E. coli

Bureau of Laboratories Commercial Media QC Record

Lowenstein-Jensen (LJ) Agar Slants

Manufacturer: ☑ Remel ☐ BD ☐ Other (Enter Brand Here)

Lot number: 359082

Expiration Date: 12/23/2026

Number of Tubes Received: 1500

Date Received: 02/05/2026

Quality Control Procedures Performed:

Verify delivery (MS.05):
Acceptable Not Acceptable Initial/Date: _____

Perform visual inspection (MS.05):
Acceptable Not Acceptable Initial/Date: _____

Culture Value (TB.12):

Pre-Inoculation: 5 LJ slants are required for culture value.

Inoculation: Inoculated (Initial/Date):_____

Organism	Dilution	Inoculation Vol. (ml)	1 Week	2 Week	3 Week
M. tuberculosis TMC 102	1:100	0.1			
M. kansasii TMC 1201	1:100	0.1			
M. scrofulaceum TMC 1305	1:100	0.1			
M. intracellulare TMC 1403	1:100	0.1			
M. fortuitum TMC 1529	1:100	0.1			

Incubation: Incubate all inoculated media at 36 ± 1° C with 5-10% CO_2 for up to 3 weeks.

Post-Incubation: Growth after 1-3 weeks.
Acceptable Not Acceptable Initial/Date: _____

List any deviations and follow-up:

Media is: Acceptable Not Acceptable Initial/Date: _____

Manager Review: _____

Media Quality Control – Michigan

MGIT

- Randomly select 3 tubes of each new lot for growth testing.
- Add 0.8 ml of PANTA to each tube.
- Inoculate one tube with 0.5 ml of a 1:50 dilution of a 0.5 McFarland broth using the following Mycobacterial organism:

  M. tuberculosis TMC 102

- Inoculate one tube with 0.5 ml of a 1:5000 dilution of a 0.5 McFarland broth using each of the following Mycobacterial organisms:

  M. kansasii TMC 1201

  M. fortuitum TMC 1529

- Incubate the tubes in the MGIT 320 or 960 instrument.
- Record results on the New MGIT Growth Tube F-787 form.
- Expected results: Refer to the F-787 form for the days to positive ranges for each organism

Bureau of Laboratories Commercial Media QC Record

MGIT Broth

Manufacturer: ☑ BD

MGIT Growth Tube Lot Number: 5225856

Expiration Date: 2/10/2027

Number of Tubes Received: 3000

Date Received: 11/24/2025

Control Procedures Performed:

Verify delivery (MS.05): Acceptable Not Acceptable Initial/Date: _____

Perform visual inspection (MS.05): Acceptable Not Acceptable Initial/Date: _____

Culture Value (TB.12):

Pre-Inoculation: 3 tubes are required for culture value.

Inoculation: Inoculated (Initial/Date): _____

MGIT Supplement Kit lot number: _____ Expiration Date: _____

Organism	Dilution	Inoculation Vol. (ml)	Date Positive	Number of days to positive	Expected results (days)
M. tuberculosis TMC 102	1:50	0.5			4-10
M. kansasii TMC 1201	1:5000	0.5			5-11
M. fortuitum TMC 1529	1:5000	0.5			1-4

Incubation: Incubate the tubes in the MGIT 320 or 960 instrument until positive.

Post-Incubation: Growth within the expected results.

Acceptable Not Acceptable Initial/Date: _____

List any deviations and follow-up:

Media is: Acceptable Not Acceptable Initial/Date: _____

Manager Review: _____

Media Quality Control - CCPHL

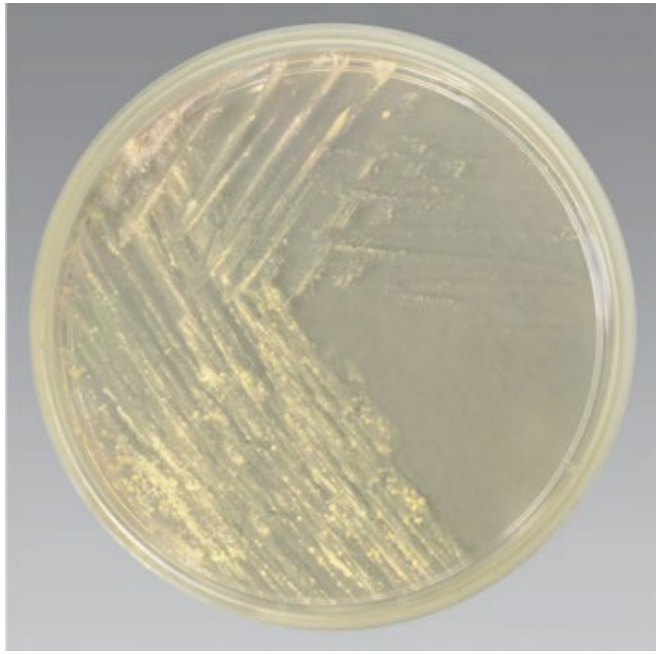
- New lot or shipment for MGIT tubes, 7H10 plates, and LJ slants
  - Liquid media QC: 1 X MTBC H37Rv, 1 X *M. fortuitum*, 1X *M. kansasii*, 1 X sterility
    - Growth must occur in expected date ranges

Organism	Expected positivity in:
M. fortuitum (1:5000)	1-3 days
M. tuberculosis (1:500)	6-10 days
M. kansasii (1:50,000)	7-11 days

  - Solid media QC: 1 X sterility, 1 X MTBC H37Rv
- Bacteriology section QCs chocolate plates

Media Quality Control - RISHL

- New lot/new shipment for MGIT tubes
  - MGIT QC – follows BD MGIT tube procedure for QC of tubes
    - *Mycobacterium tuberculosis* ATCC 25177
    - *Mycobacterium fortuitum* ATCC 6841
    - *Mycobacterium kansasii* ATCC 12478
- New lot/new shipment for 7H11 plates and LJ slants
  - Same as Michigan except do not use *Mycobacterium scrofulaceum*
  - Do not set up a plate for inhibition



Processing Batches – Michigan

- MDHHS processes in batches of 14, according to the size of our centrifuge buckets with all respiratory specimens at the front of the batch
- We use process times according to the table

Table 1

Specimen Decontamination Times & Descriptions

Source	Decontamination Time	Description
Feces	30 minutes	Hard-formed, soft, liquid Color, volume of specimen
Urine	15 minutes If >10 ml, concentration prior to decontamination required	Clear, cloudy, bloody Color, volume of specimen
Gastric	15 minutes Check pH: must be 6-8 and <72 hours old	Mucoid, serous, purulent, bloody Color, volume of specimen, pH
Sputum Bronchial washing Tracheal aspirates Wounds Abscesses (external) Feet - bone and tissues (also other sources that are mucoid or purulent)	15 minutes If >10 ml, concentration prior to decontamination required (NOT SPUTUM)	Mucoid, serous, purulent, bloody Color, volume of specimen
Body fluids Tissues	5 minutes If >10 ml, concentration prior to decontamination required	Clear, cloudy, purulent, bloody Color, volume of specimen
CSF	Direct If >1 1/2 ml, concentration required	Clear, bloody Color, volume of specimen
Blood Bone marrow	Direct lyse If >7 ml, split into multiple tubes	Volume of specimen
Animal Tissue	20 minutes	Volume of specimen
Animal Feces	45 minutes	Volume of specimen

Sources Requiring Additional LJ and Chocolate Agar Slants Incubated at 30°C

Tissue / Skin from extremities and superficial sites

Wound / Abscess / Ulcers from extremities and superficial sites

Unspecified swab source

Processing Batches - CCPHL

- Batches separated if >8-10 specimens in run
  - Depending on the lab staff processing specimen (Ie. Comfort, experience, speed, etc.)
- PT or competency specimens are performed separately

Processing Batches - RISHL

- Separated if too large (>10 specimens requiring decontamination)
- TB PCR requests processed in a separate run
- If smear positive previously within last month will add to end of a non-TB PCR run

MTBC PCR – Michigan

- MDHHS runs the Wadsworth PCR for MTBC and *M. avium* complex.
  - Each run will include the following controls:
    - Positive and negative reagent controls
    - Positive and negative extraction controls
    - Inhibition control (bicoid only)
- Validated for all sources except stool
- We run PCR on all smear postive, smear negative by request
- Test positive sediments from submitters around Michigan
- Will perform 2 PCR on smear negatives if requested

MTB PCR - CCPHL

- One PCR per specimen type per patient
  - We are in the process of validating non-sputum sources
- MTB PCR is performed after specimens are processed
- Otherwise, will require consult with TB physician
- EXCEPTION: All MTB PCR orders from our local Children's hospital will get run.
  - If we see a previous positive, we will notify provider if they want repeated testing. Sometimes they will...

MTB PCR - RISHL

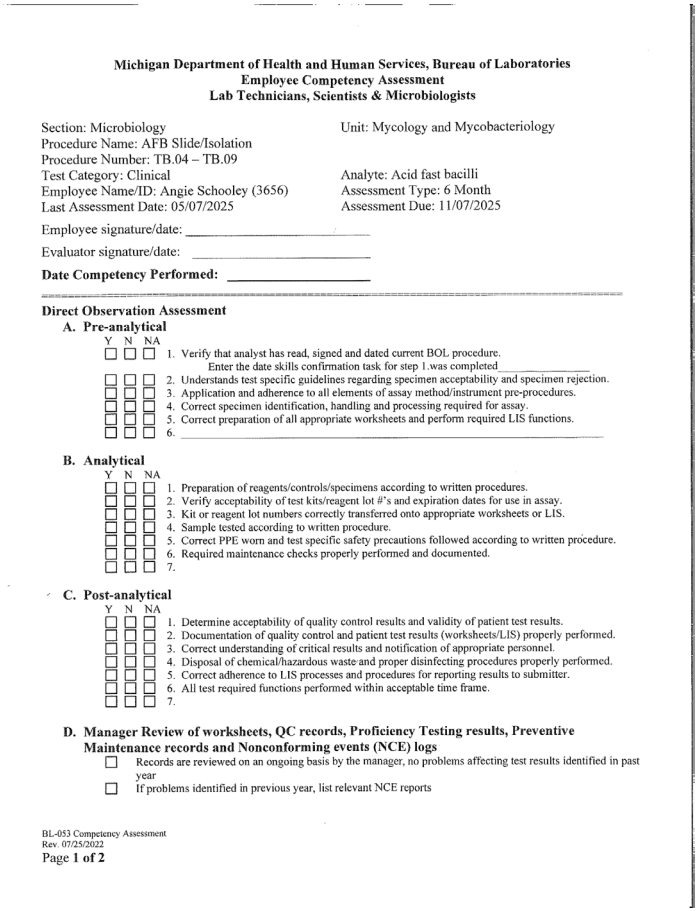
- TB PCR request ordered on requisition or add-on requests by provider, TB Clinic or TB Program (must have smear and culture also)
- Respiratory sources only (non-sputum sources includes disclaimer)
- Up to 3 TB PCRs per patient initially
- Specimens with TB PCR are done in a separate processing run

AFB QC Slide – Michigan

- MDHHS runs a positive and negative control with each staining batch
- The control material is made in house
  - The positive control slide contains autoclaved non-viable *Mycobacterium tuberculosis* TMC 102 and viable *Escherichia coli* ATCC 25922.
  - The negative control slide contains viable *E. coli* ATCC 25922.

Personnel Competency – Michigan

- Competencies and training sheets are completed on employees for all tests they are competent to perform. New test methods will have an initial competency followed by a 6 month and annual thereafter. Initial training sheets are also completed and stored for 2 years after the employee has left.



Michigan Department of Health and Human Services, Bureau of Laboratories
Employee Competency Assessment
Lab Technicians, Scientists & Microbiologists

Section: Microbiology
Unit: Mycology and Mycobacteriology
Procedure Name: AFB Slide/Isolation
Procedure Number: TB.04 – TB.09
Test Category: Clinical
Analyte: Acid fast bacilli
Employee Name/ID: Angie Schooley (3656)
Assessment Type: 6 Month
Last Assessment Date: 05/07/2025
Assessment Due: 11/07/2025

Employee signature/date: _____
Evaluator signature/date: _____
Date Competency Performed: _____

Direct Observation Assessment

A. Pre-analytical

Y	N	NA	
☐	☐	☐	1. Verify that analyst has read, signed and dated current BOL procedure. Enter the date skills confirmation task for step 1.was completed_____
☐	☐	☐	2. Understands test specific guidelines regarding specimen acceptability and specimen rejection.
☐	☐	☐	3. Application and adherence to all elements of assay method/instrument pre-procedures.
☐	☐	☐	4. Correct specimen identification, handling and processing required for assay.
☐	☐	☐	5. Correct preparation of all appropriate worksheets and perform required LIS functions.
☐	☐	☐	6. _____

B. Analytical

Y	N	NA	
☐	☐	☐	1. Preparation of reagents/controls/specimens according to written procedures.
☐	☐	☐	2. Verify acceptability of test kits/reagent lot #'s and expiration dates for use in assay.
☐	☐	☐	3. Kit or reagent lot numbers correctly transferred onto appropriate worksheets or LIS.
☐	☐	☐	4. Sample tested according to written procedure.
☐	☐	☐	5. Correct PPE worn and test specific safety precautions followed according to written procedure.
☐	☐	☐	6. Required maintenance checks properly performed and documented.
☐	☐	☐	7.

C. Post-analytical

Y	N	NA	
☐	☐	☐	1. Determine acceptability of quality control results and validity of patient test results.
☐	☐	☐	2. Documentation of quality control and patient test results (worksheets/LIS) properly performed.
☐	☐	☐	3. Correct understanding of critical results and notification of appropriate personnel.
☐	☐	☐	4. Disposal of chemical/hazardous waste and proper disinfecting procedures properly performed.
☐	☐	☐	5. Correct adherence to LIS processes and procedures for reporting results to submitter.
☐	☐	☐	6. All test required functions performed within acceptable time frame.
☐	☐	☐	7.

D. Manager Review of worksheets, QC records, Proficiency Testing results, Preventive Maintenance records and Nonconforming events (NCE) logs

☐ Records are reviewed on an ongoing basis by the manager, no problems affecting test results identified in past year
☐ If problems identified in previous year, list relevant NCE reports

BL-053 Competency Assessment
Rev. 07/25/2022
Page **1 of 2**

Problem Solving Assessment

Attach or write in an example of problem-solving on the part of this employee (example: NCE reported by employee, Q&A quiz, etc.)

Question(s):

Answer(s):

Problem Solving Assessment Results:

Satisfactory ☐
Unsatisfactory ☐
Action taken:

Blind Sample Testing:

Did results match expected results?

☐ Yes
☐ No (investigation and follow-up as a nonconforming event is required.)

Type of blind samples:

Batch/Run identifier:
☐ Previously analyzed sample
☐ Internal blind sample
☐ Old Proficiency Testing (PT) sample
☐ Current PT survey.
If using current proficiency survey indicate **Survey name:**

Overall Assessment Result:

Satisfactory ☐
Unsatisfactory ☐
Action taken:

Manager signature*/date:

*Electronic signature is acceptable

BL-053 Competency Assessment
Rev. 07/25/2022
Page **1 of 2**

AFB QC Slide and Personnel Competency - CCPHL

- AlphaTec QC1 AFB slide per each AR and KY run
  - Recently switched to BD AFB slides to due some false positive negative wells on the AlphaTec slides
- Personnel Competency
  - Initial, six months, one year
  - Training checklist per assay
  - https://www.cms.gov/regulations-and-guidance/legislation/clia/downloads/clia_compbrochure_508.pdf

Evaluation	Evaluation should include the following:	Assessment completed by:	Date Assessment completed:	Competency Demonstrated	Comments
Direct observation of routine patient testing	○ Observation of specimen handling ○ Observation of specimen processing ○ Observation of specimen testing			○ Yes ○ Needs further assessment	
Monitoring the recording and reporting of test results	○ Review of worksheets for incorrect/incomplete results ○ Comparison of worksheets to final test reports ○ Evaluation of turn-around times for reports ○ Evaluation of compliance with policies and procedures			○ Yes ○ Needs further assessment	
Review of intermediate test results and records	○ Review of worksheets ○ Review of QC records ○ Review of preventive maintenance records			○ Yes ○ Needs further assessment	
Direct observation of instrument maintenance and function checks	○ Observation of proper performance of instrument maintenance/function checks			○ Yes ○ Needs further assessment	
Assessment of test performance through blinded sample (proficiency testing or previously analyzed specimens)	○ Review of proficiency test results			○ Yes ○ Needs further assessment	
Assessment of problem solving skills	○ Review problem logs ○ Review QC failures ○ Determine root cause of issue			○ Yes ○ Needs further assessment	

AFB QC Slide and Personnel Competency - RISHL

- RISHL
  - Alpha Tec AFB QC1 slide with every staining run (FA and Kinyoun)
    - Positive control well *Mycobacterium scrofulaceum*
    - Negative control well *Escherichia coli*
  - Personnel Competency
    - Initial, six months, one year and then yearly (AFB processing) and 2x/year (AFB smear)

Contamination Rates – Michigan

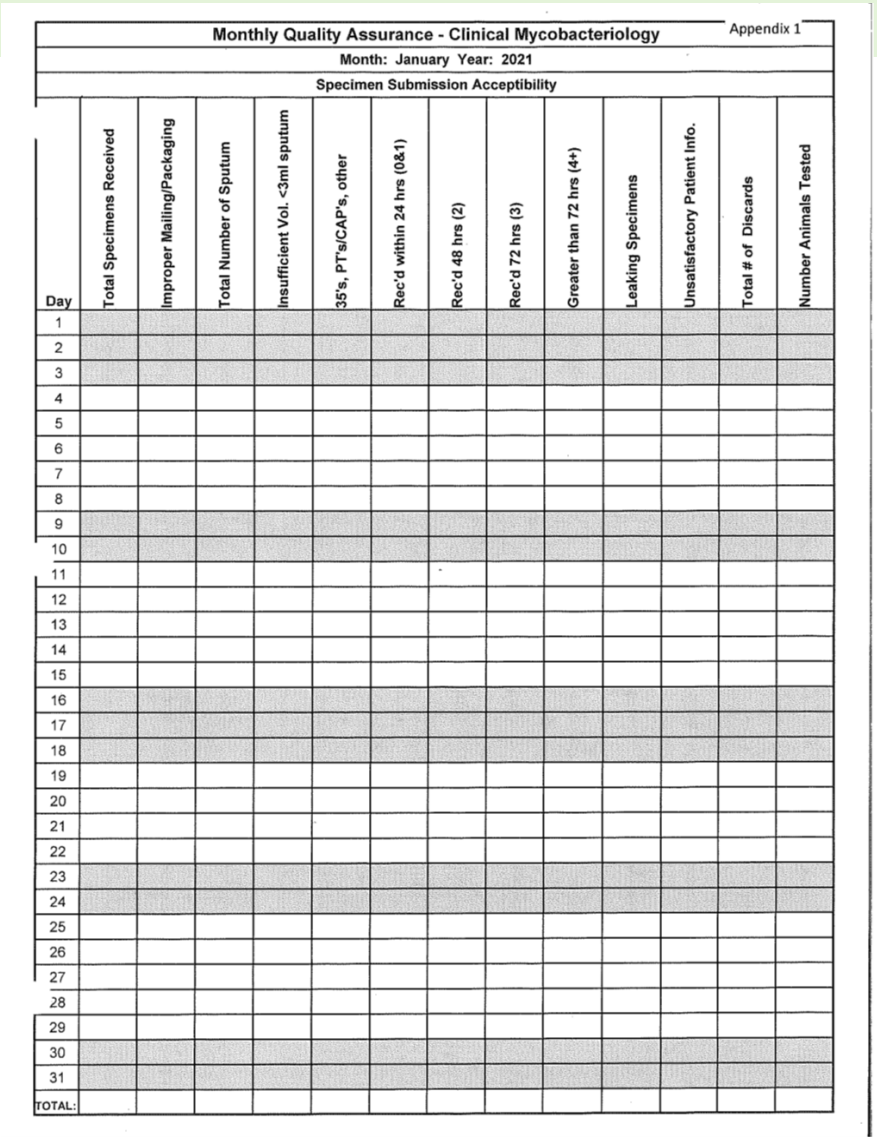
Contamination Rate Monitoring
Year: 2025

Month	Number Specimens Processed	Number Isolation Exam Unsat	% Contamination	Action Taken
January	709	8	1.13%	none
February	639	6	0.94%	none
March	869	14	1.61%	none
April	852	17	2.00%	none
May	701	9	1.28%	none
June	647	12	1.85%	none
July	613	20	3.26%	none
August	645	13	2.02%	none
September	571	21	3.68%	none
October	938	24	2.56%	none
November	561	8	1.43%	none
December	659	11	1.67%	none

Turnaround Times – Michigan

The following testing parameters are monitored to assure specimen quality, proper testing conditions, prompt result reporting, safe testing conditions, and rapid follow-up on errors.

- Clinical Specimen Submission
- Improper mailing / packaging
- Insufficient volume of specimen (<3ml of sputum)
- Specimens received from date of collection at 1, 2, 3 and >3 days
- Leaking specimens
- Unsatisfactory patient information
- Total number of specimen discards
- Daily delivery time



Monthly Quality Assurance - Clinical Mycobacteriology — Appendix 1

Month: January Year: 2021

Specimen Submission Acceptibility

Day	Total Specimens Received	Improper Mailing/Packaging	Total Number of Sputum	Insufficient Vol. <3ml sputum	35's, PT's/CAP's, other	Rec'd within 24 hrs (0&1)	Rec'd 48 hrs (2)	Rec'd 72 hrs (3)	Greater than 72 hrs (4+)	Leaking Specimens	Unsatisfactory Patient Info.	Total # of Discards	Number Animals Tested
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
12													
13													
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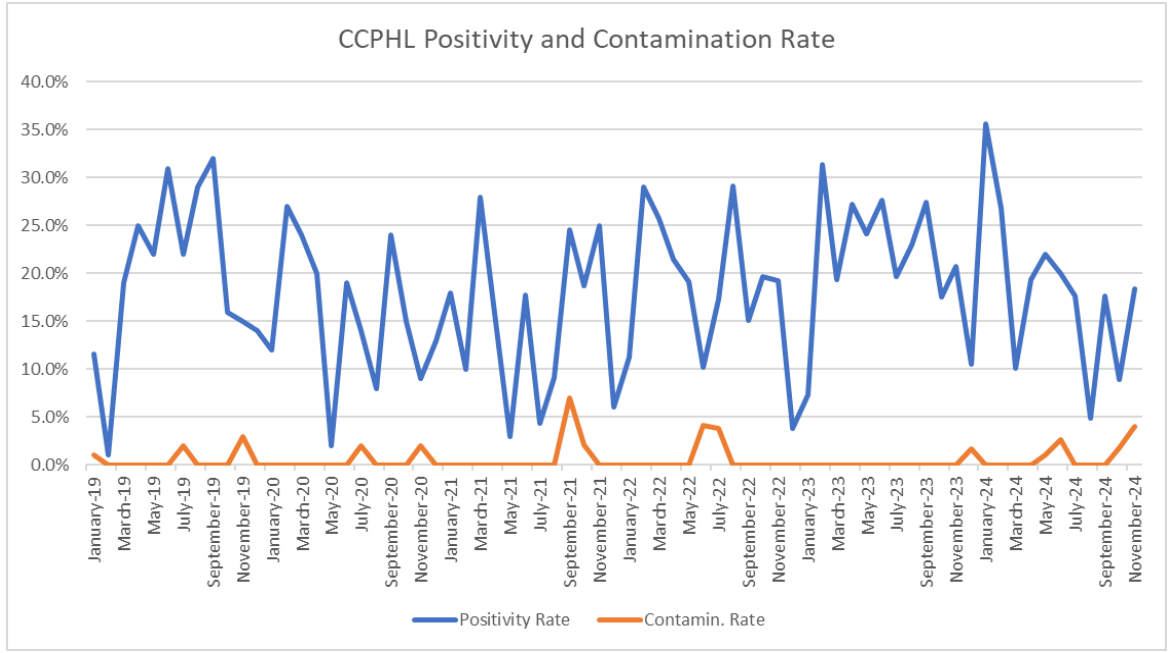
26													
27													
28													
29													
30													
31													
TOTAL:													

Contamination Rates and Turnaround Times - CCPHL

- Contamination and positivity rate is checked quarterly (ideally monthly)
- Smear and MTB PCR results within 24 hours of collection
- Negative cultures in 6 weeks
- Positive cultures in ~4-8 weeks depending on growth
- DSTs in ~2-4 weeks after MTBC ID, depending on growth



Contamination Rates and Turnaround Times - RISHL

- Contamination rates calculated every 6 months (moving to change to every month)
  - Calculate individual rates for MGIT tubes and 7H11 plate
  - Calculate overall reported contamination rate
- TAT
  - Smear - 1 business day (from receipt in lab)
  - TB PCR - 2 business days (from receipt in lab or from add-on request)

	Months	Year		
AFB Contamination Rate:	Jan-Jun	2025		
			Percent (%)	Comment
Total AFBC (per TB grant stats)		1586		
Contaminated 7H11 plates:		79	5.0	
Contaminated MGIT tubes:		82	5.2	
Overall Contamination Rate (reported as cont)		64	4.0	


CONTRA COSTA
HEALTH


MDHHS
Michigan Department of Health & Human Services


RHODE ISLAND
DEPARTMENT OF HEALTH

Thank you!


APHL | ASSOCIATION OF PUBLIC HEALTH LABORATORIES