Mycobacteriology Service Implementation

Module 2
Version 2019

Sheryl R. Stuckey, MLS (ASCP)
Manager, Microbiology Laboratory
Holy Cross Hospital
Silver Spring, Maryland

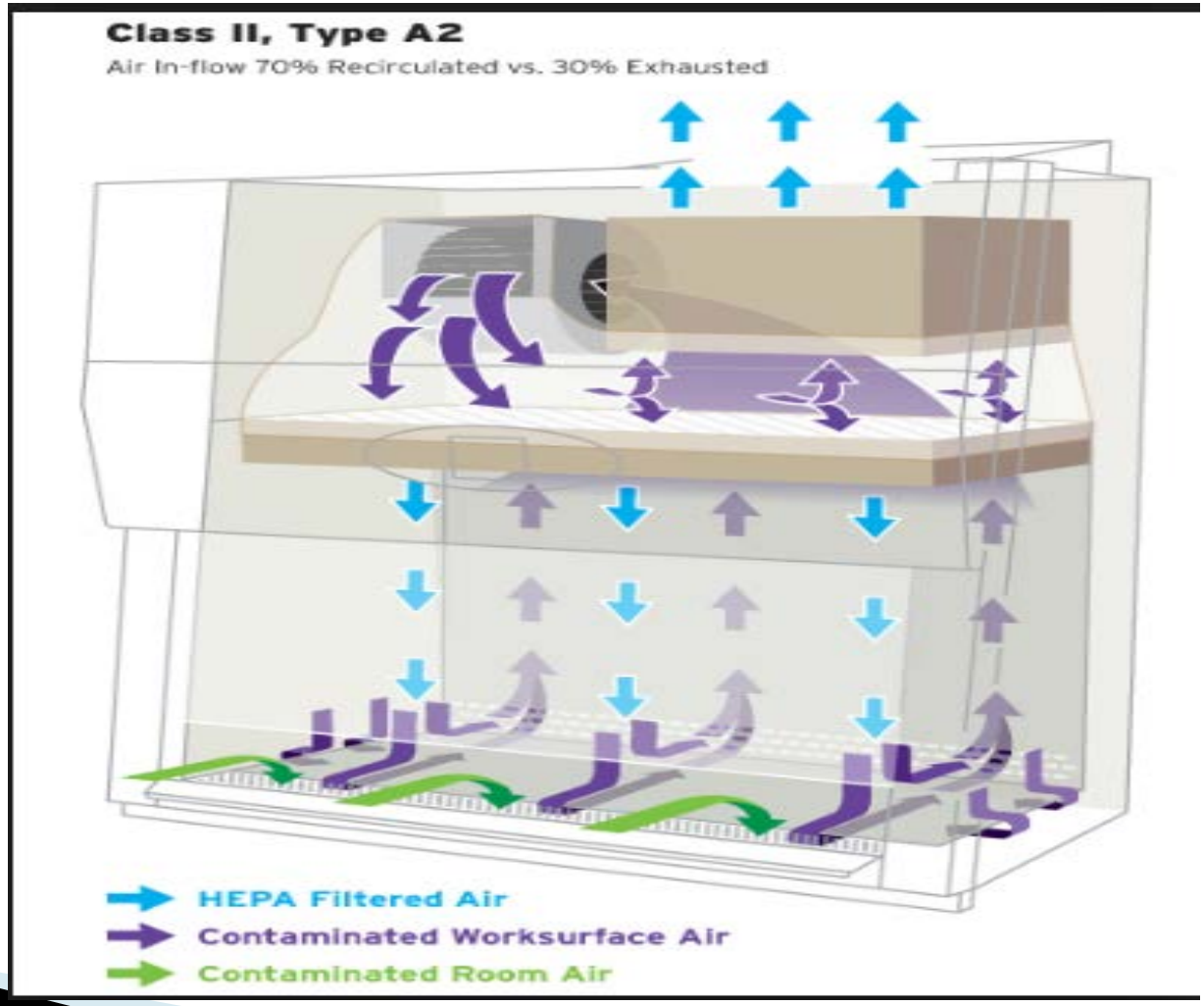
YOU ARE ASSIGNED TO PROVIDE TB SERVICES

- Now what?

Equipment

- Biological Safety Cabinet (BSC) – Class II A2
  - This is responsible for maintaining the room exhaust – ducted to outside via canopy (thimble connection)
  - Redundant exhaust fans on the roof
  - 70% recirculation through HEPA filters
  - Check exhaust airflow, lights and electrical functions daily and before each use
  - Performance certified at least annual (See label)
  - Wipe down workspace with approved disinfectant at least before and after use

Nuaire Class II, Type A2 BSC



Equipment

- Refrigerated Centrifuge (≤20°C)
  - Centrifugation creates heat friction temperatures that may kill some mycobacteria
  - Uses weighted and matched sealed carriers for biocontainment (by 50 ml tube)
  - Verify centrifugation uses ‘g’ forces instead of rpms
  - **ALWAYS OPEN SEALED CARRIERS IN THE BSC**
  - Performance certified at least annual (See label)
  - Wipe down workspace with approved disinfectant at least before and after use

Equipment

- Slide Warmer(≥65°C) –
  - Slides heat-fixed for at least 2 hours (viability, consistency of smear, workflow may affect this)
  - Check temperature just before use
- BACTEC MGIT 960 with EpiCenter –
  - Perform daily checks as prescribed by the manufacturer
  - Perform monthly check/cleaning of the instrument filters
- Autoclave – Gravity Steam without Vacuum
  - Perform sterility check each day of use
  - Document parameters met each use (tape on machine)

Routine Duties

- Check/document airflow indicators
- Check/Document all atmospheres/ temperatures for the room and equipment
- Check inventory of supplies to be used for the day's work
- Verify receiving and plating logs
- Stain, read and report smears
- Check new cultures for rapid growers and contamination
- Check centrifuge carrier O-rings
- Perform specimen processing
- Pull positive/negative MGIT tubes and print/file unloaded reports
- Process positive MGIT tubes for Sendout, if needed

Daily

- Wipe down the interior of the centrifuge and the weighted carriers
- Review cultures and forward reports:
  - Prelim: Culture in Progress
  - Interim: No growth – 3 weeks
  - Final: No growth – 6 weeks

Weekly

Specimen Collection, Handling and Transport

Tuberculosis
Not Just A Respiratory Problem

- Sputum (Expectorated or induced)
- Bronchoalveolar lavage (BAL)
- Bronchial wash/brush
- Transtracheal Aspirate

- Tissue
- Body fluids
- Blood Stool
- Gastric Lavage
- Urine
- **Endobronchial Ultrasound Sample (EBUS)**

**May be tissue or fluid

Respiratory Specimens

Non-Respiratory Specimens

Respiratory Specimens (Pulmonary)

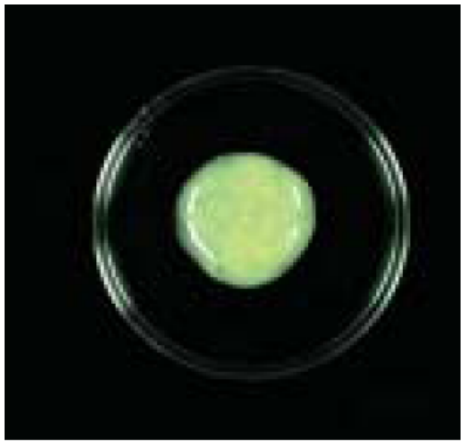
- Sputum (Expectorated/Induced)
- Bronchoalveolar Lavage
- Bronchial Washing
- Bronchial Brush
- Transtracheal aspirate

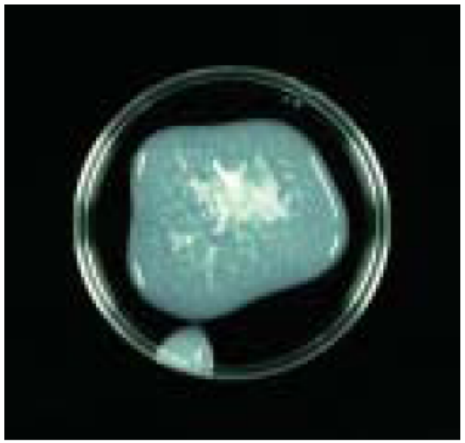
Sputum

- Expectorated- generated from deep productive cough; 1st morning best specimen
- Induced – produced with hypertonic saline to irritate lungs and force cough when patient non-productive

Sputum Quality

Thick, Mucopurulent





Watery (acceptable if induced)

Hemoptysis (Bloody Sputum)

Salivary

What are we up against?
The Normal Respiratory Flora Factor

The amount of normal respiratory flora doubles every 15 – 20 minutes.

The amount of *Mycobacterium tuberculosis* doubles every 12 – 24 hours

Common Respiratory Flora	*Mycobacterium tuberculosis*

Collection Guidance – Pulmonary Specimens Other Than Sputum

Bronchoalveolar Lavage,
Bronchial washing,
Endotracheal aspirate,
Transtracheal aspirate:

Collect a minimum volume of 3 mL in a sputum trap or sterile screw cap container

Place the brush in a sterile leak-proof container with a maximum of 5 mL of sterile saline

Aspirates/Washings	Bronchial brush

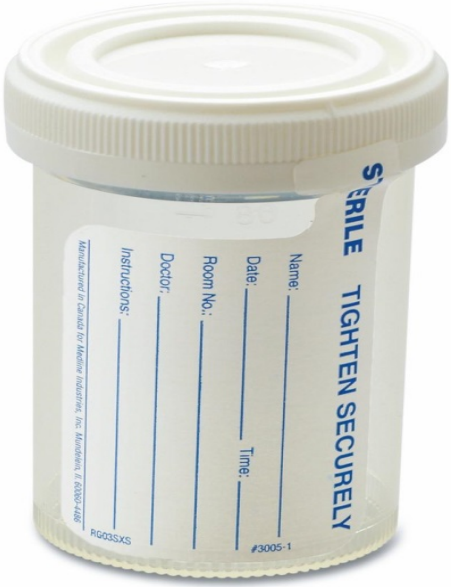
Collection Containers

Lukens Trap

Sputum Trap

Collection Containers

Sputum Collection Container



Sterile Screw Cap Container

Non-Respiratory Specimens (Extrapulmonary Specimens)

- Tissue
- Body Fluids
- Blood
- Stool
- Gastric Lavage
- Urine
- Endobronchial Ultrasound Samples (EBUS)

Non-Respiratory Specimens (Extrapulmonary)

- Specimens may be collected from non-sterile body sites (gastrointestinal tract, urine, external or trauma tissue)
- Specimens may be collected from sterile body sites or under presumed sterile conditions (CSF, sterile body fluid, OR specimens)
- Specimens may be collected in a manner that exposes them to contamination (Blood cultures)

Non-Respiratory Specimens (Extrapulmonary)

- Specimens should be collected in sterile leak-proof containers
- Specimens should be transported to the laboratory immediately upon collection; ideally within 24 hours with proper storage
- Gastric lavage samples must be buffered within one hour of collection (Acid kills mycobacteria)
- Growth of M. tuberculosis may be inhibited if blood specimens contain EDTA
- Swabs should be rejected (mycobacteria are hydrophobic)
  - EXCEPTION: Laryngeal swab made of calcium alginate wool (This will dissolve in the digestant)

Extrapulmonary Collection Guidance

Specimen from Non-Sterile Body Sites	Recommended Collection Time	Volume Requirements	Collection Frequency	Transport	Recommended for Isolation of MTBC?
Gastric Aspirate	Early morning before patient eats and while still in bed	5–10 ml is optimal; maximum volume is 15 ml	One specimen per day on three consecutive days	Room temperature; if delayed >1 hour, neutralize with 100 mg sodium carbonate	Yes
Urine	First morning specimen (void midstream)	10–15 ml minimum; prefer up to 40 ml	One specimen per day on three consecutive days	If delayed >1 hour, refrigerate	Yes
Stool	No recommendation	Minimum volume is 1 gram	No recommendation	Refrigerate if delayed >1 hour, do not freeze	Mainly for diagnosis of disseminated MAC disease in patients with AIDS

17

Extrapulmonary Collection Guidance

Specimen from Normally Sterile Body Sites	Volume Requirements	Transport	Recommended for Isolation of MTBC?
Cerebral Spinal Fluid	10 ml is optimal; minimum volume is 2-3 ml	As soon as possible at room temperature; do not refrigerate	Usually paucibacillary; culture may have limited sensitivity
Other Body Fluids (pleural, peritoneal, pericardial, synovial)	10-15 ml is optimal; minimum volume is 10 ml	If delayed, refrigerate	Yes
Tissues or Lymph Nodes	As much as possible; add 2-3 ml sterile saline	As soon as possible at room temperature (no formalin, preservatives, or fixatives)	Yes
Blood	10ml preferred, minimum 5 ml. Collect in SPS or heparin tube, no EDTA	At room temperature, do not refrigerate or freeze	Mainly for diagnosis of disseminated MAC disease in patients with AIDS

18


to be continued