TB Clinical and Reference Laboratory

Outreach Workers Training April 2019

DCLS

Learning Objectives

- Understand the collection procedure for expectorated sputum specimens.
- Understand the labeling and shipping requirements for sputum specimens.
- Understand collection interval requirements
- General understanding of laboratory testing and reporting intervals.
- Understand the difference between DNA Probe and NAAT test.

Testing results are only as good as the quality of specimen collected

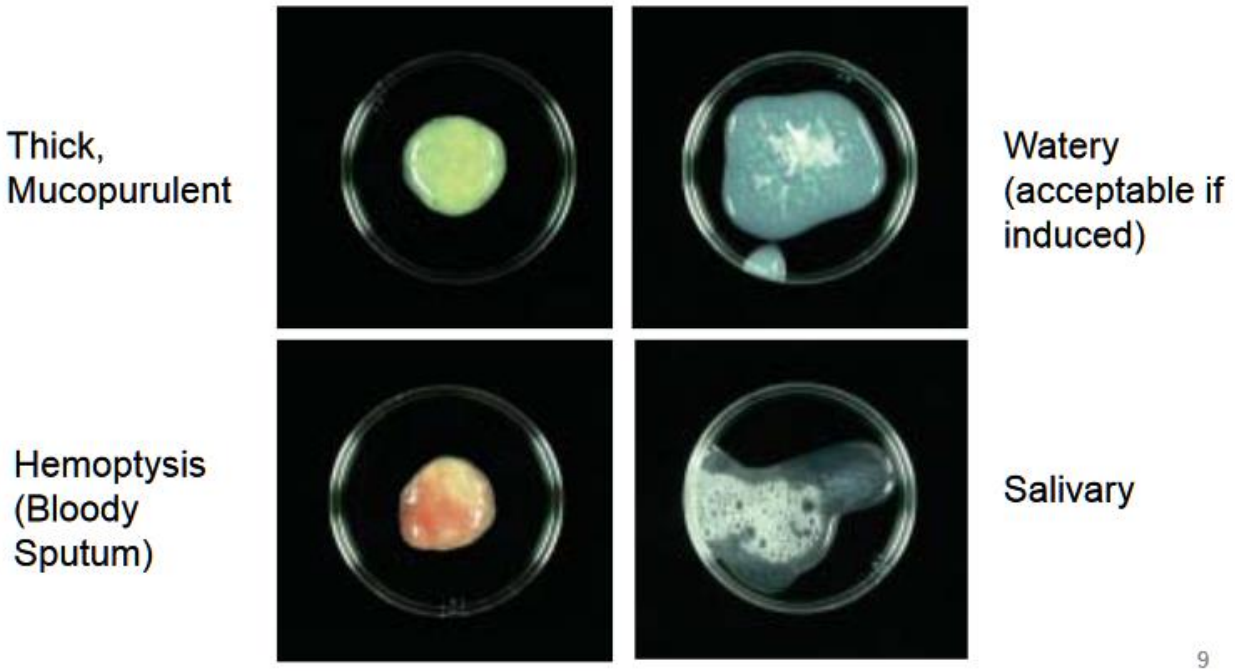
Respiratory

- **Sputum**
  - Expectorated
  - Induced
- Bronchoalveolar Lavage
- Bronchial Wash
- Bronchial Brushing
- Transtracheal aspirate

Non-Respiratory

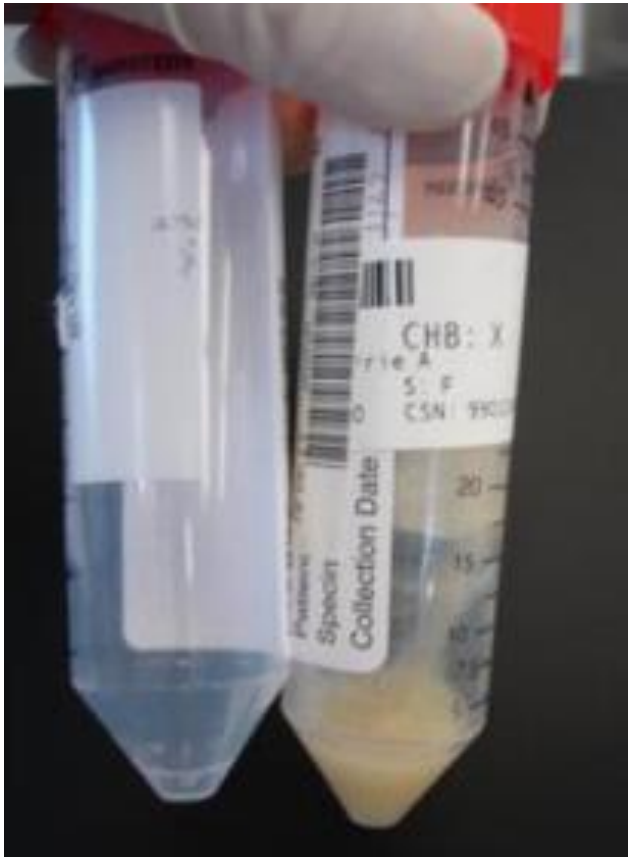
- Blood
- Bone Marrow
- Body Fluids
- Tissue
- Urine
- Gastric Lavage

Specimen Collection: Sputum

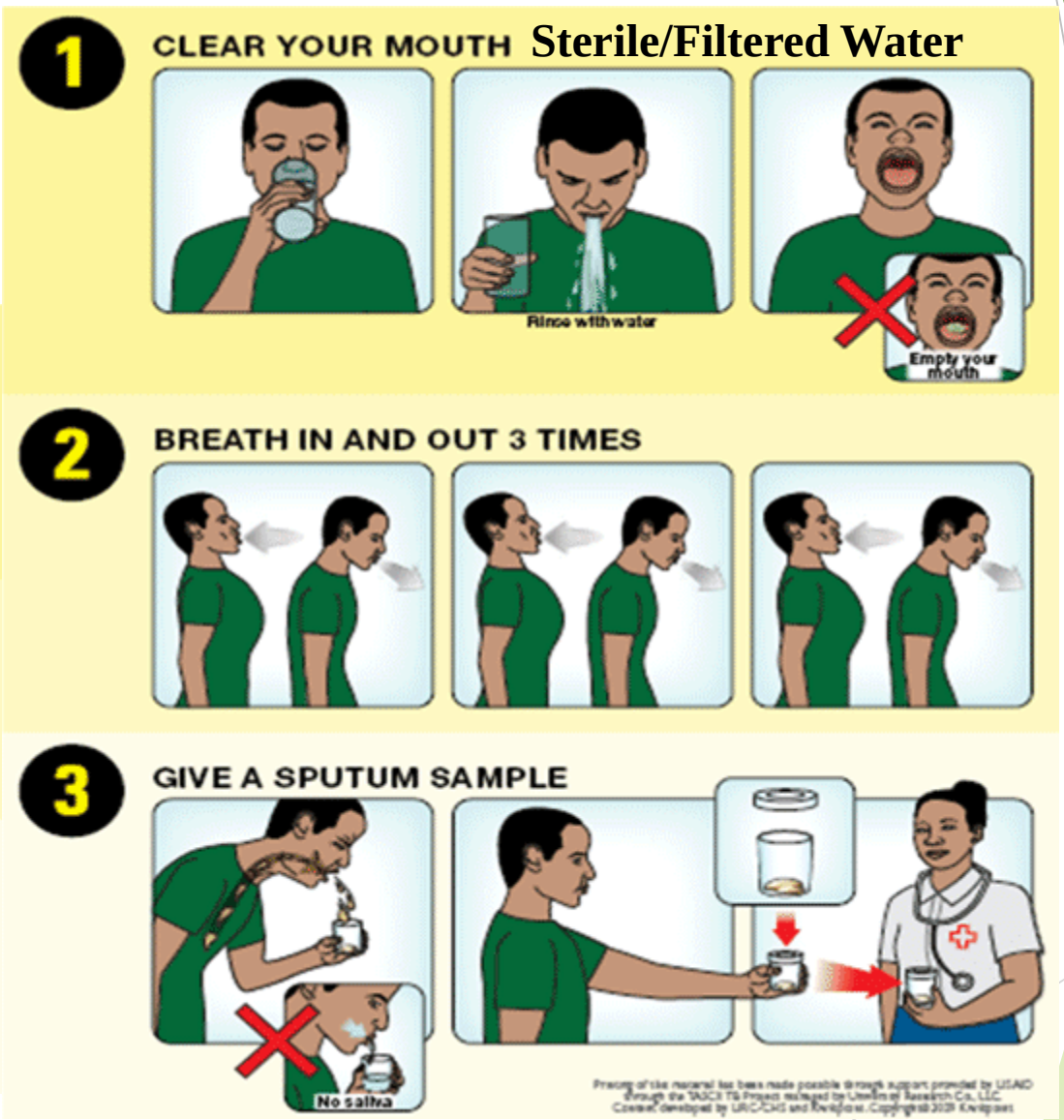


Sputum is generated by a DEEP productive cough.

Specimen Collection: Sputum



- Establishes an initial diagnosis of TB
- Monitor the infectiousness of the patient
- Determine effectiveness of treatment


1
CLEAR YOUR MOUTH
Sterile/Filtered Water
Rinse with water
Empty your mouth
2
BREATH IN AND OUT 3 TIMES
3
GIVE A SPUTUM SAMPLE
No saliva

Specimen Labeling:

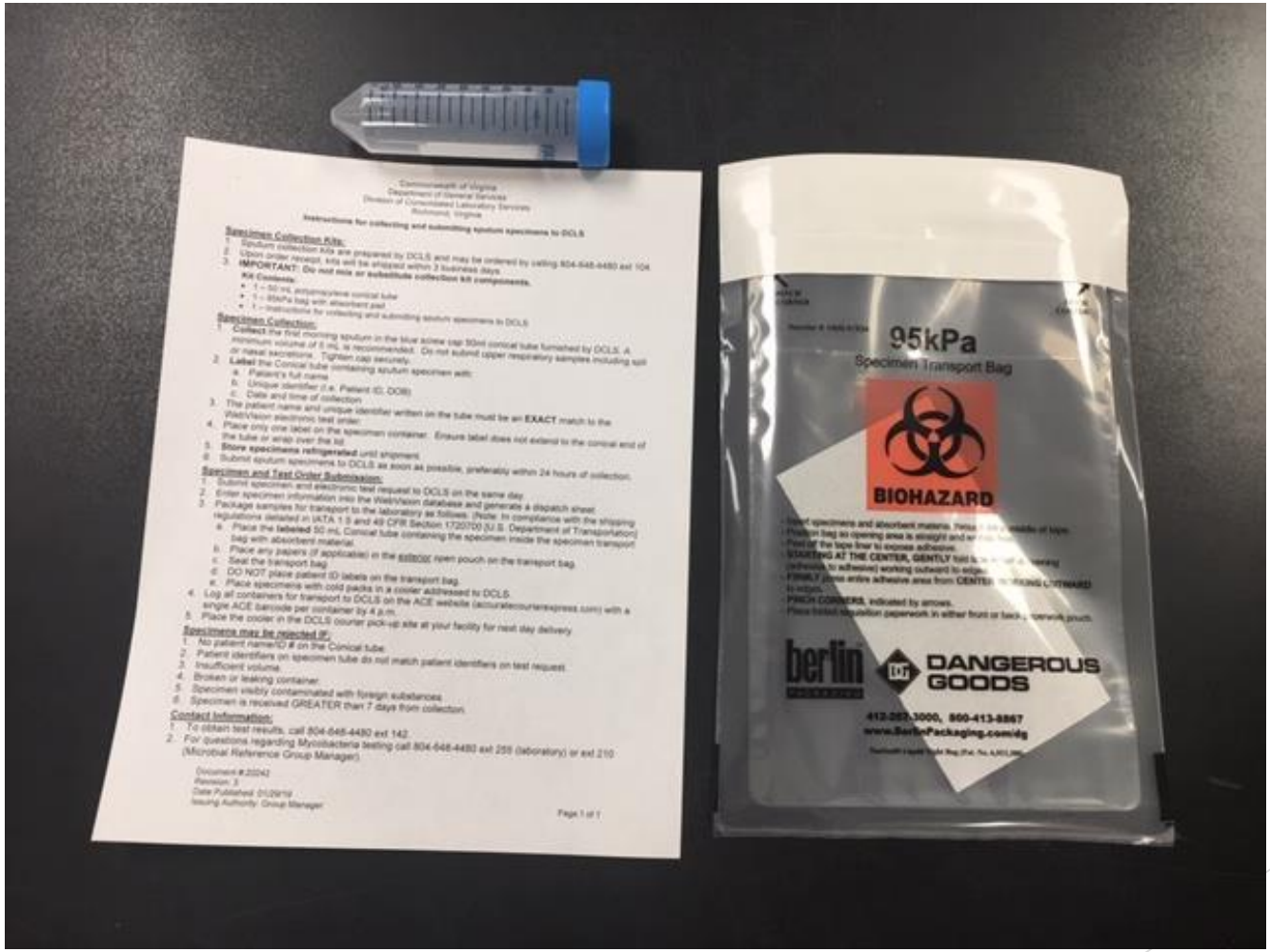
- Full Patient Name
- Unique Sample Identifier (WebVision patient ID, DOB etc).
- Collection date and time

EXACT

match to the Web Vision form!!

Specimen must be dispatched into WV in order for DCLS to accept and process the specimen.

Package Specimen for Transport



One conical tube per biohazard bag

Specimen Transport

Appropriate transport method.

Not Approved

Reminder: Biohazard bag must be sealed prior to transport!

Transport to the Laboratory: Respiratory specimens

4°

Goal

Collection Instructions Form

Commonwealth of Virginia
Department of General Services
Division of Consolidated Laboratory Services
Richmond, Virginia

Instructions for collecting and submitting sputum specimens to DCLS

Specimen Collection Kits:

1. Sputum collection kits are prepared by DCLS and may be ordered by calling 804-648-4480 ext 104.
2. Upon order receipt, kits will be shipped within 3 business days.
3. **IMPORTANT: Do not mix or substitute collection kit components.**

   Kit Contents:
   - 1 – 50 mL polypropylene conical tube
   - 1 – 95kPa bag with absorbent pad
   - 1 – Instructions for collecting and submitting sputum specimens to DCLS

Specimen Collection:

1. **Collect** the first morning sputum in the blue screw cap 50ml conical tube furnished by DCLS. A minimum volume of 5 mL is recommended. Do not submit upper respiratory samples including spit or nasal secretions. Tighten cap securely.
2. **Label** the Conical tube containing sputum specimen with:
   a. Patient's full name
   b. Unique identifier (i.e. Patient ID, DOB)
   c. Date and time of collection
3. The patient name and unique identifier written on the tube must be an **EXACT** match to the WebVision electronic test order.
4. Place only one label on the specimen container. Ensure label does not extend to the conical end of the tube or wrap over the lid.
5. **Store specimens refrigerated** until shipment.
6. Submit sputum specimens to DCLS as soon as possible, preferably within 24 hours of collection.

Specimen and Test Order Submission:

1. Submit specimen and electronic test request to DCLS on the same day.
2. Enter specimen information into the WebVision database and generate a dispatch sheet.
3. Package samples for transport to the laboratory as follows: (Note: In compliance with the shipping regulations detailed in IATA 1.5 and 49 CFR Section 1720700 [U.S. Department of Transportation]
   a. Place the **labeled** 50 mL Conical tube containing the specimen inside the specimen transport bag with absorbent material.
   b. Place any papers (if applicable) in the exterior open pouch on the transport bag.
   c. Seal the transport bag.
   d. DO NOT place patient ID labels on the transport bag.
   e. Place specimens with cold packs in a cooler addressed to DCLS.
4. Log all containers for transport to DCLS on the ACE website (accuratecourierexpress.com) with a single ACE barcode per container by 4 p.m.
5. Place the cooler in the DCLS courier pick-up site at your facility for next day delivery.

Specimen Rejection Reasons

- No patient name/identifying information
- Patient name/identifier discrepancy between specimen and test request
- Insufficient sample volume
- Specimen leaked during transit
- Specimen visibly contaminated with foreign substance
- Specimen too old; >7 days from collection date
- Specimen with no test request form or test request with no specimen

Specimen Processing

Respiratory specimens are inoculated onto a LJ, MGIT and receive a Fluorochrome smear

Acid Fast Bacilli

LIMITATION

- Not specific for mycobacteria
- Cannot differentiate *M. tuberculosis* from other nontuberculosis mycobacteria.
- Lacks sensitivity compared to culture
- Negative result does not rule out mycobacterial infection

Fluorochrome Stain Results

Report It

- When no AFB are observed:

  "NEGATIVE - No acid-fast bacilli seen"

- When AFB observed:

  "POSITIVE 3+ acid fast bacilli seen (10-90 afb per 10 fields at 250x)"

- Positive smear reported by phone same day sample received.

PRELIMINARY REPORT

Microscopy Report **Date Released: 03/18/2019**

Fluorochrome : POSITIVE - 1+ acid fast bacilli seen (1-9 afb per 10 fields at 250x)

Results phoned to

Contact Name	Date	Called by	Comments
ALEXANDRIA HEALTH DEPARTMENT	03/18/2019	Savannah McReynold	LEFT MESSAGE FOR EMILY A. TO CALL BACK FOR SMEAR RESULT

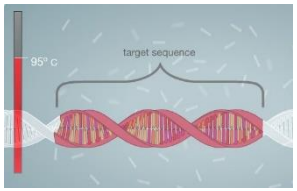
Virginia Department of Health, Tuberculosis and Newcomer Health Program Recommended Sputum Sample Collection Schedule for Monitoring Smear and Culture Conversion in Pulmonary Cases

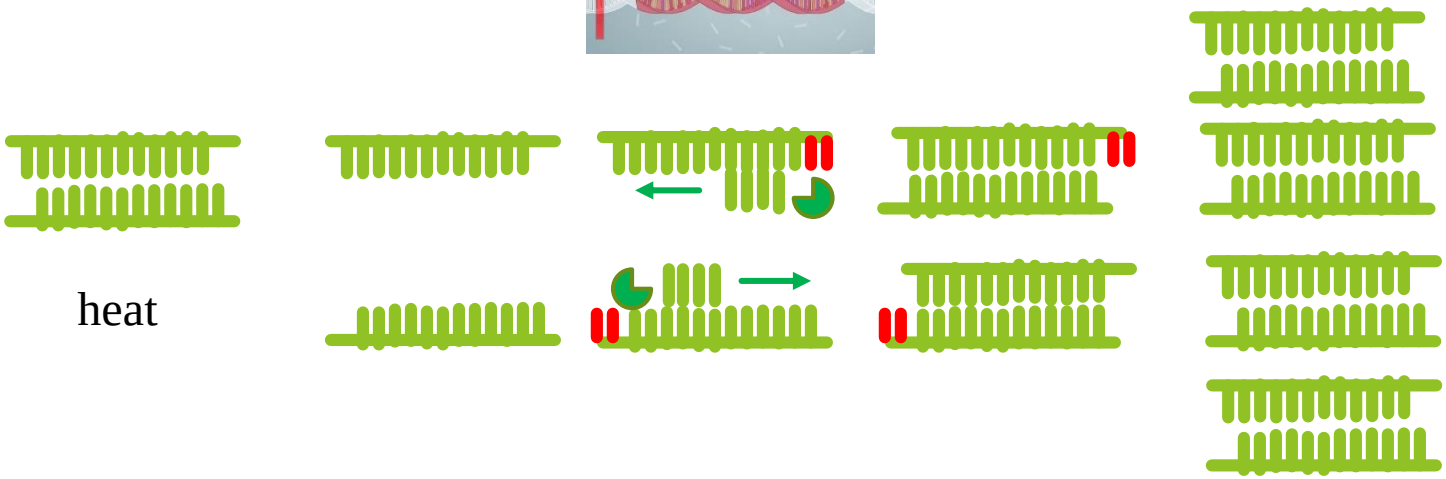
Purpose	Monitoring	Frequency	Number of specimens	Comments
Determine infectiousness **and** Confirmation of TB disease	Initial contact with client	Collect 3 consecutive specimens	Minimum of 3 samples, with one collected in the early morning. If diagnosis was **confirmed** before the client is reported, collect 3 additional specimens to determine if infectious.	At least one specimen collection should be observed / coached by HD staff. At minimum, samples should be at least 8 hours apart.
Establish the earliest date a client can be considered non-infectious and can be removed from isolation	Smear conversion or smear improvement	Collect one sputum specimen every 7–10 days; with maximum of 3/month One specimen should be collected 55-60 days after treatment initiation If it is urgent to remove from isolation, upon the first negative smear follow with collecting one every other day. If any have a positive smear resume 7-10 day frequency	Total number of specimens will vary from client to client. When there is evidence of increasing difficulty with spontaneous sputum production collect a specimen every 7 days, not every 10 days	Single specimens should be observed by HD staff. Collecting a specimen 55 – 60 days after treatment initiation provides valuable information about treatment response **Additional criteria to release from isolation** "Controlling Tuberculosis in the United States," 11/4/2005, Vol. 54, No. RR- 12, Page 9, Box 3
Monitor for response to treatment **and** Determine the need for extension of treatment	Culture conversion	One sample every 7 – 10 days, with maximum of 3/month, until 2 consecutive sputum cultures are negative with no positive culture results thereafter. **Continue monthly collection until treatment completion for:** Rifamycin resistance; MDR/XDR-TB; HIV+	Until 2 consecutive sputum cultures are negative with no positive culture results thereafter.	Single specimens should be observed by HD staff. If unable to produce sputa spontaneously. Several 30 minute induction attempts on different days, including early AM, should be undertaken before deciding that a client can no longer produce sputum.

Cepheid GeneXpert

1. Automates specimen processing
2. Nucleic Acid Amplification
3. Detection of the target sequence
    a. MTBC
    b. RIF Resistance
4. Test sputum specimen

Nucleic Acid Amplification= PCR





This cycle happens again and again until there are millions of copies of the DNA of interest.

GeneXpert Accepted Specimen Types

- Expectorated or Induced Sputum
- DCLS performs testing on DCLS processed sputum sediments only. Minimum volume requirement 0.5ml
- First time smear positive patients are automatically run on GeneXpert
- Smear negative will be run upon request form VDH TB Control if other specimen criteria are met.

NAA

Advantages

- Rapid 2-3 hours
- Direct from Clinical specimen
- Increased sensitivity over AFB stain

Disadvantages

- Specimen may contain amplification inhibitors (false negative)
- Detects non-viable MTBC
- Negative test does not exclude possibility of isolating MTBC from sputum culture
- GeneXpert has not been validated for samples from pediatric patients

GeneXpert Specimen Rejection

The sample *WILL NOT* be run on GeneXpert IF:

- The sample does not meet the TB drug criteria (LESS THAN 3 DAYS)
- The patient is pediatric (less than 18 y)
- Specimen visibly contaminated with foreign substances
- Insufficient volume

Report It

PRELIMINARY REPORT

GENEXpert **Date Released :** 5/31/16 16:22

Mycobacterium tuberculosis complex DNA detected by direct specimen Nucleic Acid Amplification Test.
No rpoB gene mutations detected by direct specimen Nucleic Acid Amplification Test; probably Rifampin susceptible. Conventional drug susceptibility testing will follow.
Comment : Results from the MTB/RIF test should be interpreted in conjunction with other laboratory and clinical data. If test results do not match clinical signs and symptoms, additional testing may be warranted . A result of "Mycobacterium tuberculosis complex DNA Not Detected " does not exclude the possibility of isolating a Mycobacterium tuberculosis complex organism from the specimen . Additionally, a result of "No rpoB gene mutations detected; probably Rifampin susceptible " does not exclude the possibility of Rifampin resistance. Test results may be affected by inhibitors and variability in specimen collection and transport .

Results phoned to

Contact Name	Date	Called by	Comments
LOUDOUN COUNTY H D - RESEARCH PLACE	05/31/2016	Randy Oglesby	LEFT MESSAGE FOR ERLIN TO CALL FOR GENEXPERT RESULT
LOUDOUN COUNTY H D - RESEARCH PLACE	06/01/2016	Randy Oglesby	GAVE GENEXPERT RESULT TO SARAH W

Culture

LJ: solid media

MGIT: liquid media

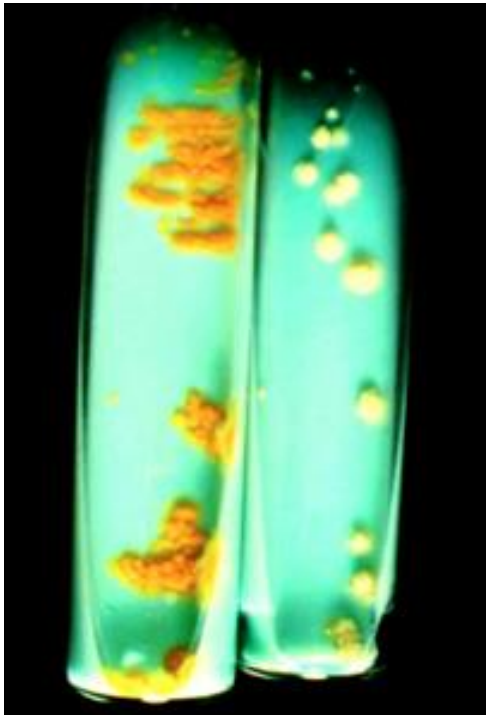
MTBC Growth rate: 12-28 days

Colony Morphology

M. tb

M. avium complex

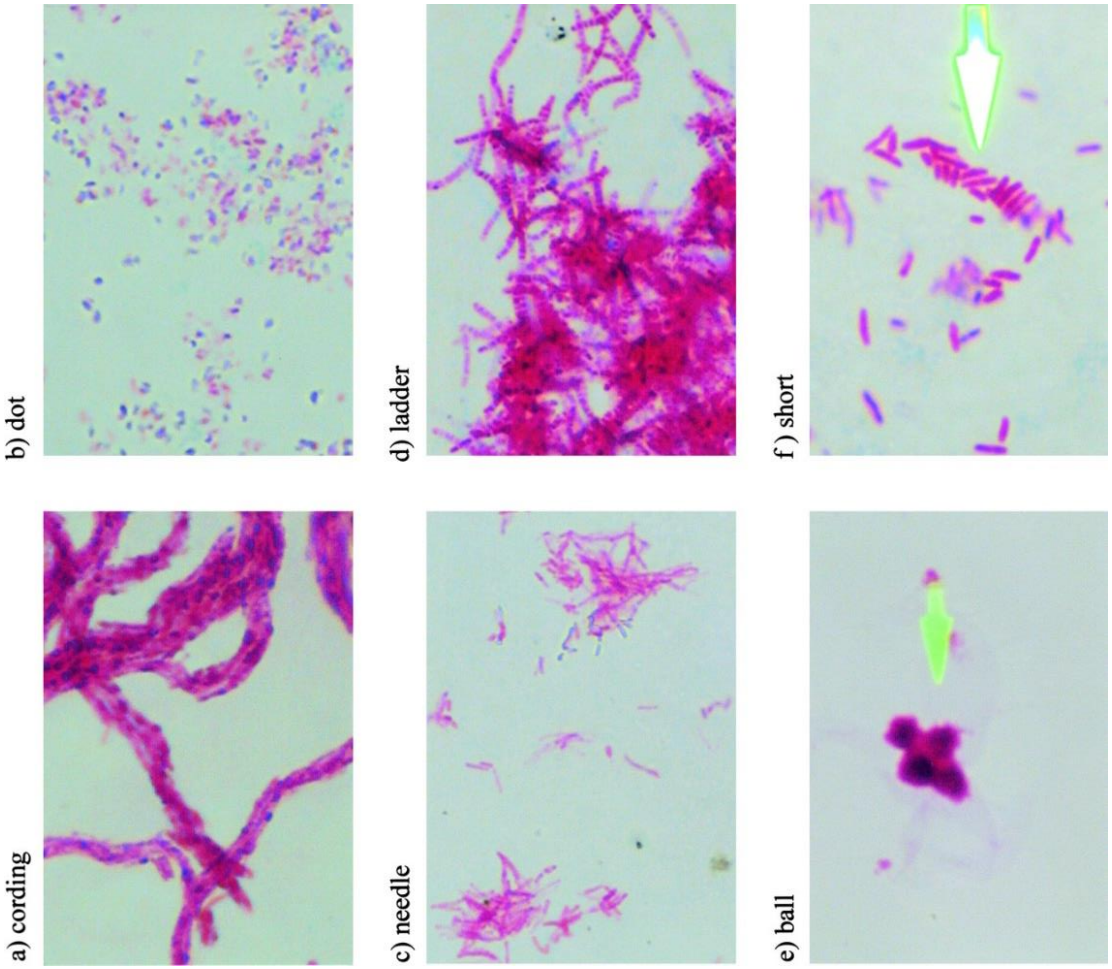
M. gordonae



M. kansasii

M. marinum

Kinyoun Stain



All Positive cultures are inoculated onto new media and read for 3 weeks

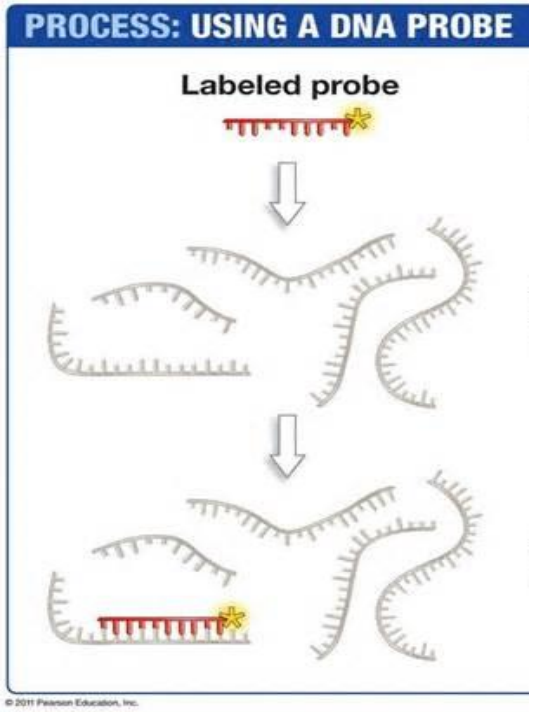
Another Preliminary Report

- If positive culture growth is confirmed as AFB by Kinyoun stain, laboratory reports:

  “Acid–fast bacilli observed: Identification to follow”

Identification – DNA Probe

- Test uses complimentary DNA to detect specific species of mycobacteria
  - MTBC and MAC
  - Test requires growth of the bacteria
- Does not amplify the DNA (only detects presence or absence)



If the probe finds a match that is complimentary, a detectable signal is generated.

Positive Probe Results

- *M. avium complex*
- *M. tb complex*
  - MTBC positive probe results are called to the HD.

PRELIMINARY REPORT

Mycobacterial DNA Probe **Date Released :** 6/8/16 10:25

M.tb complex probe : Positive
Drug susceptibility testing to follow .
Mycobacterium tuberculosis complex includes Mycobacterium tuberculosis, Mycobacterium bovis, and Mycobacterium africanum, all of which cause the clinical syndrome, tuberculosis . All laboratory results should be interpreted in conjunction with clinical findings .

Results phoned to

Contact Name	Date	Called by	Comments
LOUDOUN COUNTY H D - RESEARCH PLACE	06/07/2016	Savannah McReynolds	GAVE M.TBC PROBE RESULT TO SARAH WASHINGTON.

Antimycobacterial Susceptibility Testing (AST)

- Bactec MGIT 960
  - 4-13 day qualitative test

- Based on the growth of the MTBC isolate in the presence of a drug vs. growth in the absence of the drug

First Line:

- Streptomycin 1.0 ug/mL
- Isoniazid 0.1 ug/mL
- Rifampin 1.0 ug/mL
- Ethambutol 5.0 ug/mL
- Pyrazinamide 100 ug/ml

If resistant first line (except Streptomycin) reflex to…..

Second Line:

- Capreomycin 3.0 ug/mL
- Ofloxacin 1.5 ug/mL
- Ethionamide 5.0 ug/mL
- Isoniazid 0.4 ug/mL

AST

- Sensitive, Presumptive Resistant, Resistant, or Pending
- Name and concentration of drug
- Resistant first line drugs initiate reflex to second line drug testing and shipment of isolate to CDC for additional drug studies
- All resistant drug results are reported as "Presumptive Resistant, Confirmation to Follow" until results are confirmed

Report It

PRELIMINARY REPORT

Drug Susceptibility Report

1st Line DST **Date Released : 06/29/2016**

Streptomycin 1.0 ug/mL : SENSITIVE

Isoniazid 0.1 ug/mL : SENSITIVE

Rifampin 1.0 ug/mL : SENSITIVE

Ethambutol 5.0 ug/mL : SENSITIVE

Pyrazinamide 100 ug/mL : SENSITIVE

FINAL REPORT

Final Conclusion **Date Released :** 7/8/16 11:57

4+ growth Mycobacterium tuberculosis complex

Non Tuberculous Mycobacteria (NTM)

- NTM also cause significant human disease
- Almost all of these species are found in the environment and are opportunistic pathogens in humans
- Clinicians are ultimately responsible for determining the importance of a non-tuberculosis mycobacterial (NTM) isolate
- Recovery of environmental mycobacterial from clinical specimens does not always signify pathogenesis
  - *M. mucogenicum:* common in tap water and in catheter related infections
  - Almost never causes chronic lung disease.
  - Usually considered a contaminant in sputum

FINAL REPORT

Final Conclusion **Date Released : 09/21/2016**

Mycobacterium fortuitum group identified by 16S rRNA gene sequence analysis .

Disclaimer: This test has not been cleared or approved by the U .S. Food and Drug Administration . The results from this assay should not be used independently to make decisions regarding the management of patient care or public health .

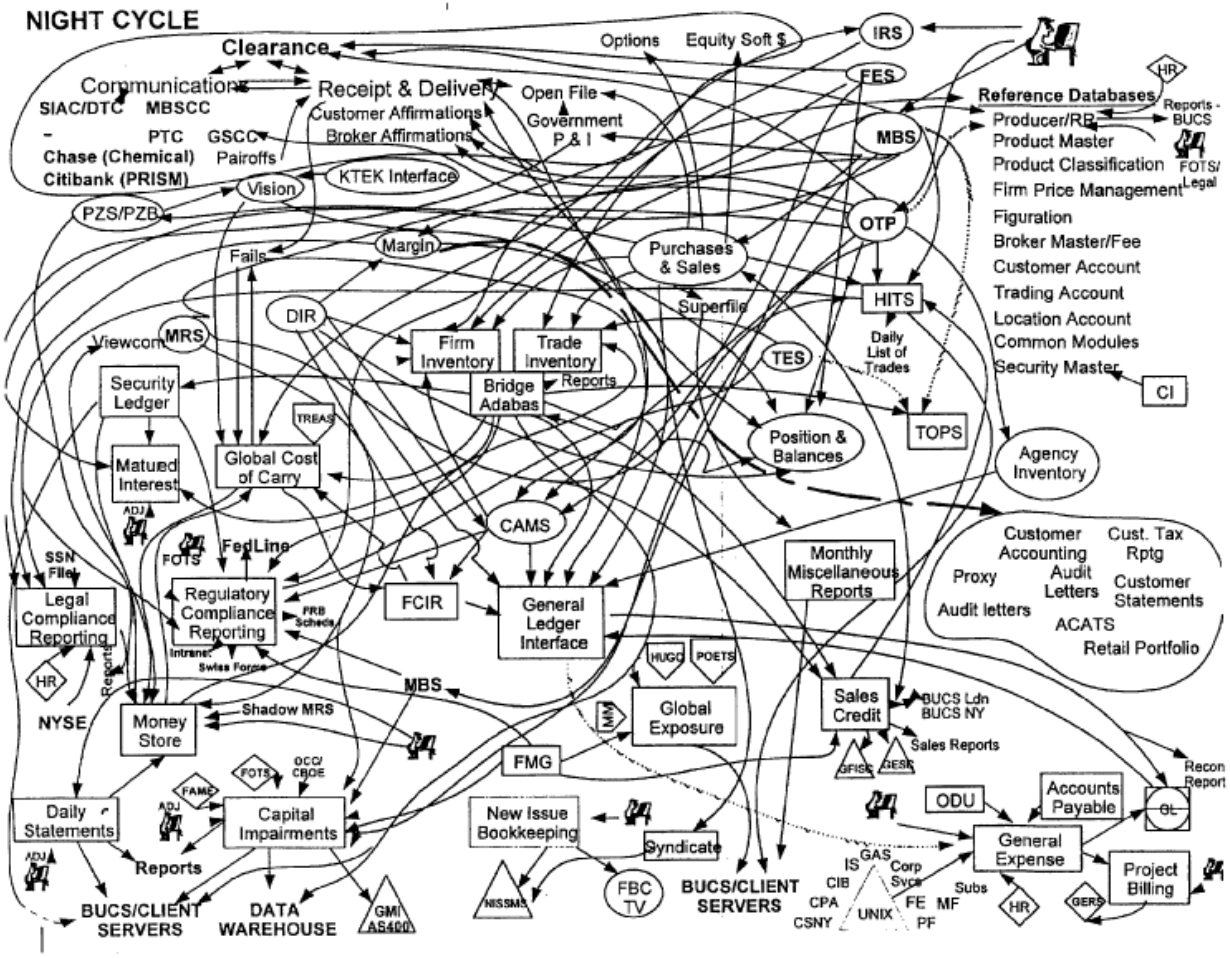
What if no growth occurs?

- Original MGIT and LJ are kept and read for 6 weeks and reported as:

Final Conclusion	Date Released :
NO ACID FAST BACILLI ISOLATED ON CULTURE	

It sounds like this…..

But it's really like this….



DCLS TB Laboratory Team

Rana, Terri, Savannah, Kathleen

Questions?
Call us at the DCLS!
804-648-4480 ex 255

Acknowledgements

VDH

Tuberculosis Elimination Laboratory Cooperative Agreement
LABTB603GY18