Review of TB Laboratory Testing AND Review of TB Infectivity and Recommendations to Reduce Transmission

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Review of TB Laboratory Testing

Importance of Specimen Collection

- Laboratory specimen collection is the only way to definitively diagnose TB and allow for drug resistance testing.
- When TB is suspected, aggressive attempts should be made to ensure proper specimen collection so tuberculosis can be confirmed or ruled out.
- Specimen collection should be done before treatment is initiated.
- Without laboratory specimens, patients may have to be treated for 6-12 months costing public resources and causing possible drug reactions.

Specimen Types

Sputum

- Natural sputum is the recommended. Must be material brought up from the lungs.
- Collect at least 3 separate specimens, preferably on 3 consecutive days.
- Saliva or nasopharyngeal discharge is not appropriate.
- Do not pool specimens.

Induced Sputum

- Recommended only for patients unable to naturally produce a sputum.
- Collected through aerosol of sterile hypertonic saline, usually produced by a nebulizer.
- These specimens should be labeled as “induced” sputums so they are not mistaken for saliva.

Specimen Types cont.

Gastric Aspiration

- May be necessary for patients who cannot produce sputum (i.e. children).
- Usually collected early morning while patient still in bed.
- Usually requires an in-patient stay.

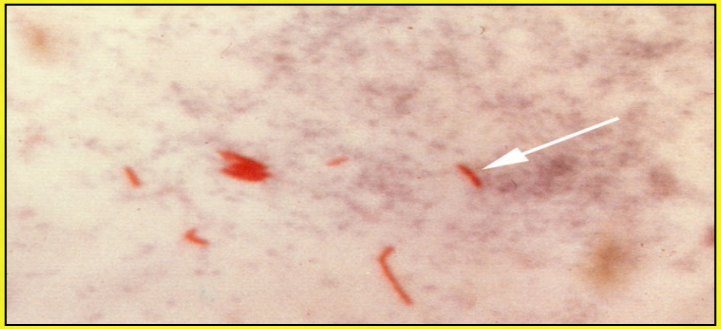
Bronchial Washing or Broncho Alveolar Lavage (BAL)

- Used when a diagnosis of pulmonary TB has not been established from sputum.
- Appropriate infection control procedures should be used while collecting these specimens.

Other Specimens

- **Urine** – first morning, midstream specimen preferred.
- **Blood** – use appropriate tubes (check with laboratory).
- **CSF** – AFB smear will usually be negative.
- **Tissue** – must be processed as a live specimen. Do not put in formaldehyde or paraffin.

1. AFB Microscopy



- AFB = acid-fast bacilli
- *Mycobacteria* species are called acid-fast bacilli because they are rod-shaped bacteria that can be seen under the microscope following a staining procedure in which the bacteria retain the color of the stain after an acid wash (acid-fast).
- Provides the first evidence that *Mycobacteria* is present in specimen as opposed to other types of bacteria.
- Test is completed within 24 hours of specimen receipt.
- Most basic lab test available for TB; used throughout the world.
- 2 types of stains: Ziehl-Neelsen or fluorochrome (auramine-O)
- Concentration (liquefied specimen is centrifuged & sediment used for staining) increases the sensitivity of test and is the preferred method.

AFB Microscopy Results

- AFB results are reported by the State Lab as the following values:

Fluorochrome negative	
+/-	(Suspicious)
+1	(Rare AFB)
+2	(Few AFB)
+3	(Moderate AFB)
+4	(Numerous AFB)

A lab report with "Positive AFB" or "Acid Fast Bacilli seen" refers to a reference isolate submitted after growth observed at originating laboratory so no direct AFB testing done.

- A negative AFB result does not rule out TB disease (wait on culture).

- A positive AFB result does not confirm TB disease but does indicate either *M. tuberculosis* or another non-tuberculin *Mycobacteria* species (NTM).

- Positive AFB results in a confirmed TB case provide an estimate of the # of bacilli in specimen. *Therefore AFB smears are vital in assessing the patient's infectiousness for culture + TB cases.*

2. Mycobacterial Culture

- All TB specimens are processed for culture automatically.
- Culturing after AFB microscopy is necessary because:
  1) Culture is more sensitive (can detect as few as 10 bacteria/1 ml of material).
  2) To identify the organism growing.
  3) To complete drug susceptibility testing.
  4) To complete genotyping.
- 2 types of culture – solid and liquid media.
- Solid media culturing takes 3-8 weeks.
- Liquid media is faster (BACTEC®, MGIT®). Allows for rapid growth within 1-3 weeks. This is the method currently used by SD State Public Health Laboratory.
- All liquid culture specimens are backed up with solid media culture but no results are issued if results match liquid media results.

Mycobacterial Culture Results

- **If no growth in liquid media, cultures are reported as negative after 6 weeks.**
- **If growth is identified, a DNA molecular probe is used to identify the species of the organism.**
- **Current commercially available DNA probes:**

  M. tuberculosis complex **(including *M. tuberculosis, M. bovis, M. africanum, M. microti, M. canetti, M. caprae, M. pinnipedii, M. suricattae* and *M. mungii*)**

  M. avium complex

  M. intracellulare

  M. kansasii

  M. gordonae

- **There are hundreds of *Mycobacterium* bacterial species that do not have a DNA probe so identification must be made by solid media culturing, biochemical testing or may not be done at all if no public health purpose is served by identifying the species. These results would be reported as: "Preliminary culture suggestive of *Mycobacterium*, not tuberculosis".**

M. Tuberculosis complex

- ***Mycobacterium tuberculosis complex*** **is defined as a genetically related group of *Mycobacterium* species that can cause TB in humans or other organisms. A positive result in humans is a confirmed case of tuberculosis.**

M. tuberculosis complex:

Reservoir for *M. tuberculosis:*	Humans
Reservoir for *M. bovis:*	Humans, cattle, deer, llamas, pigs
Reservoir for *M. africanum:*	Humans in West African
Reservoir for *M. microti:*	Voles and other small field rodents
Reservoir for *M. canetti:*	Unknown, 2 human cases reported in Horn of Africa
Reservoir for *M. caprae:*	Unknown
Reservoir for *M. pinnipedii:*	Seals
Reservoir for *M. suricattae:*	Unknown
Reservoir for *M. mungii:*	Unknown

- **Therefore a positive culture result for *M. tb complex* does not specifically identify that the culture is positive for *M. tuberculosis* however by default it is assumed to be the organism causing disease in humans.**

3. Drug Susceptibility Testing

- Determines whether the TB bacteria is resistant to first-line TB medication so appropriate, curative treatment can be provided.
- Must have a positive culture to be completed.
- Results completed within 1-2 weeks of the positive culture.
- Performed on all initial *M. tuberculosis* positive patient specimens by a CDC funded lab in CA.
- Follow-up susceptibility testing should be done if patient is culture positive after 2 months of treatment.
- Second-line drug susceptibility done by request only and requires TB program approval.
- Susceptibility testing for NTM (non-tuberculin mycobacterial) is not performed at the State Lab and only forwarded if provider agrees to pay for testing.

4. GeneXpert (PCR)

- The generic name for this type of testing is NAAT (nucleic acid amplification testing).
- The GeneXpert by Cepheid is the PCR test currently used by the SD State Lab. It is an amplified test for the detection of *M. tuberculosis* in both smear-positive and smear-negative clinical specimens. **It is the only PCR test that detects mutations for Rifampin resistance.**
- Only approved for pulmonary specimens.
- Must be performed on specimens collected within 3 days.
- Results available the same day.
- Does not replace the need for AFB smear and culture.
- A positive GeneXpert PCR result is considered diagnostic confirmation of TB.
- A negative GeneXpert PCR result does not exclude a diagnosis of TB.
- <u>The test should only be used in patients when the clinician has an moderate or high suspicion of TB disease</u>.

5. Genotyping (DNA Fingerprinting)

- Genotyping is a useful tool for TB control because it can:
  1) Confirm lab cross-contamination
  2) Assist in the investigation of outbreaks and contact investigation
  3) Determine if TB cases are due to re-infection or reactivation
  4) Monitor patterns of *M. tuberculosis* in communities
- TB genotyping is specialized testing done only at 2 CDC funded labs in the US (California & Michigan).
- 3 types of testing available:
  1) spoligotype
  2) MIRU (mycobacterial interspersed repetitive unit analysis)
  3) RFLP (IS6110-based restriction fragment length polymorphism analysis)
- Current testing consists of a spoligotype and MIRU 1 and MIRU 2 for all specimens. In order for there to be a "match", there must be identical results to all 3 tests. RFLP is only done to confirm results from other tests if needed.
- Since 2004, all culture positive TB cases in South Dakota have had genotyping completed.
- Some genotyping matches are investigated by DOH field staff to identify epi-links.

6. Quantiferon Gold Intube®

- The generic name for this type of testing is IGRA (Interferon Gamma Release Assay). QFT is one of 2 commercially available IGRA tests (the other is TBSPOT-TB).
- Will be available from SD State Health Lab soon.
- Whole blood test used anytime that the TB skin test is used.
- If patient is infected with *M. tuberculosis*, the white blood cells will release IFN-gamma in response to contact with TB antigens. The test measures the amount of IFN-gamma released after incubation of blood with the antigens (*M. tb* proteins ESAT-g and CFP-10).

Advantages

- Requires a single patient visit to draw blood.
- Not subject to reader bias.
- Not affected by BCG.
- Not affected by infections with most non-tb mycobacterium. Exceptions are *M. kansasii, M. marinum, M. szulgai.*
- Does not boost response – no need for 2-step testing.

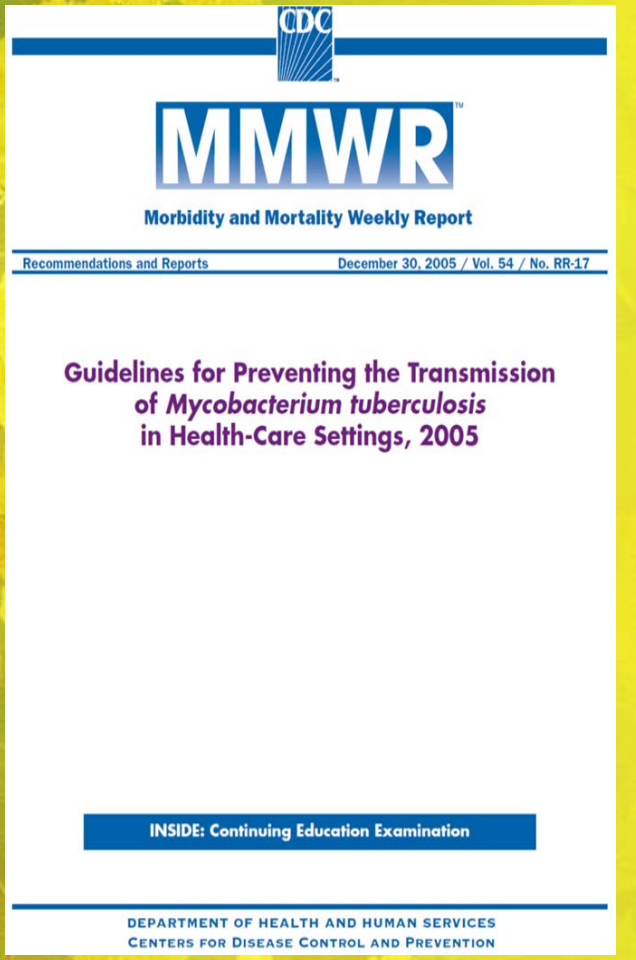
Disadvantages

- Blood samples must be transported to lab within 16 hours of collection or incubated onsite.
- Errors in collecting or transporting blood specimens or in running and interpreting the assay can decrease the accuracy.
- There is limited data on the use in children <17 and immunosuppressed persons.

Review of TB Infectivity and Recommendations to Reduce Transmission

TB Infection Control Measures

TB Infection control measures should be based on a careful assessment of risk for transmission of TB in the facility. The goals of effective TB infection control are:


CDC

MMWR

Morbidity and Mortality Weekly Report

Recommendations and Reports December 30, 2005 / Vol. 54 / No. RR-17

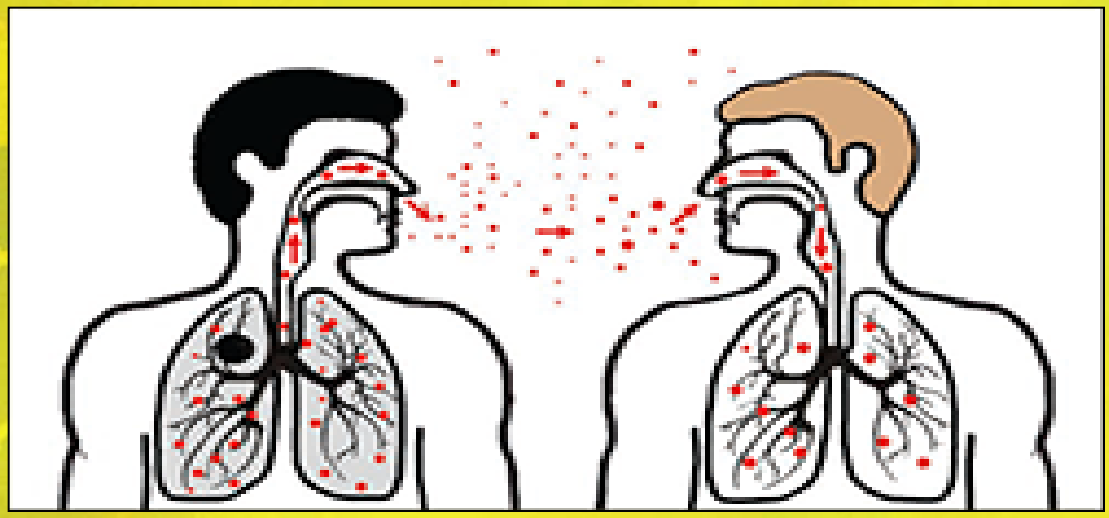
Guidelines for Preventing the Transmission of *Mycobacterium tuberculosis* in Health-Care Settings, 2005

INSIDE: Continuing Education Examination

DEPARTMENT OF HEALTH AND HUMAN SERVICES
CENTERS FOR DISEASE CONTROL AND PREVENTION

1. **Detect TB disease early and promptly;**
2. **Isolate those who have or suspected of having TB disease (airborne precautions);**
3. **Treat people who have or are suspected of having TB disease.**

Overview of Transmission of TB



- *M. tuberculosis* is carried in airborne particles called droplet nuclei which are 1-5 microns in size.
- Droplet nuclei are expelled when a person with infectious TB coughs, sneezes, shouts, sings (i.e. forceful exhalation).
- Droplet nuclei must remain suspended in the air to be infectious.
- Transmission occurs when a person inhales droplet nuclei which must travel to the alveoli of the lungs to cause infection.
- Persons most at risk of transmission are those with the closest proximity to the infectious person and who have prolonged contact.

Symptoms of Pulmonary TB Disease

- Persistent cough (3 weeks or longer)
- Chest pain
- Bloody sputum
- Weight loos or loss of appetite
- Fever or chills
- Night sweats
- Malaise
- Fatigue

Determining if a patient is a suspect TB case should consider multiple factors including symptomology, radiology results, sputum results, TB risk factors and other factors as determined by the patient's physician.

Factors that Increase the Infectiousness of a TB patient

The infectiousness of a TB patient is directly related to the number of droplet nuclei expelled into the air including:

- **Productive cough**
- **Positive AFB sputum smear**
- **Cavitation on chest radiograph**
- **Respiratory tract disease with involvement of the lung or airway including the larynx**
- **Failure to cover the mouth and nose when coughing**
- **Incorrect or short duration of anti-tuberculosis treatment**
- **Undergoing cough-inducing or aerosol-generating procedures (i.e. sputum induction, bronchoscopy)**

TB Patients Less Likely to be Infectious

- Pleural TB
- Extra-pulmonary TB
- TB in Children (especially young children)
- Confirmed TB cases on appropriate treatment who have been rendered non-infectious per the CDC recommendations

There are always exceptions!

General Principles of Transmission of TB

Transmission of TB is most likely to result from:

1. **Unsuspected pulmonary cases not receiving treatment**
2. **Diagnosed cases receiving inadequate treatment**
3. **Diagnosed cases early in the course of treatment**

Effective TB treatment rapidly decreases the patient's infectiousness because it reduces cough, sputum production, # of organisms in sputum and the viability of the organisms in the sputum. <u>However the duration of therapy required to render a person non-infectious varies between patients</u>.

CDC Criteria for Determining When a TB Case is Noninfectious

Patients with confirmed pulmonary TB can be considered noninfectious when they meet <u>ALL</u> of the following criteria:

1. **Three consecutive negative AFB sputum smears collected in 8 to 24 hour intervals (at least 1 being an early morning specimen);**

AND

2. **Symptoms improved clinically (for example they are coughing less, no longer have a fever);**

AND

3. **Received <u>appropriate treatment regimen</u> for at least 2 weeks or longer.**

CDC Recommendations for Discharge to Home of TB Patients Who are Still Infectious

If a hospitalized patient with suspected or confirmed TB is deemed medically stable (including patients with +AFB sputum), the patient can be discharged from the hospital before converting the +AFB sputum to negative if ALL the following parameters have been met:

1. **A specific plan exists for follow-up care with the local TB-control program;**
2. **The patient has been started on a standard multi-drug TB treatment regimen and DOT has been arranged;**
3. **No infants or children aged <4 or persons with immunocompromising conditions are present in the household;**
4. **All immunocompetent household members have been previously exposed to the patient;**
5. **The patient is willing to not travel outside of the home except for health-care associated visits until the patient has negative sputum smear results.**

Patients with suspected or confirmed infectious TB disease should not be released to health-care settings or homes in which the patient can expose others who are at high risk for progressing to TB disease if infected (i.e. children <4, immunosuppressed).

APHL Consensus Statement on the Use of GeneXpert to Remove Patients from Airborne Infection Isolation (A.I.I.) February 2015

APHL

Consensus statement on the use of Cepheid Xpert MTB/RIF® assay in making decisions to discontinue airborne infection isolation in healthcare settings

Morbidity and Mortality Weekly Report

Revised Device Labeling for the Cepheid Xpert MTB/RIF Assay for Detecting *Mycobacterium tuberculosis*

Division of Microbiology Devices, Office of In Vitro Diagnostics and Radiological Health, Center for Devices and Radiological Health, Food and Drug Administration

FDA News Release

New data shows test can help physicians remove patients with suspected TB from isolation earlier

Purpose: To provide guidance on the use of GeneXpert to discontinue airborne infection isolation (A.I.I.) for persons with suspected, infectious pulmonary TB.

FDA Approval of GeneXpert for A.I.I.

Either one or two sputum specimens can be used as an alternative to examination of serial acid-fast stained sputum smears to aid in the decision to discontinue A.I.I. for patients with suspected pulmonary TB.

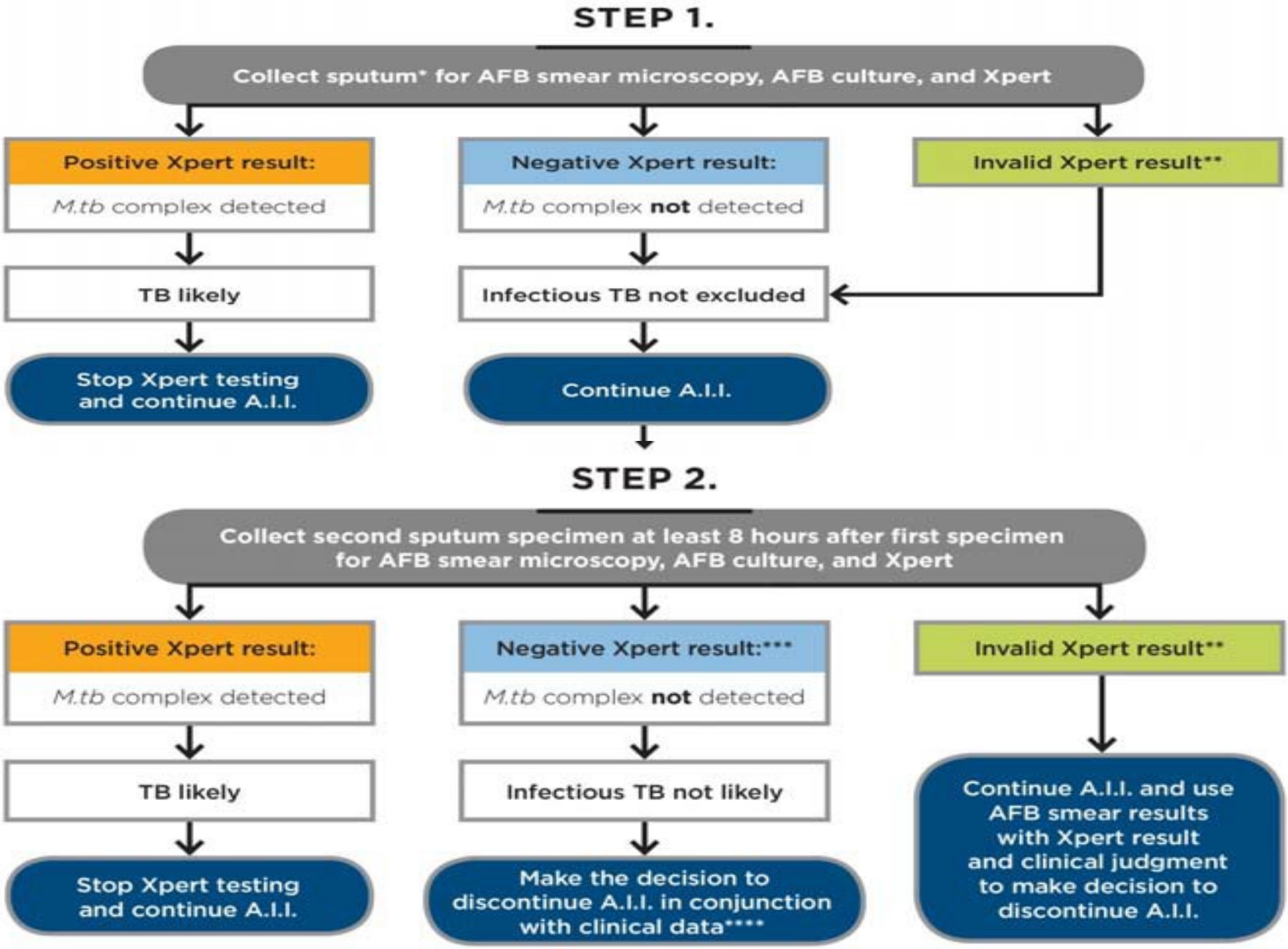
Consensus Statement

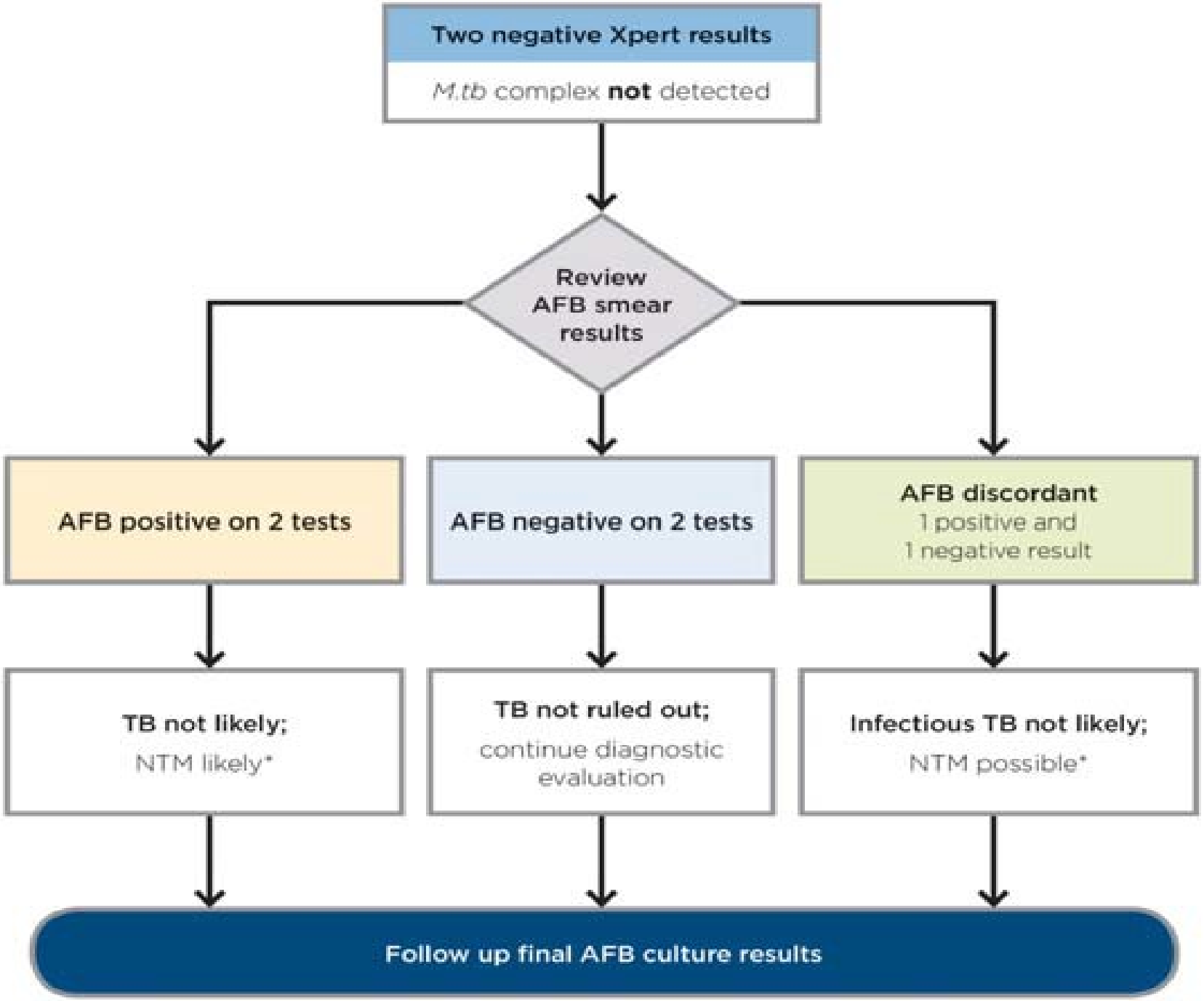
Does not address the diagnosis of TB.

Does not address when a confirmed TB case can be removed from respiratory isolation or released from the hospital .

Does help predict the infectiousness of a patient.

Does help determine clinical appropriateness to be removed from isolation.


STEP 1.
Collect sputum* for AFB smear microscopy, AFB culture, and Xpert
Positive Xpert result:
M.tb complex detected
TB likely
Stop Xpert testing and continue A.I.I.
Negative Xpert result:
M.tb complex not detected
Infectious TB not excluded
Continue A.I.I.
Invalid Xpert result**
STEP 2.
Collect second sputum specimen at least 8 hours after first specimen for AFB smear microscopy, AFB culture, and Xpert
Positive Xpert result:
M.tb complex detected
TB likely
Stop Xpert testing and continue A.I.I.
Negative Xpert result:***
M.tb complex not detected
Infectious TB not likely
Make the decision to discontinue A.I.I. in conjunction with clinical data****
Invalid Xpert result**
Continue A.I.I. and use AFB smear results with Xpert result and clinical judgment to make decision to discontinue A.I.I.


Two negative Xpert results
M.tb complex not detected
Review AFB smear results
AFB positive on 2 tests
AFB negative on 2 tests
AFB discordant
1 positive and
1 negative result
TB not likely;
NTM likely*
TB not ruled out;
continue diagnostic evaluation
Infectious TB not likely;
NTM possible*
Follow up final AFB culture results

Contact Information

TB Program

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