TECHNICAL ASSISTANCE: TB 9
January 30, 2002

Laboratory Services for Tuberculosis

I. TITLE: *Fast Track* laboratory protocols for the most rapid and accurate laboratory diagnosis of tuberculosis including drug susceptibility testing for all tuberculosis patients at the Florida Department of Health (DOH), Bureau of Laboratories, Mycobacteriology Laboratory.

II. TYPE OF STANDARD: Service

III. OUTCOME:

A. Acid-fast smear microscopy results provided to healthcare provider within 24 hours of receipt for >90% of all specimens.

B. Nucleic acid amplification (MTD/Gen-Probe, Inc.) results provided to the healthcare provider within 24 hours of positive smear result on >90% on all first-time, acid-fast bacilli (AFB)-positive clients.

C. Susceptibility testing results (rifampin result serves as a surrogate marker) completed between 15-30 days from specimen receipt to reporting for >90% of all clients initially diagnosed with *M. tuberculosis*.

D. Clinical Chemistry and Hematology results provided within 24 hours of collection for >90% of all specimens received for these analyses.

IV. PERSONNEL:

A. Laboratory personnel are licensed in accordance with State of Florida, Department of Health, Division of Medical Quality Assurance. These include Clinical Laboratory Directors, Supervisors, Technologists, and Technicians.

B. Other personnel are determined by appropriate local jurisdictions in accordance with levels of care provided.

V. COMPETENCIES:

Laboratory professionals who supervise or perform laboratory analyses must be licensed according to Florida Statute 483. Furthermore, all new employees are subject to an orientation and training during their first few months of employment (documentation required). A supervisor reviews all test results and a stringent ongoing quality assurance program is practiced in all areas of the mycobacteriology laboratory. All personnel are evaluated annually for competency and documentation of this is provided in the quality assurance program record.

The mycobacteriology laboratory is enrolled in a proficiency-testing program provided by the College of American Pathologists, participates in two self-assessment modules of the Centers for Disease Control and Prevention, and serves as a reference laboratory for the New York State and the Puerto Rico Proficiency Testing Program. These entail AFB smears, nucleic acid amplification, growth detection, identification, and drug susceptibility testing.

VI. AREAS OF RESPONSIBILITY:

A. LABORATORY FACILITIES:

1. **Mycobacteriology.** The Jacksonville laboratory operates 7 days a week, including holidays and the West Palm Beach Laboratory (located at the A.G. Holley State Hospital campus in Lantana) operates Monday-Saturday.

2. **Clinical Chemistry.** The Jacksonville and West Palm Beach Laboratories perform testing for clinical chemistries Monday-Friday.

3. **Hematology.** The Jacksonville and West Palm Beach Laboratories perform testing for hematology Monday through Saturday.

B. LABORATORY REQUEST FORMS

1. The laboratory request form is DH 1847.

2. To ensure the laboratory testing is accurate, and timely, the submitter must:
   a. Type or print legibly on all copies of the form.

   b. Complete all areas on the form (**required**):
      1) Medicare/Medicaid/3rd party payer information and number
      2) Social security number
      3) Client's name (last, first)
      4) Client's address
      5) Date of birth, race, sex, and ethnicity
      6) Program component
      7) Date of specimen collection; time required for hematology specimen collection.
      8) Specimen type
      9) Send report to: include the physician's name and complete address.
      10) ICD9 Diagnostic codes
      11) Test requested

3. Laboratory request forms need to be complete to ensure proper testing is performed.

C. SPECIMEN COLLECTION FOR AFB SMEAR, NUCLEIC ACID AMPLIFICATION, GROWTH DETECTION, IDENTIFICATION, AND SUSCEPTIBILITY TESTING:

1. Sputum collection procedure:

   a. Respiratory specimens are collected from deep, productive coughs in the early morning on 3 consecutive days. The client should rinse the mouth with water before collecting sputum and should take four deep breaths, inhaling deeply with the first two, blowing air out on the third, and inhaling deeply and coughing on the fourth. Repeat this procedure as needed to produce sputum. The client should hold the sputum collection cup to the lower lip and release the specimen into the container, pressing the rim of the container to the lower lip to minimize the chance of contaminating the outer surface of the container.

      Note: The volume of sputum must be 5 to 10 mL for best results.

   b. The sputum specimen must be collected in the sterile, plastic, 50 mL, conical, centrifuge tube provided by the Bureau of Laboratories. It is important to use the correct centrifuge tube, as they may break if not of a sufficient rating for g-force when centrifuged. The client should be instructed to screw the lid on tightly to prevent leakage.

   c. The tube must be placed inside a metal inner mailing container with absorbent material. The laboratory slip must be placed around the outside of the metal container (not around the centrifuge specimen tube) to protect the slip in case of leakage.

   d. The metal container must be placed inside a properly addressed, cardboard outer mailing container with a C*linical Specimen* label for timely shipping to the laboratory.

      All lids must be tightened to protect shipping personnel.

   e. Mailing kits for mycobacterial testing are available from the state laboratories:
      Jacksonville Laboratory
      1217 Pearl Street
      Jacksonville, FL 32202
      Phone: (904) 791-1630
      Suncom: 866-1630
      Fax: (904) 791-1637

West Palm Beach Branch Laboratory
A.G. Holley Complex
East End, Lantana Rd.
Lantana, FL 33462
Phone: (561) 540-1170
Suncom: 256-1170
Fax: (561) 540-1172

f. The specimens **must** be shipped as soon as possible (within 24 hours) for the shortest turnaround time and the best recovery of mycobacteria. Delays in shipping to the laboratory will result in delayed smear, nucleic acid amplification, and may result in the specimen being contaminated due to the growth of more rapidly growing bacteria and fungi.

2. Extrapulmonary specimens

Please contact the laboratory before obtaining extrapulmonary specimens (see section C.1.e. for telephone numbers) for questions on the collection and shipping of extrapulmonary specimens. These include blood, biopsy specimens, urines, stool, body fluid specimens, gastric washings*, etc.

*Gastric washings require that they be neutralized with a pinch of sodium bicarbonate before *overnight* shipping for better recovery of mycobacteria.

D. HANDLING AND SHIPMENT OF AFB SPECIMENS:

1. The client should be instructed to refrigerate specimens after collection and to deliver them to the county health department as soon as possible. The specimen should be shipped on the same day as collected, if possible.

2. Clinic staff should refrigerate the specimens before and during transport whenever possible to reduce overgrowth by normal flora.

3. The specimens should be sent to the state laboratory using the US Postal service or other reliable overnight courier. **Specimens should preferably be sent using a <u>trackable</u> system in order to ascertain prompt delivery of the package.** Any delay in delivery of specimens to the laboratory will result in delayed results.

<u>Note</u>: Both the Jacksonville and West Palm Beach Mycobacteriology Laboratories can accept deliveries and mail on Saturday, and Jacksonville receives shipments on Sunday and holidays. If specimens are shipped on Friday, County Health Departments and other submitters should request Saturday delivery.

E. INTERPRETATION AND REPORTING OF AFB LABORATORY RESULTS:

1. Smears results, as well as nucleic acid amplification (NAA) and identification results are reported daily on all AFB specimens, with positive results returned by fax the same day to the submitting facility. A hard copy is also mailed to the county health department of the client's county of residence, as well as to the Bureau of TB and Refugee Health reporting unit (with the exception of smear results). County health departments with the HCMS system will receive information electronically daily.

2. All newly diagnosed AFB-smear positive specimens submitted to the state laboratory will ***automatically*** have nucleic acid amplification (MTD/Gen-Probe, Inc.) performed. MTD is FDA-approved for use on AFB positive or negative respiratory specimens. **However, for AFB negative respiratory specimens *prior arrangement through the TB Physician's Network (I-800-4TB-INFO) is required.*** The MTD testing is performed Monday-Saturday in Jacksonville. This testing can be performed on Sunday and holidays upon request. Results are called and faxed to the submitter.

   The appropriate number of specimens to test with NAA will vary depending on the clinical situation, the prevalence of TB, the prevalence of nontuberculous mycobacteria (NTM), and laboratory proficiency. Based on available information, the following algorithm from the *MMWR Notice to Readers: Update: Nucleic Acid Amplification Tests for Tuberculosis* [July 7, 2000/49(26); 593-594] is a reasonable approach to NAA testing of respiratory specimens from clients with signs or symptoms of active pulmonary TB for whom a presumed diagnosis has not been established.

   Algorithm

   a. Collect sputum specimens on 3 different days for AFB smear and mycobacterial culture.
   b. Perform NAA test on the first sputum specimen collected, the first smear-positive sputum specimen, and additional sputum specimens as indicated below.
      1) If the first sputum specimen is smear-positive and NAA-positive, the client can be **presumed to have TB** without additional NAA testing. However, unless concern exists about the presence of NTM, the NAA test adds little to the diagnostic work-up.
      2) If the first sputum is smear-positive and NAA-negative, a test for inhibitors should be done.
         a) Additional specimens (not to exceed a total of three) should be tested. The client can be **presumed to have NTM** if a second sputum specimen is smear-positive, NAA-negative, and has no inhibitors detected.

b) If inhibitors are detected, the NAA test is of no diagnostic help. Additional specimens (not to exceed a total of three) can be tested with NAA.

3) If sputum is smear-negative and MTD-positive†, additional specimens (not to exceed three) should be tested with MTD. The client can be **presumed to have TB** if a subsequent specimen is MTD-positive.

4) If sputum is smear-negative and MTD-negative†, an additional specimen should be tested with MTD. The client can be presumed not to be infectious if all smear and MTD results are negative.

Note: The clinician must rely on clinical judgment in decisions regarding the need for antituberculous therapy and further diagnostic work-up because negative NAA results do not exclude the possibility of active pulmonary TB.

c. If the indicated repeat NAA testing fails to verify initial NAA test results, the clinician must rely on clinical judgment in decisions regarding the need for antituberculous therapy, further diagnostic work-up, and isolation.

d. **Ultimately, the client's responses to therapy and culture results are used to confirm or refute a diagnosis of TB.**

†The Gen-Probe MTD assay is approved for use on smear-negative specimens; the Roche Amplicor assay is only approved on smear-positive specimens. MTD is the assay used by the state laboratory system.

3. All AFB specimens are cultured using either the BACTEC 12B vials or MGIT liquid media and a solid Lowenstein-Jensen slant to provide the best culture yield.

4. The state laboratories use polymerase chain reaction restriction analysis (PRA) or Accuprobe for the identification of *M. tuberculosis* complex* and Accuprobes, PRA or high performance liquid chromatography (HPLC) for the identification of NTM, e.g. *M. avium* complex, *M. kansasii*, etc.

*_M. tuberculosis_ complex includes *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, *M. canettii* and *M. microti.*

5. Negative culture results are reported by the end of 6 weeks.

6. Smear positive/culture negative specimens will be held an additional 4 weeks with an amended report if necessary.

F. TESTING PROCEDURE FOR MYCOBACTERIAL DRUG SUSCEPTIBILITIES

1. Only the Jacksonville Laboratory performs drug susceptibility testing.

All cultures of ***M. tuberculosis*** **and** ***M. tuberculosis*** **complex** received and identified by the State Laboratories or any other laboratory and designated as a "new case" by the Bureau of TB and Refugee Health, will **automatically** have susceptibility testing done, based on a search of the Bureau of Laboratories database. **Note:** Isolates from new clients will be retested if a subsequent specimen is still positive after 60 days since the first collection. This testing is able to be determined at the state laboratory based on specimens received within its system.

All other *M. tuberculosis* complex cultures identified by the State Laboratories or any other laboratory will be tested only on the request of the physician. These will **not** automatically be tested.

2. All *M. tuberculosis* complex cultures are tested by the radiometric (BACTEC) method. At present, the drugs used are:

Primary drugs:

Streptomycin	2.0 μg/ml
Isoniazid	0.1 μg/ml
Isoniazid	0.4 μg/ml
Rifampin	2.0 μg/ml
Ethambutol	2.5 μg/ml
Pyrazinamide	100.0 μg/ml at pH 6.0

Secondary drugs:

Isolates resistant to Isoniazid at 0.4 μg/ml

Isoniazid	2.0 μg/ml
Isoniazid	4.0 μg/ml
Ethionamide	1.25 μg/ml

Isolates resistant to Streptomycin at 2.0 μg/ml

Streptomycin	6.0 μg/ml

Isolates resistant to Ethambutol at 2.5 μg/ml

Ethambutol	7.5 μg/ml

3. *M. tuberculosis* complex isolates resistant to any **two** primary drugs are screened against the following secondary drugs:

Ethionamide	1.25 μg/ml
Kanamycin	5.0 μg/ml
Clofazamine	0.5 μg/ml
Capreomycin	1.25 μg/ml
Rifabutin	0.5 μg/ml
Ofloxacin	2.0 μg/ml

4. All cultures submitted for susceptibility testing are checked for purity by smear and by subculture to a purity plate. Furthermore,

this plate allows the checking for characteristic colonial morphology of the organism. A mixed culture of 2 or more mycobacterial species or mixed culture of acid-fast and non-acid fast organisms can lead to false drug resistance. Drug resistant isolates are confirmed as part of the quality assurance program.

5. Any isolate resistant to PZA is tested by HPLC and biochemicals to identify the species within the *M. tuberculosis* complex. An isolate of this type may be *M. bovis* or *M. bovis* BCG, which will be differentiated from *M. tuberculosis* in this case.

6. The Bureau of Laboratories will not perform routine drug susceptibility testing of ***M. avium* complex** isolates. This is based on a recommendation by the American Thoracic Society (ATS) found in the *American Journal of Respiratory and Critical Care Medicine*, volume 156, number 2, August 1997, p. S1-S25. *M. avium* complex mycobacteria show a high degree of "in vitro" resistance to antituberculosis drugs. "Susceptibility testing with rifabutin and the antituberculosis drugs is not recommended. Routine testing against clarithromycin should not be performed, but that test should be performed on isolates from clients who have failed prior macrolide therapy or prophylaxis".

   Note: These cultures can be sent from the state laboratory to a reference laboratory for this testing when requested by the physician.

7. **Nontuberculous mycobacteria (NTM) organisms other than *M. avium* complex** will be forwarded for susceptibility testing to the Centers for Disease Control and Prevention only at the physician's request. The ATS provides guidelines for diagnostic criteria of NTM lung disease in **symptomatic clients** with infiltrate, nodular or cavitary disease, or a high resolution computed tomography scan that shows multifocal bronchiectasis and/or multiple small nodules, based on the following criteria (specifically, *M. avium* complex, *M. abscessus,* and *M. kansasii*) in evaluation of three respiratory samples:

   - If three sputum/bronchial wash results are available from the previous 12 months, three positive cultures with negative AFB smear results or two postive cultures and one positive AFB smear;

   - If only one bronchial wash is available, positive culture with a 2+,3+ or 4+ AFB smear or 2+,3+, or 4+ growth on solid media;

   - If sputum/bronchial wash evaluations are nondiagnostic or another disease cannot be excluded, transbronchial or lung biopsy yielding a NTM or biopsy showing mycobacterial

histopathologic features and one or more respiratory specimens are positive for an NTM.

Using these criteria, susceptibility testing for rapidly growing mycobacteria (*M. abscessus, M. fortuitum, M. chelonae*) may be tested upon request at the Jacksonville state laboratory. Additionally, *M. avium* complex isolates from clients meeting the ATS criteria, may be tested upon request through the TB Physician's Network (1-800-4TB-INFO), for clarithromycin.

Tests will not be done on NTM normally recognized as non-pathogenic (i.e., *M. gordonae*), except on cultures considered clinically significant.

8. All cultures identified elsewhere and submitted only for susceptibility testing must indicate the species identified. These isolates should be pure cultures. A completed laboratory request form DH 1847 should be sent with the isolate. BACTEC 12B vials as well as other commercial media are accepted for testing.

9. For additional information, contact Yvonne Hale at:
Jacksonville Mycobacteriology Laboratory
1217 Pearl Street
Jacksonville, FL 32202
Phone: (904) 791-1630 or SC 866-1630
Fax: (904) 791-1633 or SC 866-1633
E-mail: yvonne_hale@doh.state.fl.us

G. DNA FINGERPRINTING TESTING PROCEDURE FOR RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP), ON ISOLATES OF *M. TUBERCULOSIS* COMPLEX

1. RFLP testing is performed on isolates of *M. tuberculosis* only on request from the Bureau of TB and Refugee Health, county, and submitter.
This testing is performed for the following reasons:
    - Suspect false-positive result
    - Epidemiological studies
    - Subtyping of a client's isolates

2. Since October 1995, cultures submitted to the Florida Bureau of Laboratories as first time isolates are stored. Additional cultures may also be stored as well.

3. RFLP testing takes a minimum of 4 weeks to perform, longer if the isolate has been frozen. The culture must currently be grown up to a certain amount prior to testing. In the future, a PCR based typing (spoligotyping) is anticipated to be implemented, requiring minimal amounts of growth and a 2-day procedure for testing.

4. Testing using RFLP is done only for client benefit. Requests should be called into the Mycobacteriology Laboratory, Bureau of Laboratories, Jacksonville at (904) 791-1630.

H. SPECIMEN COLLECTION, HANDLING AND TRANSPORT FOR CLINICAL CHEMISTRY AND HEMATOLOGY (BUN, CREATININE, BILIRUBIN, SGOT AND CBC)

1. For testing BUN, creatinine, bilirubin and SGOT, 5-10 mL of venous blood should be collected in a red top Vacutainer-type collection tube or serum separation tube (SST). **Only clear serum (3 ml or more) or centrifuged SST should be sent to the laboratory.** Laboratory Request Form DH 1847 should be completed and included with the specimen. This specimen should be kept refrigerated at 2-8° C until it is shipped.

   Specimens **must be** centrifuged and separated. Hemolyzed blood will NOT be satisfactory and a new specimen must be drawn.

2. For testing complete blood count (CBC) with platelets, 5-10 mL venous blood should be collected in a lavender top Vacutainer-type collection tube. It should be gently mixed for one minute. Blood should be stored in the refrigerator at 2-8° C and transported to the laboratory on ice **within 24 hours** (test must to be run within 24 hours of collection.) Laboratory Request Form DH 1847 should be completed and included with the specimen.

I. INTERPRETATION FOR CLINICAL CHEMISTRY AND HEMATOLOGY RESULTS

Clinical Chemistry and Hematology normal values are listed on the laboratory report forms with client's results.

J. EVALUATION OF LABORATORY ACTIVITIES

The laboratory staff responsible for testing the specimens submitted will evaluate the completeness, accuracy of the client form and timeliness of the specimens submitted for testing.

The submitter is responsible for reviewing laboratory test results and will evaluate the completeness and accuracy of the laboratory report and timeliness of the laboratory results received.

K. DOCUMENTATION OF LABORATORY ACTIVITIES

The laboratory staff responsible for laboratory testing will document all aspects of laboratory testing on Department of Health record forms. Examples of this documentation may include the laboratory request form [DH 1847], internal test result forms, and monthly quality assurance

forms. Confidentiality policies and procedures will be adhered to at all times.

The county health department nurse case manager responsible for the completeness of the TB medical record will periodically review the medical record to ensure that appropriate laboratory tests have been ordered, performed, documented and the results posted in the medical record.

VII. REFERENCES:

American Thoracic Society. 1997. Diagnosis and treatment of disease caused by nontuberculous mycobacteria. *Am. J. Respir. Crit. Care Med.* 156:S1-S25.

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Centers for Disease Control and Prevention. 1995. Essential Components of a Tuberculosis Prevention and Control Program *MMWR* 44:RR-11.

Centers for Disease Control and Prevention. 2000. Update: Nucleic Acid Amplification Tests for Tuberculosis. *MMWR* 49:593-4.

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Metchock BG, Nolte FS, Wallace RJ. 1999. *Mycobacterium* in Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH (eds). Manual of Clinical Microbiology, American Society for Microbiology, Washington DC, 7th edition, pp. 399-437.

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