



The following individuals contributed to the preparation of this  
edition of *Mycobacteriology tuberculosis: Assessing Your  
Laboratory*

Phyllis Della-Latta, Ph.D.  
Columbia Presbyterian Medical Center

Loretta Gjeltena, MA, MT(ASCP)  
National Laboratory Training Network

Ken Jost  
Texas Department of State Health  
Services

Beverly Metchock, Dr.PH.  
Centers for Disease Control and  
Prevention

Glenn D. Roberts, Ph.D.  
Mayo Clinic

Max Salfinger, MD  
Florida Bureau of Laboratories

Dale Schwabb, Ph.D., D(ABMM)  
Quest Diagnostics

Julie Tans-Kersten  
Wisconsin State Laboratory of  
Hygiene

Anthony Tran, MPH, MT(ASCP)  
Association of Public Health  
Laboratories

David Warshauer, PhD.  
Wisconsin State Laboratory of  
Hygiene

Gail Woods, MD  
University of Texas Medical Branch

Kelly Wroblewski MPH, MT(ASCP)  
Association of Public Health  
Laboratories

## MYCOBACTERIUM TUBERCULOSIS: ASSESSING YOUR LABORATORY

### **Background:**

Tuberculosis is a serious re-emerging infectious disease. An estimated 10-15 million United States (US) citizens have latent tuberculosis infections, and without detection and treatment, approximately 10% of these individuals will develop tuberculosis at some point in their lives. Costly tuberculosis outbreaks still occur. The growing threat of multi-drug resistant (MDR) and extensively-drug resistant (XDR) TB not only leads to increased cases of tuberculosis but also increased costs. Tuberculosis-related healthcare costs approach \$1 billion each year in the US.

Quality laboratory testing is essential in order to reach the goal of TB elimination in the US.

Improvements in laboratory testing must be maintained and translated into expedited and improved treatment and control. It is imperative that the laboratory community provides healthcare providers and TB controllers with accurate smear, culture, drug susceptibility and molecular results within acceptable turn-around times while providing a safe work environment for laboratorians. *Mycobacterium Tuberculosis: Assessing Your Laboratory* is intended to be used as a self-assessment tool to provide information on best-practices in the laboratory and an opportunity to thoroughly review your procedures, assign priorities, and adopt a plan to update and improve your laboratory practices if needed.

### **Intended Use:**

*Mycobacterium Tuberculosis: Assessing Your Laboratory* is intended for any public health, clinical, or commercial laboratory that performs TB testing in the United States. It is designed to be a self-assessment tool, meaning that scores will not be compiled and any information is intended for your laboratory's own self-improvement.

The tool consists of a series of 94 questions divided into the following sections:

- General Specimen Collection and Handling
- Safety
- General Laboratory Practice
- Smears from Clinical Specimens
- Public Health and Epidemiology
- Specimen Processing and Decontamination
- Inoculation and Growth Detection
- Susceptibility Testing
- Direct Detection

It is advised that laboratories only answer the questions in the sections that correspond with services that their mycobacteriology laboratory provides. Each question has an accompanying guidance section that contains information that may be helpful in improving the quality of your laboratory operations in that particular area. For best results, it is suggested that several individuals within your laboratory participate in the self-assessment process.

The regulations included in this tool are not exhaustive and following the recommendations in the Guidance Sections will not necessarily make your laboratory compliant with CLIA, CAP, JACHO, or any other accrediting agency.

### **Interpreting Your Results:**

All questions will be answered with “YES” “NO” or “NOT APPLICABLE” responses. An affirmative (“yes”) answer indicates acceptable laboratory practices in a given area. A negative (“no”) answer indicates that there is room for improvement in this area and the Guidance Section for these questions should be reviewed carefully.

Several questions have been identified as “critical questions”. A negative answer to any one of these questions indicates a significant gap in the safety or quality of testing within your laboratory. Multiple negative answers to non-critical questions may indicate significant deficiencies in the quality of your mycobacteriology services. Additionally, two levels of “critical questions” have been identified:

**Red Questions** – A negative answer to a red question indicates a severe gap in safety or quality. It is suggested that laboratories that are flagged on red questions voluntarily suspend all or part of their mycobacteriology services immediately and until the deficiency can be remedied.

**Yellow Questions** – A negative answer to a yellow question indicates a gap in the quality systems of the laboratory. It is suggested that laboratories that are flagged on yellow questions immediately and carefully review their safety, testing, and quality assurance protocols. Steps should be taken to improve whatever deficiencies exist as soon as possible.

It is recommended that laboratories with a high number of negative answers or negative answers on any “critical question” take the suggested steps to improve the safety, accuracy, and turn-around time of their mycobacteriology testing. *Mycobacterium Tuberculosis: Assessing Your Laboratory* may be used as often as you wish and it may be helpful to retake the assessment every 6 months to one year to determine your laboratory’s progress.

# **MYCOBACTERIUM TUBERCULOSIS: ASSESSING YOUR LABORATORY**

## **Part I: Questions**

### **General Specimen Collection and Handling**

1. Does your Mycobacteriology Laboratory have an up-to-date reference manual (electronic or written) for your providers that includes specimen requirements, testing methods, and testing algorithms?
2. Does your Mycobacteriology Laboratory provide instructions for specimen collection, storage, and transport that are easily understood by the person collecting the patient specimen?
3. As part of your Quality Assurance (QA) Program, does your Mycobacteriology Laboratory communicate regularly with providers to ensure that adequate specimens are obtained and to promote an understanding of quality assurance parameters?
4. Does your Mycobacteriology Laboratory obtain the following information on the submission form:
  - a. Patient identification information examples include: name, registration number and location, or a unique confidential specimen code if an alternate audit trail?
  - b. Patient gender?
  - c. Patient date of birth or age?
  - d. Name and address (if different than receiving laboratory) or other suitable identifiers of the legally authorized person ordering the test?
  - e. Tests to be performed?
  - f. Source of the specimen, when appropriate
  - g. Date and time of collection?
  - h. Clinical information when appropriate?
5. Does your Mycobacteriology Laboratory include instructions to the provider for specimen labeling?
6. Does your Mycobacteriology Laboratory include instructions to the provider for the volume and type of specimen required?
7. Does your Mycobacteriology Laboratory include instructions to the provider for packaging the specimen for delivery or transport?
8. Does your Mycobacteriology Laboratory provide sputum collection containers that include a sterile, leak-proof, and clear 50 ml plastic conical centrifuge tube with screw cap closures?
9. Does your Mycobacteriology Laboratory provide shipping containers for specimen transport via US Postal Service, commercial carrier, or courier?
10. Does your Mycobacteriology Laboratory have personnel certified to package and ship Category A Infectious Substances and Category B Biological Substances?

11. Does your Mycobacteriology Laboratory or the originating laboratory monitor the number of specimens collected per patient as part of the Quality Assurance (QA) program?
12. Does your Mycobacteriology Laboratory monitor delivery time to ensure that less than 24 hours have elapsed between specimen collection and its arrival at the laboratory?
13. Does your Mycobacteriology Laboratory verify that the patient's name and/or identification number on each specimen container matches that on the submission form?
14. Does your Mycobacteriology Laboratory furnish the healthcare provider with a copy of the laboratory's:
  - a. Criteria for rejecting specimens?
  - b. Reporting policy?
15. Does your Mycobacteriology Laboratory report unsatisfactory specimens to the healthcare provider within 24 hours of receipt?
16. Does your Mycobacteriology Laboratory review and record the number of specimens rejected and the reason for rejection as part of the quality assurance program?
17. Does your Mycobacteriology Laboratory include the following information on the specimen report form:
  - a. The patient's name and identification number OR a unique patient identifier and identification number?
  - b. Name and address of your laboratory?
  - c. Name and address of the testing laboratory, if different from the reporting laboratory?
  - d. The test report date?
  - e. The test performed?
  - f. The specimen source, when appropriate?
  - g. The test result and, if applicable, the units of measurement, interpretation, or both?
  - h. Any information regarding the condition and disposition of specimens that do not meet the laboratory's acceptability criteria.

## **Safety**

18. Does your Mycobacteriology Laboratory follow a written biosafety plan that:
  - a. Defines safe laboratory practices?
  - b. Includes procedures for handling spills and other emergencies?
19. Does your Mycobacteriology Laboratory have a written respiratory protection program?
20. Does your Mycobacteriology Laboratory provide annual fit testing if N-95 or N-100 respirators are used?

21. Does your Mycobacteriology Laboratory decontaminate all personal protective equipment and laboratory waste before it leaves the mycobacteriology laboratory area?
22. Does your Mycobacteriology Laboratory require employees to review the biosafety plan annually?
23. Does your Mycobacteriology Laboratory follow a written chemical hygiene plan that defines safe laboratory practice?
24. Does your Mycobacteriology Laboratory perform a risk assessment within the mycobacteriology work area or laboratory?
25. Does your Mycobacteriology Laboratory monitor the Mantoux tuberculin skin test (TST) or whole blood interferon gamma release assay (IGRA) conversion rate of personnel as part of a risk assessment plan?
26. Does your Mycobacteriology Laboratory provide the following for *new* employees:
  - a. A two-step Mantoux tuberculin skin test (TST) or IGRA?
  - b. A medical evaluation, including a chest radiograph, if the TST or IGRA is positive?
27. Does your Mycobacteriology Laboratory provide or ensure for *all* employees:
  - a. An annual tuberculin skin test (TST) or IGRA on tuberculin or IGRA negative employees?
  - b. A medical evaluation if the TST or IGRA converts to positive or if symptoms of tuberculosis are exhibited?
  - c. Medical evaluation, follow-up, and counseling for any known exposure event or TST/IGRA conversion?
  - d. Maintenance of a permanent record of skin testing or IGRA results?
  - e. A periodic (at least annual) symptom review for people with a history of latent TB infection (LTBI) or prior tuberculosis?
28. Does your Mycobacteriology Laboratory use a Class I, II, or III Biological Safety Cabinet (BSC) that has been certified at least annually?
29. Does your Mycobacteriology Laboratory work with **clinical specimens** in at least a Biosafety Level 2 (BSL-2) laboratory?
30. Does your Mycobacteriology Laboratory work with **cultures** suspected or confirmed to contain *M. tuberculosis* complex in a BSL-3 laboratory?
31. Does your Mycobacteriology Laboratory provide safety training on aerosol prevention techniques for all employees before assigning work with mycobacterial specimens or cultures?
32. Does your Mycobacteriology Laboratory perform all manipulations on mycobacterial specimens and cultures that may generate aerosols *only* in a BSC?

33. Does your Mycobacteriology Laboratory use a centrifuge equipped with aerosol-free carriers with O-rings?
34. Does your Mycobacteriology Laboratory limit access into the laboratory when clinical specimens are being processed or when working with mycobacterial cultures?
35. Does your Mycobacteriology Laboratory have a one-pass (non-recirculating) ventilation system?
36. Does your Mycobacteriology Laboratory monitor the environmental conditions in the isolation room at least annually to determine the number of air exchanges and the negative pressure status?
37. Does your Mycobacteriology Laboratory provide personal protective equipment that includes laboratory coats or gowns, gloves, respiratory, and face protection?

### **General Laboratory Practice**

38. Does your Mycobacteriology Laboratory monitor and evaluate the overall quality of the general laboratory systems?
39. Does your Mycobacteriology Laboratory participate in an approved proficiency testing program?
40. Does your Mycobacteriology Laboratory follow standard operating procedures and maintain the results of quality control for each test procedure for at least two years?
41. Does your Mycobacteriology Laboratory monitor and evaluate the overall quality of analytical systems?
42. Does your Mycobacteriology Laboratory ensure that accurate laboratory reports are sent to the submitter and maintain all patient reports and test records for a minimum of two years?
43. Does your Mycobacteriology Laboratory record the date and time positive results are telephoned, faxed, or electronically reported to health care provider(s) and public health officials?

### **Smears from Clinical Specimens**

44. Does your Mycobacteriology Laboratory process at least 15 acid-fast smears per week?
45. If your laboratory performs direct, unconcentrated smears, does your laboratory ensure that a concentrated smear is also performed?
46. Does your Mycobacteriology Laboratory use fluorochrome stain as the primary acid-fast stain for smears made from patient specimens?

47. Does your Mycobacteriology Laboratory check reactivity of fluorochrome stain for each batch of smears by staining and examining known acid-fast and non-acid-fast organisms?
48. Does your Mycobacteriology Laboratory report an approximation of the number of acid-fast organisms viewed on the slide by using a standard semi-quantitative scale?
49. Does your Mycobacteriology Laboratory telephone, fax, or electronically report all positive acid-fast smear results to both the patient's health care provider and the public health department as soon as results are known, and within 24 hours from the specimen receipt?
50. If your laboratory performs only smear microscopy, does your Mycobacteriology Laboratory send *all* specimens to a full service laboratory for culture within 24 hours of collection?
51. Does your Mycobacteriology Laboratory use indicators, such as turn-around-time, to monitor the quality of performance of laboratories to which you refer specimens for nucleic acid amplification, culture, identification, and/or drug susceptibility testing?

### **Public Health and Epidemiology**

52. Does your Mycobacteriology Laboratory receive and review a report of the prevalence and resistance patterns of *M. tuberculosis* complex isolated from your geographic area?
53. Does your Mycobacteriology Laboratory ensure universal genotyping by sending all initial *M. tuberculosis* complex isolates to the appropriate public health laboratory?
54. Does your Mycobacteriology Laboratory have access to laboratory training through your Public Health Laboratory?
55. Does your Mycobacteriology Laboratory have access to a state or regional Mycobacteriology Laboratory network that can provide surveillance data, training, and other resources?

### **Specimen Processing and Decontamination**

56. Does your Mycobacteriology Laboratory process and culture at least 20 specimens per week?
57. Does your Mycobacteriology Laboratory take steps to eliminate cross-contamination between cultures?
58. Does your Mycobacteriology Laboratory routinely process and culture specimens within 24 hours of receipt in the laboratory?
59. Does your Mycobacteriology Laboratory use a refrigerated centrifuge(s) at a relative centrifugal force (RCF) of at least 3000 x g for 15 to 20 minutes to process mycobacterial specimens for culture?

60. Does your Mycobacteriology Laboratory prepare, stain, and examine acid-fast smears from all specimens (except blood) sent for mycobacterial culture?

### **Inoculation and Growth Detection**

61. Does your Mycobacteriology Laboratory inoculate all specimens into a standardized broth system for the primary culture?
62. Does your Mycobacteriology Laboratory inoculate specimens other than blood to at least one solid medium?
63. Does your Mycobacteriology Laboratory inoculate a negative control with each batch of cultures that are inoculated?
64. Does your Mycobacteriology Laboratory use a continuously monitoring broth system or check broth cultures for evidence of growth every 2-3 days for weeks 1-3, and weekly thereafter for at least six weeks?
65. Does your Mycobacteriology Laboratory examine cultures on solid media for evidence of growth weekly for 6-8 weeks?
66. Does your Mycobacteriology Laboratory use a microscope or hand lens to examine solid media for earlier visualization of mycobacterial growth?
67. Does your Mycobacteriology Laboratory perform an acid-fast smear from:
- a. Broth cultures indicating growth?
  - b. Selected colonies on solid medium at an early stage growth?
68. Does your Mycobacteriology Laboratory subculture all broth cultures exhibiting acid-fast growth to solid medium?
69. Does your Mycobacteriology Laboratory use or ensure the use of a rapid method to confirm or rule out the presence of *M. tuberculosis* complex from positive cultures?
70. Does your Mycobacteriology Laboratory monitor the turnaround time of test results to ensure that 80% of *M. tuberculosis* complex isolates from primary patient specimens are identified within 21 days of specimen collection?
71. Does your Mycobacteriology Laboratory retain positive *M. tuberculosis* complex cultures for at least one year and other *Mycobacterium spp.* for a minimum of three months?
72. Does your Mycobacteriology Laboratory, as part of your laboratory QA program, correlate the smear positive and negative results with culture positive and negative results to evaluate smear/culture quality?
73. Does your Mycobacteriology Laboratory document the rate of contamination of culture media inoculated with digested/decontaminated sediment as a way of monitoring the specimen preparation and decontamination process?

74. Does your Mycobacteriology Laboratory subculture colonies with differing morphology for use in species identification?
75. Does your Mycobacteriology Laboratory identify a broad range of nontuberculous mycobacteria?

### **Susceptibility Testing**

76. Does your Mycobacteriology Laboratory perform or ensure susceptibility tests on all initial isolates of *M. tuberculosis* complex?
77. Does your Mycobacteriology Laboratory ship the initial positive culture (broth or solid media, whichever grows first) to a reference laboratory as soon as *M. tuberculosis* complex is detected if susceptibility testing is not performed in your laboratory?
78. Does your Mycobacteriology Laboratory determine or ensure susceptibility testing of *M. tuberculosis* complex isolates to primary drugs using a broth-based system?
79. Does your Mycobacteriology Laboratory use a strain of *M. tuberculosis* complex that is susceptible to all anti-mycobacterial agents being tested as a control once each week that patient isolates are tested?
80. Does your Mycobacteriology Laboratory perform or ensure second-line drug susceptibility testing for *M. tuberculosis* complex when appropriate?
81. Does your Mycobacteriology Laboratory follow CLSI guidelines regarding the first-line and second-line drugs tested?
82. Does your Mycobacteriology Laboratory repeat susceptibility testing if the patient is culture-positive after three months of therapy or shows clinical evidence of failure to respond to therapy?
83. Does your Mycobacteriology Laboratory confirm or ensure confirmation of drug resistance?
84. Does your Mycobacteriology Laboratory follow a different algorithm if there is suspicion of drug resistance [any drug resistance, multi drug resistance (MDR) or extensive drug resistance (XDR)] as indicated on the request form?
85. Does your Mycobacteriology Laboratory telephone, fax, or electronically transmit a drug susceptibility report to the health care provider as soon as results are available and follow with a written report within 24 hours?
86. Does your Mycobacteriology Laboratory telephone, fax, or electronically transmit a drug susceptibility report to the Tuberculosis Control Program in the state where the patient resides as soon as the results are available?

87. Does your Mycobacteriology Laboratory report at least 80% of initial *M. tuberculosis* complex drug susceptibility test results within 28 days of specimen collection?
88. Does your Mycobacteriology Laboratory monitor the turnaround time for reporting primary drug susceptibility test results on *M. tuberculosis* complex isolates to ensure reports are sent within 28 days of specimen collection?
89. Does your Mycobacteriology Laboratory participate in a proficiency testing program for drug susceptibility testing?

### **Direct Detection**

90. Does your Mycobacteriology Laboratory perform or ensure access to (via a reference laboratory) to nucleic acid amplifications (NAA) testing for direct detection of *M. tuberculosis* complex in AFB smear-positive respiratory initial diagnostic specimen?
91. Does your Mycobacteriology Laboratory perform or ensure access (via a reference laboratory) to NAA testing for direct detection of *M. tuberculosis* complex in AFB smear-negative respiratory specimens from patients at high risk for TB?
92. Does your Mycobacteriology Laboratory perform or provide access (via a reference laboratory) to a *M. tuberculosis* complex NAA test that allows detection of inhibitors?
93. Does your Mycobacteriology Laboratory telephone, fax, or electronically report result of *M. tuberculosis* complex NAA testing results within 48 hours of receipt for 75% of specimens tested in the laboratory?
94. Does your Mycobacteriology Laboratory perform or ensure access (via a reference laboratory) to molecular detection of drug resistance, especially to rifampin and isoniazid?

## **MYCOBACTERIUM TUBERCULOSIS: ASSESSING YOUR LABORATORY**

### **PART II: Guidance**

#### **Specimen Handling and Collection**

Regulations implementing the Clinical Laboratory Improvement Amendments (CLIA) of 1988 specifically address laboratory responsibility in the area of patient test management<sup>1</sup>.

**Patient Test Management For Moderate or High Complexity Testing:** The laboratory must employ and maintain a system that provides for proper patient preparation; proper specimen collection, identification, preservation, transportation and processing; and accurate result reporting. The laboratory system must ensure optimum specimen integrity and identification throughout the pre-analytical, analytical, and post-analytical processes.

The efficacy of the laboratory smear examination, nucleic acid amplification assays, culture and identification procedures and susceptibility testing from clinical specimens depends upon the collection and transport of quality specimens. The laboratory staff should provide written or electronic information that will promote high quality specimens. These policies and procedures must ensure positive identification and optimal integrity of the specimen from collection to reporting without delay. A specimen collected and transported haphazardly could potentially yield suboptimal or misleading results.

**For Referral Specimens:** A laboratory must refer specimens for testing only to a laboratory possessing a valid certificate authorizing the performance of testing in the specialty or subspecialty of service for the level of complexity in which the referred test is categorized. The referring laboratory must specify its requirements for turnaround times and tests to be performed on the specimens submitted.

The referring laboratory must retain or be able to produce an exact duplicate of the testing laboratory's report.

The authorized person who orders a test or procedure must be notified by the referring laboratory of the name and address of each laboratory location at which a test was performed.

#### **General Specimen Collection and Handling**

**1. Does your Mycobacteriology Laboratory have an up-to-date reference manual (electronic or written) for your providers that includes specimen requirements, testing methods, and testing algorithms?**

Laboratories should provide submitters' with a reference manual that contains the following information about each laboratory test available:

- Laboratory contact information
- Test description and methodology
- Availability of test
- Expected turn-around time
- Recommended uses

- Contraindications
- Specimen requirements
- Collection instructions (patient preparation, collection container, preservation etc.)
- Specimen handling and transport
- How to fill out the test request form, required information
- Criteria for specimen rejection
- Result format
- Test limitations
- Reflex testing under certain conditions (for example: if MTBC isolated, first-line drug susceptibility testing automatically performed)<sup>2</sup>.

**2. Does your Mycobacteriology Laboratory provide instructions for specimen collection, storage, and transport that are easily understood by the person collecting the patient specimen?**

The health care professional responsible for collecting mycobacteriology specimens should be informed by the laboratory of the necessity for extreme care in the collection and handling of specimens<sup>1</sup>. The results of tests, as they affect patient diagnosis and treatment, can be directly related to the quality of the specimen collected and delivered to the laboratory. It is imperative for the laboratory to develop a working relationship with the health care professionals who collect patient specimens so that information can be freely exchanged.

Patients should be instructed by the attending medical personnel in methods and importance of proper specimen production and collection. See Appendix A for more information on proper specimen collection procedures.

Specific instruction to patients should include information on the difference between sputum and saliva or nasopharyngeal secretions; the necessity for a deep, productive cough; and rinsing the mouth with water before collecting a sputum specimen. Information should be provided to the patient on the volume of sputum specimen needed.

Additionally, the patient should be informed of the possibly infectious nature of his or her secretions, and the need to tightly close the collection container after the specimen is collected. The specimen should not contaminate the outside of the tube or collection container.

Specimens are preferably collected under the direction of a trained health care professional. Because of the infectious nature of tuberculosis and the danger to the health care professional, guidelines have been developed that specifically address the necessity for control of conditions under which specimens are collected.

Written laboratory instruction to providers should include the following:

- Collect a series of at least three sputum specimens at least eight hours apart and at least one of which is an early morning specimen. If two of the first three sputum smears are positive, three specimens are enough to confirm the diagnosis. A few patients shed mycobacteria in small numbers and only irregularly; for these patients, obtaining additional specimens may be indicated.

- Collect specimens before chemotherapy is started; even a few days of drug therapy may kill or inhibit sufficient numbers of mycobacteria to prevent isolation.
- Collect at least 1 sputum specimen monthly to monitor treatment until at least 2 sequential monthly specimens are culture-negative<sup>3</sup>.

The efficacy of the laboratory procedure used to perform nucleic acid amplification assays and to culture mycobacteria from clinical specimens depends on the manner in which the specimen is obtained and handled. Therefore, following collection, specimens should be transported as quickly as possible to the laboratory, preferably within 30 minutes. Specimens delayed longer than 30 minutes before transport should be refrigerated. See M48A (Table 2) for a comprehensive list of specimen types and recommendations for collection and transportation<sup>4</sup>.

To see the exact wording of the corresponding CLIA regulation, refer to CLIA 493.1241.

**3. As part of your Quality Assurance (QA) Program, does your Mycobacteriology Laboratory communicate regularly with providers to ensure that adequate specimens are obtained and to promote an understanding of quality assurance parameters?**

Processing inadequate specimens wastes financial and personnel resources and delays the potential diagnosis of tuberculosis<sup>5</sup>.

Personal communication with providers underscores the importance of an acceptable specimen and helps to educate the health care professional about the laboratory specimen requirements and test parameters (e.g. specificity, sensitivity, positive predictive values, and negative predictive values). Communication enhances the health care team approach and reinforces the value of each individual's contribution to total patient care<sup>2</sup>.

**4. Does your Mycobacteriology Laboratory obtain the following information on the submission form:**

- a. Patient identification information examples include: name, registration number and location, or a unique confidential specimen code if an alternate audit trail?**
- b. Patient gender?**
- c. Patient date of birth or age?**
- d. Name and address (if different than receiving laboratory) or other suitable identifiers of the legally authorized person ordering the test?**
- e. Tests to be performed?**
- f. Source of the specimen, when appropriate**
- g. Date and time of collection?**
- h. Clinical information when appropriate?**

The information listed is required by CLIA and must be present or solicited from the requestor when not included<sup>1</sup>. Quality Assurance guidelines require that you monitor your program regularly to ensure the availability of this information on each specimen submitted.

The information required by your individual laboratory or TB Control Program may be more extensive. It is important to seek the assistance of the state Tuberculosis Control Program and infectious disease specialists when designing a new form. The TB laboratory, TB Control and healthcare providers must work together to establish request forms which serve all parties involved, benefiting patient care and the control of tuberculosis. Ideally, the following information should be included with the request in order to optimize scarce resources in the current healthcare environment and to optimize the value of the TB laboratory's contribution:

- The date when anti-TB treatment was started and drug regimen patient is receiving. (If applicable)
- Whether or not drug resistance suspected (e.g. patient exposure to MDR or XDR-TB, foreign born, previously treated for TB, etc).

Additionally, the TB laboratory is charged with informing its partners of the requirements for optimum testing, such as sample volume, rapid transportation, test limitations, etc. This becomes challenging for commercial laboratories serving the entire country, whereas state public health laboratories can fine tune their operation with the state TB control program.

Instruction to the provider should encourage completing the specimen submission form as a part of the institutional or hospital information system<sup>1</sup>. To prevent possible contamination from a leaking specimen, the form should be separated from the specimen container (e.g. in a plastic specimen bag with a separate pocket provided for the specimen form).

The laboratory must perform tests only upon written or electronic request of an authorized person; oral requests are permitted only if the laboratory subsequently obtains written authorization for testing within 30 days. Attempts to obtain written authorization must be documented.

To see the exact wording of the regulation, refer to CLIA 493.1241.

##### **5. Does your Mycobacteriology Laboratory include instructions to the provider for specimen labeling?**

The labeling procedure that a laboratory follows must ensure positive identification and optimum integrity of the patient specimen from collection to reporting. Laboratory written policy, in keeping with CLIA regulation, can define an acceptable labeling requirement and should be included in provider instructions<sup>1</sup>. Providers should be expected to comply with this instruction. Any deviation from the laboratory requirement for proper labeling is reason to reject the specimen.

To see the exact wording of the regulation, refer to CLIA 493.1232/42.

##### **6. Does your Mycobacteriology Laboratory include instructions to the provider for the volume and type of specimen required?**

CLIA regulations state that a laboratory should define and include policies on specimen acceptability within provider instructions<sup>1</sup>. Provider instructions should include a request for individual sputum volume of not less than 3mL<sup>5</sup>. However, the optimal volume is 5-10mL<sup>6</sup>. Less

than 3mL may not provide the optimal opportunity for recovery of mycobacteria. Low quality (i.e. expectorated sputum that appears to be saliva) or an inadequate quantity of sputum specimen should be noted on the laboratory report. The requirement for submitting acceptable volumes of different specimens should be included in the health care provider instructions.

To see the exact wording of the regulation, refer to CLIA 493.1242.

**7. Does your Mycobacteriology Laboratory include instructions to the provider for packaging the specimen for delivery or transport?**

CLIA regulations state that a laboratory should define and include policies on specimen delivery and/or transport within provider instructions<sup>1</sup>. Specimens should be collected in appropriate tubes that are sterile, clear, plastic, and leak-proof, (preferably a 50-mL screw capped centrifuge tube that can withstand 3000 x g). Specimens should be delivered in transport containers that:

- Protect the staff and environment from possible exposure in case of leakage
- Separate the form from the specimen
- Ensure the safety of anyone handling the specimen during transport

To see the exact wording of the regulation, refer to CLIA 493.1242.

Specimen forms may be handled by staff that are not protected by personal protective equipment. Therefore, it is very important to keep the form separated from the specimen itself, to keep it uncontaminated. Any form that may have been contaminated by the specimen should be contained and treated as medical waste.

Federal and international regulations must be met when using the postal service or commercial courier service to send samples containing etiologic agents. Proper packaging reduces the number of broken specimens, contains and absorbs leaking specimens, and helps ensure the safety of personnel handling them<sup>4</sup>.

Please refer to the following references for a shipping service's specific requirements:

- Title 49 Code of Federal Regulations, Transportation Parts 100 and 185. Available electronically and from U.S. Government Printing Office, [www.access.gpo.gov](http://www.access.gpo.gov)
- International Air Transport Association Dangerous Goods Regulations (46<sup>th</sup> ed.) 2005. Available for purchase from IATA 800-716-6326 or [www.iata.org](http://www.iata.org)
- International Air Transport Association Infectious Substances and Diagnostic Specimens Shipping Guidelines (5<sup>th</sup> ed.) 2004. Available for purchase from IATA 800-716-6326 or [www.iata.org](http://www.iata.org)
- USPS regulations. Domestic Mail Manual C023: <http://pe.usps.gov>
- U.S. Department of Transportation (DOT): [www.dot.gov](http://www.dot.gov) [www.tsi.dot.gov](http://www.tsi.dot.gov) (Transportation Safety Institute)

- International Civil Aviation Organization (ICAO): [www.icao.int](http://www.icao.int)

**8. Does your Mycobacteriology Laboratory provide sputum collection containers that include a sterile, leak-proof, and clear 50 mL plastic conical centrifuge tube with screw cap closures?**

The vial or tube for collecting a mycobacteriology specimen must be sterile, clear, plastic, and leak-proof (i.e. 50-mL screw capped centrifuge tubes) for ease of handling, consistency, and safety. These tubes are designed with screw caps for watertight closure.

50-ml plastic, disposable centrifuge tubes that can withstand 3000 x g are recommended. They are optimal for collection and processing specimens for these reasons:

- They eliminate the need for transport to another tube and prevent the opportunity for mislabeling
- They allow space for adequate mixing of the specimen and the digesting agent
- They allow the specimen to be processed within the specimen container
- They can be centrifuged without transfer or aerosol formation
- The specimen volume can be easily determined

Sputum collection kits that contain appropriate 50-mL tubes are available from various scientific supply distributors.

All centrifugation should be performed using safety cups to enclose the centrifuge tubes. Overfilled tubes may result in leakage during the centrifugation<sup>3</sup>.

**9. Does your Mycobacteriology Laboratory provide shipping containers for specimen transport via US Postal Service, commercial carrier or, courier?**

Although no regulation specifically addresses the necessity for providing transport material to providers, doing so helps control the uniformity and correct parameters for shipping (such as the correct type of centrifuge tubes). The containers for submission should be appropriate for the transport system in place. Laboratory designed and distributed specimen submission forms specific for the mycobacteriology program ensure the accessibility of the information necessary for proper testing, reporting and follow-up.

**10. Does your Mycobacteriology Laboratory have personnel certified to package and ship Category A Infectious Substances and Category B Biological Substances?**

(DOT 49 CFR part 172.704) The Department of Transportation requires training for all persons involved in the transportation of hazardous materials and infectious substances in commerce.

**Category A** “infectious substances” specifically include *M. tuberculosis* complex cultures and have extensive regulations for shipping; these cultures are no longer accepted by the US Postal Service for transportation. Clinical specimens are classified as **Category B** “biological substances” and also have special regulations for shipping<sup>7</sup>. See the resources indicated in the guidelines for question 7 for more information.

**11. Does your Mycobacteriology Laboratory or the originating laboratory monitor the number of specimens collected per patient as part of the Quality Assurance (QA) program?**

Monitoring the number and succession of specimens received on each patient identifies collection problems that otherwise might go unnoticed. Since some patients shed mycobacteria intermittently and only in small numbers, collecting a greater number of specimens increases the likelihood of obtaining a positive culture<sup>8,9</sup>. The number of bacilli in a specimen varies from patient to patient and from day to day. Since guidelines recommend the collection of three specimens per patient<sup>3</sup>, monitoring the number of specimens submitted per patient will improve the quality of the laboratory program and increase the interaction between hospital and laboratory staff. Communication of such information with the provider(s) requesting tests will result in more appropriate testing.

Specific monitoring activity can be based on reviewing a certain number or percentage of specimens by patient per week or month, depending on the number and quality of laboratory specimens received. If many single, unrelated sputum specimens are received, it would be important to remind your providers of the value of multiple specimens. This QA activity should be recorded and maintained with other QA records.

**12. Does your Mycobacteriology Laboratory monitor delivery time to ensure that less than 24 hours have elapsed between specimen collection and its arrival at the laboratory?**

To ensure that specimens are processed quickly and accurately, they must be received in a timely manner. The CLIA Quality Assurance (QA) guidelines require “accurate, reliable, and prompt reporting of test results<sup>1</sup>.” Patient treatment can be significantly enhanced by a timely acid-fast smear report.

Specimens should be delivered to the laboratory as soon as possible after collection so the smear, nucleic acid amplification, and culture are performed as soon as possible. Holding specimens while waiting for more specimens to accumulate is not recommended as it further delays a process that requires three to four weeks from beginning to completion.

The date and time of collection should be required on the specimen submission form, and the date and time of receipt should be noted by the laboratory. A monthly or weekly recording and regular monitoring of these times by sample for a measured number or percent of specimens will serve as QA documentation. When necessary, this documentation can be used to work with health care providers and/ or clients to reduce the time of specimen delivery to the laboratory.

In the interest of speeding delivery or transport, CDC recommends the following: “Promote the rapid delivery of specimens to the laboratory on a daily basis, even if this requires pickup of individual specimens to guarantee arrival within 24 hours<sup>10</sup>.” A change in the transport system can only be effected through close cooperation with the provider and a commitment from the laboratory management team.

**13. Does your Mycobacteriology Laboratory verify that the patient’s name and/or identification number on each specimen container matches that on the submission form?**

Your laboratory must have available and follow a documented policy for proper labeling of specimens<sup>1</sup>. Specimens that are being processed, transferred and/or subcultured must be labeled appropriately to ensure the integrity of the specimens.

The specimen container should be labeled with the patient's name and/or number. The name and number on the container should match that on the accompanying specimen request form. Each specimen should be checked before processing to ensure that it is properly identified. Specimens that are not properly identified should be rejected.

To see the exact wording of the regulation, see CLIA 493.1232.

**14a. Does your Mycobacteriology Laboratory furnish the healthcare provider with a copy of the laboratory's criteria for rejecting specimens?**

The healthcare provider must be given a copy of the laboratory's criteria for rejection of specimens<sup>1</sup>. The laboratory must report any information regarding the condition and disposition of specimens that do not meet the laboratory's criteria for acceptability. Unsatisfactory specimens must be reported to the provider as soon as possible.

Better cooperation will be obtained if the healthcare provider understands the laboratory's rejection policy. Listed below are several typical reasons for specimen rejection (the list is not exhaustive):

- Specimen not labeled.
- Name on specimen and requisition form do not match.
- Specimen leaking.
- Specimen in non-sterile container.
- Gastric specimen more than two hours old or not neutralized.
- Prolonged delivery time.

To see the exact wording of the regulation, see CLIA 493.1242.

**14b. Does your Mycobacteriology Laboratory furnish the healthcare provider with a copy of the laboratory's reporting policy?**

CLIA regulations require that an adequate system be in place to report test results in a timely, accurate, and confidential manner. If a specimen is unsatisfactory, the reason(s) for rejection must be reported. The laboratory must develop and follow a written procedure for reporting life-threatening or "critical" laboratory results or values<sup>1</sup>.

To see the exact wording of the regulation see CLIA 493.1291.

Initial positive critical-results, as defined by your laboratory, should be telephoned, faxed, or electronically reported to the health care provider as soon as they are available, so that patient management procedures can begin immediately. Delay in this crucial step can result in the dissemination of tuberculosis from infected individuals to his or her contacts in the community or health care setting. A single undiagnosed, multiple drug resistant (i.e. MDR-TB) case can have a major impact on the control of TB in the community.

The following format for reporting positive acid-fast microscopy<sup>10</sup> and TB culture results<sup>11</sup> is recommended:

- a. Report positive microscopy results within 24 hours of specimen receipt.
- b. Report results confirming the identification of *M. tuberculosis* complex as soon as available, but within 21 days of specimen collection.
- c. Report drug susceptibility results as soon as available, but within 28 days of specimen collection.
- d. Report all final positive or negative test results in writing or electronically as a cumulative report that includes previously reported preliminary results.
- e. Follow all telephoned or faxed reports with a written report on the same or next work day. Confidentiality of patient results is a special concern when telephoning or faxing results. The laboratorian and health care provider should communicate to establish a protocol that secures confidentiality under all circumstances.

For more information on critical results, please refer to Appendix B.

**15. Does your Mycobacteriology Laboratory report unsatisfactory specimens to the healthcare provider within 24 hours of receipt?**

It is recommended that laboratories report specimen rejection to the health care provider as soon as possible. Immediate notification of an unsatisfactory specimen may allow the provider to collect another sample. A delayed unsatisfactory report postpones the collection and examination of a replacement specimen, and may result in deferred or inadequate care of the patient. Immediate attention to the quality of the specimen delivers a message to the provider that an unsatisfactory specimen is important enough to merit special handling and calling the submitter.

**16. Does your Mycobacteriology Laboratory review and record the number of specimens rejected and the reason for rejection as part of the quality assurance program?**

A written QA program will establish a routine review that requires a focused surveillance of the quality of specimens received for laboratory testing. If there is a pattern of submission of unsatisfactory specimens, or if one provider has an unusual number of unsatisfactory reports, action should be taken in the form of communication with or training of the specific provider. Although some specimens may still be rejected, the laboratory can improve the quality of specimens received by eliminating any misunderstanding providers may have concerning how to properly submit specimens.

Monitor unsatisfactory specimens by recording individual specimens rejected over time (i.e. for 1 month). Record the number, the source, and the reason for rejection. Repeat the surveillance quarterly, notifying providers with a compliance problem of ways they can improve specimen quality<sup>13, 14</sup>.

**17. Does your Mycobacteriology Laboratory include the following information on the specimen report form?**

- a. **The patient's name and identification number OR a unique patient identifier and the identification number?**
- b. **Name and address of your laboratory?**
- c. **Name and address of the testing laboratory, if different from the reporting laboratory?**
- d. **The test report date?**
- e. **The test performed?**

- f. **The specimen source, when appropriate?**
- g. **The test result and, if applicable, the units of measurement, interpretation, or both?**
- h. **Any information regarding the condition and disposition of specimens that do not meet the laboratory's acceptability criteria?**

These data elements are the minimal information required by CLIA regulations to appear on a specimen report form<sup>1</sup>. Your laboratory may seek input from healthcare providers and TB Control for additional relevant information to be included on your report form.

To see the exact wording of the regulation see CLIA 493.1291.

Laboratories having a single Health Care Financing Administration (HCFA) certificate for multiple sites/locations must have a system in place to identify which tests were performed at each site.

The laboratory must provide information that is necessary for proper interpretation of the results. Therefore, the numbers of organisms viewed per field and the type of stain used will be of interest to the physicians when reporting smear results. *M. tuberculosis* complex susceptibility reports of "susceptible" or "resistant" should include a record of the method used, (i.e., broth or agar proportion method), and also the anti-tuberculosis drugs tested and the concentration of each drug used.

Additional information that may be added to the specimen report form but is not required by CLIA includes:

- The date and time the specimen was received in the laboratory
- Test methods as appropriate

## **Safety**

### **SAFETY IN THE MYCOBACTERIOLOGY LABORATORY IS EVERYONE'S RESPONSIBILITY!!**

Although providing a safe work environment in the laboratory is the responsibility of management and administration, it is the responsibility of the worker to practice safe work habits, follow established safety procedures, and help protect the safety of himself/ herself and others. Potentially infectious aerosols are the greatest hazard in the mycobacteriology laboratory. Infectious aerosols can be created in the laboratory by any of the following manipulations<sup>15</sup>:

- Pouring liquid culture or supernatant fluids
- Using fixed volume automatic pipettes
- Mixing fluid cultures with pipettes
- Using a high-speed blender for homogenizing
- Dropping tubes or flasks of broth cultures
- Breaking tubes during centrifugation

- Agitating specimens during processing
- Letting drops of microbial suspension fall from a pipette onto a hard work surface
- Sonicating
- Vortexing
- Spreading inoculum on solid medium

This list includes many of the common manipulations that may produce aerosols, but is not all inclusive.

**18.**

**18a. Does your Mycobacteriology Laboratory following a written biosafety plan that defines safe laboratory practices?**

Federal “Bloodborne Pathogens” Regulations<sup>16</sup> require that a biosafety manual be prepared or adopted by every laboratory working with infectious material. The plan should describe administrative policies, work practices, facility design, and safety equipment to prevent transmission of biologic agents to laboratory workers, other persons, and the environment. Personnel must be advised of special hazards and are required to read and to follow instructions on practices and procedures<sup>17</sup>. This plan, along with an explanation of its contents, must be available to employees.

**18b. Does your Mycobacteriology Laboratory follow a written biosafety plan that includes procedures for handling spills and other emergencies?**

Laboratory workers should know the appropriate action to take and persons to contact in an emergency involving exposure to potentially infectious materials. Because the immediate response will have an impact on the final outcome of the incident, safety drills should be conducted regularly, so that employees learn to respond appropriately to spills and potential exposures. The plan should include the following elements:

- Evacuation procedure
- Procedure for notification of staff and supervisors
- Instructions on how to evaluate the extent of contamination and exposure
- Procedure for safe re-entry and the personal protective equipment required
- Decontamination and clean-up procedure
- Accident report procedure
- Medical follow-up procedure

If your biosafety plan does not have detailed information on how to handle a laboratory emergency, information may found in the CDC Laboratory Manual, *Isolation and Identification of Mycobacterium tuberculosis: A Guide for the Level II Laboratory*, pages 140-142<sup>18</sup>.

**19. Does your Mycobacteriology Laboratory have a written respiratory protection program?**

The Occupational Safety and Health Standards require employers to provide a written respiratory protection program. 29 CFR 1910.134(c) specifies that “The employer is required to develop and implement a written respiratory program with required worksite-specific

procedures and elements for required respirator use<sup>19</sup>.” The written program shall include the following elements:

- selection process; medical evaluations
- fit testing
- procedures for use
- procedures and schedules for cleaning the respirator
- disinfecting
- storing
- inspecting
- repairing and discarding
- quantity and flow
- training in respiratory hazards
- training in use limitations
- maintenance and procedures for regularly evaluating the effectiveness of the program.

**20. Does your Mycobacteriology Laboratory provide annual fit testing if N-95 or N-100 respirators are used?**

The Occupational Safety and Health Standards require employers ensure annual fit testing if N-95 or N-100 respirators are used. 29 CFR 1910.134(f) specifies that “Before an employee may be required to use any respirator with a negative or positive pressure tight-fitting face piece, the employee must be fit tested with the same make, model, style and size of the respirator that will be used<sup>19</sup>.” The employer shall ensure that employees using a tight-fitting face piece respirator pass an appropriate qualitative or quantitative fit test. After initial fit testing, employees are required to be fit tested annually thereafter. Fit testing should also be done whenever there is a change in the respirator type, a change in employee physical condition that could affect the fit or upon observations or reports.

Powered air purification respirators (PAPR) can be used if fit testing is not available and for staff who cannot wear respirators (e.g. men with beards)<sup>19</sup>.

**.21. Does your Mycobacteriology Laboratory decontaminate all personal protective equipment and laboratory waste before it leaves the mycobacteriology laboratory area?**

Reusable laboratory clothing should be placed into covered containers or laundry bags and autoclaved before laundering. Gloves, disposable masks and other disposable clothing may be discarded when contaminated or when single usage is complete. Disposable gloves should never be reused. CDC-approved methods for decontaminating disposables include autoclaving, chemical disinfection, and incineration<sup>17</sup>.

The laboratory must have a method for decontaminating all laboratory wastes, preferably within the laboratory. Autoclave indicators (i.e. spore strips) should be used to monitor autoclave function.

**22. Does your Mycobacteriology Laboratory require employees to review the biosafety plan annually?**

Federal regulations regarding occupational exposure to infectious material require a written biosafety plan, which includes the safe handling of infectious agents. Federal regulations as they

apply to medical laboratories defer to the *CDC/NIH Biosafety in Microbiological and Biomedical Laboratories* manual<sup>17</sup>. Laboratory personnel must receive appropriate training in:

- The potential hazards associated with the work
- The necessary precautions to prevent exposure
- The exposure evaluation procedures

Personnel must receive annual updates or additional training as necessary for procedure or policy changes.

### **23. Does your Mycobacteriology Laboratory follow a written chemical hygiene plan that defines safe laboratory practice?**

Federal regulations regarding occupational exposure to chemicals require the development and implementation of a written chemical hygiene plan that specifically identifies each chemical hazard in the workplace. Material safety data sheets (MSDS) must be available in an easily retrievable format. Employees must be informed of the chemical hazards in the workplace and be prepared for emergency action if exposure occurs<sup>20</sup>.

Personnel must receive annual updates or additional training as necessary for procedure or policy changes.

### **24. Does your Mycobacteriology Laboratory perform a risk assessment within the mycobacteriology work area or laboratory?**

Assuring a safe working environment in each laboratory should be based on a risk assessment within the area of work. The risk assessment should be conducted by a group that may include laboratorians, microbiologists, hospital epidemiologists, infectious disease specialists, and/or pulmonary disease specialists. Further information on developing a risk assessment plan can be found in CDC's *Guidelines for Preventing the Transmission of Tuberculosis in Health Care Facilities* (2005)<sup>8</sup> and in CDC's *Controlling tuberculosis in the United States: recommendations from the American Thoracic Society, CDC, and the Infectious Diseases Society of America*<sup>21</sup>.

With special engineering controls and the use of personal protective equipment (PPE), the laboratory staff may become comfortable in their own environment and develop an artificial sense of safety. Risk assessment and abatement procedures should be conducted for each stage of culturing mycobacteria, from opening the specimen mailing container or delivery tray to transferring actively growing cultures.

The prevalence of multi-drug resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) in the patient population should also be taken into account. The frequency of tuberculin skin test (TST) or interferon gamma release assays (IGRA) testing of laboratory workers should be based on the level of risk within the immediate work group. Since mycobacteriology laboratory workers may be at increased risk of becoming infected with *M. tuberculosis* complex, they should be evaluated for infection periodically using TST or IGRA.

Laboratories with a history of conversion(s) within the past year should consult the State TB Control Program or the infection control professional in their institution to develop a plan of action that meets the needs of the laboratory.

In certain clinic and hospital settings, the laboratorian may be present or involved in the collection of expectorated or induced sputum specimens. Employees who have been adequately trained and fit tested with National Institute of Safety and Health (NIOSH) Type C ( $\geq 95\%$  efficiency) respirators will be protected from TB infection. Strict management procedures and reliable engineering controls are needed to reduce the risk of exposure<sup>8</sup>.

**25. Does your Mycobacteriology Laboratory monitor the Mantoux tuberculin skin test (TST) or whole blood interferon gamma release assay (IGRA) conversion rate of personnel as part of a risk assessment plan?**

If insufficient data for determining risk have been collected based on TST conversions or positive IGRA among laboratory staff, these data should be compiled, analyzed, and reviewed expeditiously. Until such data are analyzed and found to warrant a lesser risk rating, laboratory workers should be considered high risk<sup>8</sup>.

**26. Does your Mycobacteriology Laboratory provide or ensure the following for *new* employees:**

**26a. A two-step Mantoux tuberculin skin test (TST) or IGRA?**

All new employees should be evaluated at the time of employment to establish a baseline for future TST or IGRA testing. If the TST is used, a two-step method should be used to detect the boosting phenomenon that might be misinterpreted as skin test conversions. An IGRA can be used for those employees with a history of vaccination with *M. bovis* BCG.

Newly hired employees with a documented history of a positive TST, adequately treated disease, or a history of having completed adequate preventive therapy for infection, and who have no symptoms or signs of active tuberculosis, should be exempt from TST screening. Employees with a positive initial skin test should be referred for medical evaluation<sup>8</sup>.

**26b. A medical evaluation, including a chest radiograph, if the TST or IGRA is positive?**

Laboratory workers with positive TST(s) or IGRA should have a chest radiograph as part of the initial medical evaluation of their TST. If the chest radiograph is negative, repeat chest radiographs are not needed unless symptoms develop that may be due to TB. All information on initial and subsequent TB screening should become a part of the employee's permanent record and maintained confidentially<sup>8</sup>.

**27. Does your Mycobacteriology Laboratory provide or ensure for *all* employees:**

**27a. An annual tuberculin skin test (TST) or IGRA on tuberculin or IGRA negative employees?**

For laboratories with careful documentation of tuberculin skin tests for a 3-5 year period with no conversions, annual skin testing for employees is appropriate. In a laboratory where transmission of tuberculosis has recently occurred, tuberculin testing should be repeated every three months until no additional conversions have been detected for two consecutive three-month intervals<sup>8</sup>.

**27b. Medical evaluation if the TST or IGRA converts to positive or if symptoms of tuberculosis are exhibited?**

If medical evaluation suggests tuberculosis, immediate investigation and follow-up should be initiated. Tuberculin skin tests or IGRA testing of other employees should also be initiated. Employees with symptoms of tuberculosis should be examined immediately by their health care provider<sup>8</sup>.

**27c. Medical evaluation, follow-up, and counseling for any known exposure event or TST/IGRA conversion?**

All laboratory workers with newly recognized positive TST or IGRA tests should be promptly evaluated for clinically active TB. Those without clinical TB should be evaluated for possible latent TB according to published guidelines<sup>22, 23</sup>.

If an employee becomes TST or IGRA positive, a history of possible exposure should be obtained by working with TB Control/Infection Control in an attempt to determine the potential source of exposure. If a laboratory source is known, the drug susceptibility pattern of the particular strain of *M. tuberculosis* complex should be determined to implement appropriate preventive therapy for the worker.

In the event of the TST/ IGRA conversion of a laboratory employee, supervisors should:

- Review laboratory activities and practices for possible breach in standard operating procedures.
- Review and test all equipment for safe operation and review safety procedures with all employees<sup>24</sup>.
- Re-evaluate the adequacy of the safety plan.

**27d. Maintenance of a permanent record of skin testing or IGRA results?**

Results of TST or IGRA tests should be confidentially recorded both in the individual employee health records and in a retrievable aggregate database of all workers' results, so that they can be analyzed periodically to estimate the risk of acquiring new infection in the laboratory. This record is the basis of the risk assessment and the development of an effective biosafety plan. All information on initial and subsequent TB screening should become a part of the employee's permanent record and be maintained confidentially<sup>8</sup>.

**27e. A periodic (at least annual) symptom review for people with a history of latent TB infection (LTBI) or tuberculosis?**

A periodic symptom review should be conducted on each employee with a history of a past positive TST or IGRA, regardless of prior treatment. Persons with symptoms or signs of possible tuberculosis should be referred for a clinical evaluation. Employees also should be educated about symptoms of TB and should report to their supervisor if symptoms develop at any time, so that a medical evaluation can be performed<sup>8</sup>.

**28. Does your Mycobacteriology Laboratory use a Class I, II, or III Biological Safety Cabinet (BSC) that has been certified at least annually?**

**IF THE ANSWER IS "NO," REEVALUATE YOUR PROGRAM.**

***NO LABORATORY SHOULD PERFORM DIAGNOSTIC MYCOBACTERIOLOGY WITHOUT A WELL-MAINTAINED, PROPERLY FUNCTIONING BIOLOGICAL SAFETY CABINET.***

This is the single most important piece of equipment for reducing the possibility of laboratory acquired infections. If a BSC is not available or not working properly, all mycobacteriology smear preparation and culture processing must be referred to another laboratory for examination. The Occupational Safety and Health Act (OSHA), details the employer's responsibility for protecting the employee from harm<sup>19</sup>.

If you have a BSC, but are not sure how effectively it is functioning, consult with the biosafety officer or a certified technician and check to see if it has been certified within the past year. The airflow should be checked with each use to ensure it is within the correct range as indicated when it was certified<sup>17</sup>.

Please review the chart below to determine the type of BSC you have. Note that each BSC has a High Efficiency Particular Air (HEPA) filter through which egress air is filtered. If your cabinet does not have a HEPA filter, or you are not sure, call the manufacturer for more information.

COMPARISON OF BIOLOGICAL SAFETY CABINETS (12)

Cabinets

Application

<b>BSC Class</b>	<b>Face velocity (lfpm)</b>	<b>Airflow Pattern</b>	<b>Nonvolatile Radionuclides and Toxic Chemicals</b>	<b>Volatile Radionuclides and Toxic Chemicals</b>	<b>Biosafety</b>	<b>Product Protection</b>
I*, open front	75	In at front; out rear and top through HEPA filter	YES	When exhausted outdoors	2,3	NO
II, Type A1	75	70% recirculated to the cabinet through HEPA; 30% exhausted through HEPA to outside through canopy unit	YES (minute amounts)	NO	2,3	YES
II, Type B1	100	30% recirculated through HEPA; exhaust via HEPA and hard-ducted	YES	YES (minute amounts)	2,3	YES
II, Type B2	100	No recirculation; total exhaust via HEPA and hard-ducted	YES	YES (small amounts)	2,3	YES
II, Type A2	100	Similar to II, A1, but higher intake velocity and plena under negative pressure to room	YES	When exhausted outdoors (minute amounts)	2,3	YES
Class III	NA	Supply air inlets and exhaust through 2 HEPA filters	YES	YES (small amounts)	3,4	YES

\*glove panels may be added and will increase face velocity to 125 lfpm; gloves may be added with an inlet air pressure release that will allow work with chemicals/radionuclides

**Airflow characteristics of Class I (negative pressure) and Class II (vertical laminar flow)\* biological safety cabinets**

Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, February, 2007. U.S. Department of Health and Human Services, Center For Disease Control and Prevention

**Class I Biological Safety Cabinet:**

A ventilated cabinet for personnel and environmental protection with a non-recirculated inward airflow away from the operator is suitable for working with *M. tuberculosis* complex (Biosafety Level 2/3, depending on whether pure cultures are transferred or manipulated). This BSC does not provide product protection.

**Class II Biological Safety Cabinet:**

A ventilated cabinet for personnel, product, and environmental protection having an open front and inward airflow for personnel protection, downward HEPA-filtered laminar airflow for product protection and HEPA filtered exhausted airflow for environmental protection. This BSC is suitable for working with *M. tuberculosis* complex (Biosafety Level 2/3). Several types of Class II BSCs are available, including types A1, A2, B1, and B2. The difference in types depends on the filtered airflow, and the recirculation of air and the air exhaust requirements. All types are applicable for use with biohazards, including *M. tuberculosis* complex; B2 is designed for “total (100%) exhaust” and is not required for the mycobacteriology laboratory.

**Class III Biological Safety Cabinet:**

A ventilated cabinet designed for work with highly infectious microbiological agents Biosafety Level 3/4). This BSC provides maximum protection for the environment and the worker. It is a gas-tight enclosure with a non-opening view window. Both supply and exhaust air are HEPA filtered on a Class III cabinet. Long, heavy-duty rubber gloves are attached in a gas-tight manner to ports in the cabinet and allow direct manipulation of the materials isolated inside. Although these gloves restrict movement, they prevent the user's direct contact with the hazardous materials.

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

### **29. Does your Mycobacteriology Laboratory work with clinical specimens in at least a Biosafety Level 2 (BSL-2) laboratory?**

Because of the low infective dose of *M. tuberculosis* complex, clinical specimens from suspected or known cases of tuberculosis must be considered potentially infectious and handled with appropriate precautions. The 5<sup>th</sup> edition of the *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* recommends that BSL-2 practices and procedures, containment equipment, and facilities be used for the manipulation of these clinical specimens<sup>17</sup>. All aerosol-generating activities must be performed in a biosafety cabinet.

### **30. Does your Mycobacteriology Laboratory work with cultures suspected or confirmed to contain *M. tuberculosis* complex in a BSL-3 laboratory?**

The 5<sup>th</sup> edition of the *Biosafety in Microbiological and Biomedical Laboratories* states that BSL-3 practices, containment equipment, and facilities are required for laboratory activities in the growth and manipulation of cultures of any of the species in the *Mycobacterium tuberculosis* complex<sup>17</sup>.

Engineering controls include the following:

- Separate room or suite specifically designed for mycobacterial culture
- One-pass (non-recirculating) ventilation system
- At least annually certified Class I, II, or III biological safety cabinet
- Exhaust air from BSC discharged through HEPA filters or recirculated only after passing through filters certified to remove 99.97% of particulates 0.3 $\mu$ m or larger
- Negative pressure in culture area and a means to monitor negative pressure (Smoke tubes or differential pressure-sensing devices can be used to monitor negative pressure)
- Proper airflow pattern (Directional airflow should be from clean to least clean area)

Appropriate number of air exchanges per hour (Six to twelve room air exchanges per hour provide removal of 99% of airborne particulates within 30-45 minutes.)

### **31. Does your Mycobacteriology Laboratory provide safety training on aerosol prevention techniques for all employees before assigning work with mycobacterial specimens or cultures?**

Employees must receive instructions on how to handle a laboratory accident, and be instructed to report any real or suspected departure from standard operating procedures to the supervisor.

All employees with potential occupational exposure to infectious agents must participate in a training program. The training must be provided prior to the time of initial assignment to tasks where occupational exposure may take place. Refresher training should be provided at least annually.

Persons with compromised immune systems are at increased risk of TB infection and disease. Laboratories are responsible to ensure that all employees who may come into contact with *M.*

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

*tuberculosis* complex understand this. Employees with compromised immune systems should consult with their medical provider about their risk of occupational exposure to TB infection. Further consultation with their laboratory director regarding potential work reassignment may be indicated.

For further information, *see* “Guidelines for Preventing the Transmission of *Mycobacterium tuberculosis* in Health-Care Settings<sup>8</sup>.”

### **32. Does your Mycobacteriology Laboratory perform all manipulations on mycobacterial specimens and cultures that may generate aerosols *only* in a BSC?**

Studies have shown that the risk of *M. tuberculosis* infection is three to five times greater among workers in the mycobacteriology laboratory than other laboratory workers. The production of aerosols containing tubercle bacilli can pose an unrecognized hazard to workers who are not trained in safety practices. The most dangerous aerosols are those that produce droplet nuclei, particles of less than 5 $\mu$ m in size. These droplet nuclei remain suspended almost indefinitely in air unless they are removed by controlled airflow or ventilation. If droplet nuclei are not contained or eliminated, they are capable of entering a pulmonary alveolus and establishing the primary site of infection<sup>25</sup>.

All procedures that create aerosols should be performed only in a fully functioning BSC. Examples include: processing clinical specimens, preparing smears, inoculating plates or tubes, performing *in-vitro* tests, pipetting cultures or pouring supernatant liquids, mixing or diluting broth cultures or concentrates. All workers are responsible for the safety of themselves and their coworkers.

The BSC must be located in area with controlled access and in an area which facilitates as little movement of air as possible around the BSC while it is in operation. Air currents generated by the opening and closing of doors or by workers moving around can disturb the airflow of the cabinet.

The BSC should have the capacity to draw 75 to 100 linear feet of air per minute (lfpm) across the entire front opening. An anemometer may be used to check the airflow velocity across the front opening of the BSC. A strip of tissue paper taped to the front of the BSC provides a simple indication whether the direction of air movement is correct or not. The magnehelic gauge on the front of the cabinet will indicate the degree to which the HEPA filter has become loaded due to routine use and will also indicate if a leak in the HEPA filter has occurred. The space inside the BSC should be kept clean and free of racks and stored material that may limit or distort the airflow within the cabinet.

If the airflow is less than 75 lfpm, as detected by an anemometer or by the magnehelic gauge on the front of the BSC, the HEPA filters may be clogged and need replacement. Prior to replacing the HEPA filter, the BSC must be decontaminated with paraformaldehyde or another effective agent such as hydrogen peroxide vapor. This operation should be performed by a qualified service technician.

**Ultraviolet (UV) Lights:** *The CDC and NIH state that UV lamps are not recommended nor required in biological safety cabinets*<sup>26</sup>. The activity of UV lights for sterilization/decontamination is limited by a number of factors including penetration, relative

## ***Mycobacterium tuberculosis: Assessing Your Laboratory***

humidity, temperature, air movement, cleanliness, and age. The UV light has little penetrating power and is easily blocked by dust, grease, or organic material. Humidity above 70% greatly decreases the germicidal effects of UV light. Temperatures below 77-80° F reduce output of the germicidal wavelength and moving air cools the lamp below its optimum operating temperature. Dirt can block the germicidal effectiveness of the UV lamp and the effectiveness of the lamp decreases with age.

### **33. Does your Mycobacteriology Laboratory use a centrifuge equipped with aerosol-free carriers with O-rings?**

To reduce aerosol hazard from breakage during centrifugation, only aerosol-free safety cups with domed O-ring sealed closures should be used in mycobacteriology laboratories. Because tubes may leak or break, open the safety carriers and remove tubes only inside a BSC. Additionally, laboratories must ensure that centrifuge tubes are rated to the required g force.

Microcentrifuges should not be placed under the BSC for operation because air convection during operation may compromise the integrity of the BSC. Use only microcentrifuges that are equipped with aerosol containing safety cups.

Further discussion of centrifugal safety and efficiency can be found in “Public Health Mycobacteriology: A Guide for the Level III Laboratory” (p 31-35)<sup>24</sup>.

### **34. Does your Mycobacteriology Laboratory limit access into the laboratory when clinical specimens are being processed or when working with mycobacterial cultures?**

A system must be in place to limit access to the area during specimen processing or culture workup to only those who need to be present. The presence of unnecessary personnel increases the risk of exposure to infectious agents.

### **35. Does your Mycobacteriology Laboratory have a one-pass (non-recirculating) ventilation system?**

The purpose of a one-pass system is to prevent the spread of contaminated air to uncontaminated areas. The direction of air flow is controlled by creating lower (negative) pressure in the area into which flow is desired. Negative pressure is attained by exhausting air from the area at a higher rate than it is being supplied. The level of negative pressure necessary to achieve the desired air flow will depend on the physical configuration of the ventilation system and area, including the air flow path and flow openings, and should be determined by an experienced ventilation engineer on a case-by-case basis. Six to twelve air changes per hour are acceptable and provide removal of 99% of airborne particulate within 45 minutes<sup>17</sup>.

The room should have negative pressure relative to the adjacent area. The work area should contain no air sources such as open windows, unsealed ceiling tiles, or through-the-wall ventilation ducts. Doors between this room and other areas should remain closed except for entry or egress and there should be a small gap of 1/8 to 1/2 inch at the bottom of the door to provide an air flow path. A person with expertise in ventilation or industrial hygiene should work closely with the infection control committee and microbiology staff to establish air handling guidelines<sup>8</sup>.

### **36. Does your Mycobacteriology Laboratory monitor the environmental conditions in the isolation room at least annually to determine the number of air exchanges and the negative pressure status?**

## ***Mycobacterium tuberculosis: Assessing Your Laboratory***

Monitor the air handling systems at least annually, and record the findings as a part of your preventive maintenance. Documentation of professional inspection and analysis will support the laboratory findings. After any modification in the general air handling or ducting system, experts in ventilation engineering should re-inspect the mycobacteriology ventilation system to determine the air quality in the area. Record the event as any other equipment maintenance event is recorded.

In a laboratory without continual monitoring systems, a simple way to ensure that a room has negative air pressure on a daily basis is to tape short strips of tissue paper at the base of the door and on air duct grills. The directional movement of the paper strips serves as a constant indicator of the direction of the air flow<sup>27</sup>.

### **37. Does your Mycobacteriology Laboratory provide personal protective equipment that includes laboratory coats or gowns, gloves, respiratory, and face protection?**

Protective gowns designed for laboratory use must be worn while in the laboratory. The gowns preferably have back closures and fitted sleeve cuffs that fit snugly over the wrist. Personal protective equipment (PPE) is removed before leaving the laboratory for non-laboratory areas (e.g., cafeteria, library, administrative offices). Gowns, face protectors, gloves and other PPE worn in the BSL-3 must be removed and disposed of appropriately before assuming duties in the larger laboratory. All protective clothing should be either disposed of in the laboratory or laundered by the institution. Protective clothing should never be taken home by personnel<sup>17</sup>.

Face protection (goggles, masks, face shield or other splatter guards) is used when splashing or sprays of infectious or other hazardous materials to the face are possible.

The standard of practice is the use of protection provided by a HEPA filtered respirator(s) such as N95 or N100. Selection of the maximum respiratory protection for the task is important, but equally important is *using* the selected device correctly. Both supervisors and health care workers should be trained in the selection, proper use, and maintenance of respiratory protection appropriate for personal use against airborne tubercle bacilli. Annual fit testing is required if N-95 or N-100 masks are used. Fit testing is not required for Powered Air Purifying Respirators (PAPRs)<sup>28</sup>.

## **General Laboratory Practice**

### **38. Does your Mycobacteriology Laboratory monitor and evaluate the overall quality of the general laboratory systems?**

As stated in the Manual of Clinical Microbiology:

“Quality management is a system for continuously analyzing, improving, and re-examining resources, processes, and services within an organization to produce the best possible outcome. Microbiologists have, for many years, concerned themselves with the quality of events that take place within the clinical microbiology laboratory. These events include such things as ensuring the performance of equipment, reagents, stains, and media and monitoring the accuracy of tests performed in the laboratory. These activities fall under category of quality control (QC). Quality assurance (QA) broadens the scope of traditional QC to encompass processes and events in the preanalytical and postanalytical phases. QA includes such things as determining specimen quality and evaluating the competency and training of personnel. Both QC and QA can be considered aspects of quality management that also includes quality improvement (QI) or quality enhancement. Although applied successfully in industry for many years, QI and quality

## ***Mycobacterium tuberculosis: Assessing Your Laboratory***

enhancement are relatively recent concepts in the clinical laboratory setting. Instead of focusing on inspection, identifying poor performance, and taking corrective action, as in traditional QC and QA programs, QI programs emphasize thorough training and prevention and view the system for improvement opportunities rather than the people<sup>5</sup>.”

### **Confidentiality of Patient Information:**

The confidentiality of pertinent information is an area where quality is of utmost importance. If the laboratory uses a laboratory information system (i.e. an electronic reporting system), determine the security measures that have been instituted to ensure that transmitted reports go directly from the device sending reports to only the individual ordering the test or using the test results.

.A written policy for reporting by telephone, fax, or electronic mail is necessary to meet the CLIA intent for confidentiality of reporting<sup>1</sup>. The laboratory must communicate with users to obtain current contact information to ensure that written reports are accessible as soon as possible. A list of names and addresses of “authorized persons” is helpful to individuals reporting preliminary results.

To see the exact wording of the regulation, see CLIA 493.1231.

Laboratories must also have the following policies in place in each of the following areas to ensure the quality of general laboratory systems:

- Positive specimen identification and optimal integrity ( see CLIA section 493.1232)
- Documentation and investigation of problems and complaints in the laboratory (see CLIA section 493.1233), and documentation of corrective actions (see CLIA 493.1282)
- Documentation of communication efforts and investigation of communication problems between the laboratory and authorized individuals who order or receive test results (see CLIA section 493.1234)
- Quality of pre-analytic systems such as test requesting (see CLIA 493.1241), specimen submission, handling, and referral testing (see CLIA 493.1242).

### **39. Does your Mycobacteriology Laboratory participate in an approved proficiency testing program?**

Laboratories performing non-waived tests must participate in an approved proficiency testing (PT) program in each of the specialties (microbiology) and subspecialties (mycobacteriology) for which they are certified<sup>1</sup>. Preparation and examination of direct acid-fast smears are classified as moderate complexity testing. Preparation and examination of concentrated smears, culture, identification, and susceptibility testing are classified as high complexity testing.

Therefore, laboratories performing any one or combination of these tests must subscribe to an approved mycobacteriology PT program containing two testing events per year, with five specimens per event. PT programs are approved annually, so the list of approved PT programs may change from year to year. Check with your laboratory inspection agency to obtain a current list of approved PT programs. It is the laboratory’s responsibility to enroll in an approved PT program that is appropriate for the level of testing provided for patient specimens.

## ***Mycobacterium tuberculosis: Assessing Your Laboratory***

PT samples must be tested in the same manner as patient specimens, with no unusual or extraordinary consultation or attention. They must be tested with the same frequency as routine samples, and the staff may not consult with personnel from other laboratories concerning the samples. The samples may not be referred to another laboratory for testing. The individual performing the testing and the laboratory director must certify that the PT samples were integrated into the daily workload and treated the same way as patient specimens. PT records must be retained for two years<sup>29, 30</sup>.

To see the exact wording of the regulations described above, see CLIA 493.825 and 493.913.

For more guidance on proficiency testing, see CLSI document GP21-A2: Training and Competence Assessment, and CLSI document GP27-A2: Using Proficiency Testing to improve the Clinical Laboratory.

### **40. Does your Mycobacteriology Laboratory follow standard operating procedures and maintain the results of quality control for each test procedure for at least two years?**

#### **Standard operating procedures**

Written standard operating procedures for all methods and tests must be readily available and followed by all technical personnel. The director must approve, sign and date all modifications to procedures, indicating approval. If changes are made, procedures must be re-approved.

The dates of initial use and discontinuance of each procedure must be documented. A copy of a discontinued procedure must be retained for at least two years after the date of discontinuance<sup>1</sup>.

To see the exact wording of the regulation, see CLIA 493.1251.

#### **Quality control**

The laboratory must follow a quality control protocol to monitor and evaluate the quality of the analytical testing process<sup>1</sup>. All quality control readings must be documented and the records maintained for a minimum of two years.

To see the exact wording of the regulation, see CLIA 493.1253-56.

For more information on establishing quality control protocols see CLSI document GP02-A5: Laboratory Documents: Development and Control<sup>31</sup>.

### **41. Does your Mycobacteriology Laboratory monitor and evaluate the overall quality of analytical systems?**

When introducing new test methods, laboratories must establish and verify performance specifications. Laboratories must perform and document maintenance, calibration and function tests on equipment, instruments, and test systems. For each test system, the laboratory is

## ***Mycobacterium tuberculosis: Assessing Your Laboratory***

responsible for having quality control procedures that monitor the accuracy and precision of the complete analytical process<sup>1</sup>.

To see the exact wording of the regulation, see CLIA 493.1253-56.

For additional guidance on quality control policies see CLSI documents HS1-A2 “A Quality Management System Model”<sup>13</sup> and GP-26-A3 “Application of a Quality System Management Model for Laboratory Services”<sup>14</sup>.

### **42. Does your Mycobacteriology Laboratory ensure that accurate laboratory reports are sent to the submitter and maintain all patient reports and test records for a minimum of two years?**

The laboratory must have adequate systems in place to ensure test results are accurately and reliably sent to the final report destination in a timely manner<sup>1</sup>.

The original report or an exact duplicate, original test requisition, and test records (i.e. worksheets) must be retained and retrievable for a minimum of two years, or as required by accrediting agencies or state statutes. The “exact duplicate” may be saved in an electronic format, as long as it contains the exact information sent to the individual ordering the test or using the test results. Reports must be maintained in a manner that permits identification and timely accessibility.

To see the exact wording of the regulation, see CLIA 493.1291.

### **43. Does your Mycobacteriology Laboratory record the date and time positive results are telephoned, faxed, or electronically reported to health care provider(s) and public health officials?**

Written procedures for reporting and documenting life-threatening results and/or critical values, such as AFB smear-positive smears, to health care providers and public health networks should be developed. (Refer to Appendix B for examples of critical values) Logging telephone calls of critical values or maintaining a copy of fax results is an integral part of the quality assurance plan. Documentation is also necessary to develop and maintain accurate networks to facilitate communication of data. Since telephone results of critical values directly impact patient care, the following information must be provided and recorded or entered into the computer with the results when telephone reports are given<sup>1</sup>

:

- a. Person making the call should identify him/herself
- b. Name of the health care provider being called
- c. Patient’s name
- d. Type of specimen source
- e. Date specimen collected
- f. Laboratory results
- g. Date and time of call

To see the exact wording of the regulation, see CLIA 493.1291.

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

To assure patient confidentiality, all test results must be protected<sup>32</sup>. Laboratories must abide by the Health Insurance Portability and Accountability Act (HIPAA) of 1996.

### **Smears from Clinical Specimens**

#### **44. Does your Mycobacteriology Laboratory process at least 15 acid-fast smears per week?**

It is recognized by the American Thoracic Society (ATS) and CDC that to maintain technical proficiency in acid-fast microscopy and smear examination, microscopists should prepare a minimum of 15 smears per week. Laboratories processing less than 15 specimens per week should refer mycobacteriology specimens to another laboratory with established quality of performance.

In order to ascertain initial competency and to maintain proficiency in AFB smear preparation and examination, each microscopist should be provided with periodic proficiency tests. If each microscopist reads less than 15 specimens per week then it is suggested that at least 20% of AFB-stained smears be reviewed blindly by a supervisor or another employee. A QA program should be established to compare smear results to culture<sup>4</sup>.

*M. tuberculosis* complex may present characteristic morphology as viewed on microscopy (i.e. shape of bacteria), but other species may appear the same; therefore, do not use microscopy alone to identify individual species of mycobacteria.

#### **45. If your laboratory performs direct, unconcentrated smears, does your laboratory ensure that a concentrated smear is also performed?**

If direct smears are used for rapid diagnosis, follow up with a smear from a concentrated specimen is **necessary**. For the preparation of a direct smear, select the cheesy, necrotic, blood-tinged particles in the specimen because they are the most likely to produce positive direct smear results<sup>33,34</sup>.

In situations in which a rapid smear result is needed prior to processing the specimen for culture, concentration by cytocentrifugation can be used for the detection of acid-fast bacilli. Studies suggest that this method is comparable, or even better than smears prepared in the traditional method. An aliquot of the specimen is treated with an equal amount of 5% sodium hypochlorite, vortexed, and allowed to stand for 5 minutes. It is then cytocentrifuged and the slide is stained<sup>35</sup>.

#### **46. Does your Mycobacteriology Laboratory use fluorochrome stain as the primary acid-fast stain for smears made from patient specimens?**

The routine use of the fluorochrome stained smear for the examination of TB specimens is recommended by CDC. A fluorochrome stain is preferable to carbol fuchsin staining when screening for acid-fast bacilli in clinical specimens because of increased sensitivity and the ease of reading the smears. Fluorescent staining is examined at a lower power (250-400X) of magnification and therefore allows more rapid examination of smears, because a greater area of the slide can be examined at one time.

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

Laboratories do not need to confirm results of positive fluorochrome stains with the carbol fuchsin stain unless there is question about the result of the initial smear. Some laboratories confirm smears of newly AFB positive patients by a second reader.

In contrast, the carbol fuchsin acid-fast stain, either Ziehl-Neelsen or Kinyoun, is the recommended method for detection of positive cultures. The fuchsin-based stains are preferred for cultures for the following reasons:

1. the brightfield microscope is easier to use and maintain than the fluorescent one
2. the presence of numerous microorganisms in culture negates the need for high sensitivity afforded by the fluorochrome-stain
3. rapidly growing nontuberculous mycobacteria can fail to fluoresce in fluorochrome-stained smears
4. they allow for the visualization of non-AFB contaminants<sup>36</sup>

Detailed information concerning acid-fast stain procedures has been published. Examples include Koneman's Color Atlas and Textbook of Diagnostic Microbiology 6<sup>th</sup> edition<sup>36</sup>(Lippincott Williams & Wilkins) and the Manual of Clinical Microbiology 9<sup>th</sup> edition<sup>5</sup> (ASM Press).

### **47. Does your Mycobacteriology Laboratory check reactivity of fluorochrome stain for each batch of smears by staining and examining known acid-fast and non-acid-fast organisms?**

Mycobacteriology laboratories are responsible for assessing the performance of the fluorochrome stain for acid-fastness with each batch of smears prepared and with each new lot of reagents. Confirmation of stain performance is accomplished by reviewing acid-fast positive and acid-fast negative control slides prior to reading the patient smears.

Control slides may be prepared within the laboratory or purchased commercially. Protocols for stain preparation, storage, and smear examination of acid-fast control slides have been described<sup>37</sup>. For optimal quality control documentation, the laboratory should ensure that the stain lot numbers, expiration dates, results of the control slides, and name of the technical person performing the procedure is recorded in a log book or in computer format.

Reactivity of quality control fluorescent smears and result interpretation are summarized in the following table:

RESULTS	POSITIVE CONTROL	NEGATIVE CONTROL
Acceptable	Bright yellow-green fluorescent bacilli with auramine alone  Orange-yellow fluorescent bacilli with auramine-rhodamine	No fluorescent bacilli
Unacceptable	Either absence of or dull fluorescence  Few bacilli present	Fluorescent bacilli

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

Background fluorescence, excess artifacts or debris
-----------------------------------------------------

If results of the control slides stain meet the expected criteria, patient smears should be examined and results reported as per protocol. However, when quality control results are unacceptable, patient smears cannot be read and the run is considered unacceptable. It is helpful to consult a supervisor or other experienced technologist for confirmation of questionable results. Document quality control failures and the corrective action taken. When the problem is resolved, repeat the staining for both control and patients specimens.

Some parameters for investigating problematic results obtained with control slides are provided below:

1. Examine the fluorescence microscope for optimal brightness, and note halogen lamp usage and frequency of bulb change.
2. Ensure proper fixation of the slides. If a warmer is used to heat fix slides, be sure it is set at the correct temperature, i.e. 65<sup>0</sup> to 75<sup>0</sup> C for at least 2 hours in a BSC and maintains a uniform temperature across its surface<sup>4</sup>. Several alternative methods include: heat fixing slides at 80°C for 15 minutes<sup>38</sup> or using a method using phenol and ethanol<sup>39</sup>.
3. Examine stain reagents for signs of precipitation, debris or deterioration.
4. Re-stain control slides with a new lot number of stain reagents.
5. Ensure that staining racks are being used for smear preparation and not staining jars or dishes.

### **48. Does your Mycobacteriology Laboratory report an approximation of the number of acid-fast organisms viewed on the slide by using a standard semi-quantitative scale?**

AFB staining of clinical specimens is used to assess patient's infectiousness and to monitor therapy response, because AFB smear microscopy provides a semi-quantitative estimate of the number of bacilli being excreted. Although the number of AFB in pulmonary secretions can be directly related to the risk of transmission, the quality of the specimen is a variable that must be considered. Instructions for accurate sputum collection or direct observation of sputum collection are highly suggested. Induced sputa or bronchoscopy is considered for patients unable to produce satisfactory sputum specimens.

The quantification of the numbers of AFB per microscopic field for flurochrome and carbol fuchsin stains varies. The former are usually read at 250-400x and the latter under oil immersion at 1000X<sup>3</sup>.

The table below provides an example of the smear observation and interpretation for both the carbol fuchsin and fluorescent stain methods:

<b>AFB Number per view</b>	<b>AFB Number per view</b>	
----------------------------	----------------------------	--

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

<b>fields 1000 X oil immersion</b>	<b>fields (250 X)</b>	
None per 300 fields	None per 30 fields	No AFB seen
1-2 per 300 fields	1-2 per 30 fields	Inconclusive, repeat
1-9 per 100 fields	1-9 per 10 fields	Rare, 1+
1-9 per 10 fields	1-9 per field	Few, 2+
1-9 per field	10-90 per field	Moderate, 3+
>9 per field	>90 per field	Numerous, 4+

A quantification of the numbers of acid-fast organisms per field should be rated 1+ to 4+. The number of tubercle bacilli in pulmonary secretions is directly related to the risk of transmission<sup>33</sup>.

It is important to note that AFB smear-negative TB patients may also transmit TB and have been shown to account for approximately 17% of TB transmission<sup>40</sup>. Also extra pulmonary specimens are often smear negative. Patients are still considered contagious, regardless of smear result when the clinical index of suspicion for tuberculosis is high.

### **49. Does your Mycobacteriology Laboratory telephone, fax, or electronically report all positive acid-fast smear results to both the patient's health care provider and the public health department as soon as results are known, and within 24 hours from specimen receipt?**

The Mycobacteriology Laboratory plays a crucial role in the timely and accurate detection and reporting of first-time positive *M. tuberculosis* complex results to the test requestor and infection control practitioner of the facility and to local and state public health officials. By promptly alerting the appropriate authorities, infection control precautions, such as respiratory isolation, and adequate treatment can be instituted. In addition, communicating with public health systems expedites the initiation of appropriate public health management (including contact investigations) and outbreak investigations<sup>41</sup>.

It is the responsibility of laboratories to monitor the turnaround time for result reporting from the time of specimen collection (if available) or time of specimen receipt. Positive results are considered critical values and are expected to be reported within the timeframes listed in Appendix B as recommended by the CDC, APHL, and ATS and mandated by many city departments of health<sup>21</sup>. The health care provider may identify patients for whom negative smear results should be communicated as a critical value (e.g. a patient in respiratory isolation).

### **50. If your laboratory performs only smear microscopy, does your Mycobacteriology Laboratory send all specimens to a full service laboratory for culture within 24 hours of collection?**

Because culture is more sensitive than smear microscopy, detecting 10-100 viable mycobacteria per milliliter of sample, specimens should be sent to a full-service referral laboratory for culture. To promote rapid turn-around-times for detection and identification of mycobacteria, specimens should be forwarded to a full service laboratory as soon as possible (e.g. via overnight delivery). For specimens from patients with a high suspicion of TB, who are smear positive, or who are suspected of drug resistance; specimens should be shipped via overnight delivery<sup>10</sup>.

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

Specimens that may contain *M. tuberculosis* complex, must be packaged, labeled and shipped in accordance with specific regulations. Information about these regulations can be found at the following locations:

Title 49 Code of Federal Regulations, Transportation Parts 100 and 185. Available electronically and from U.S. Government Printing Office, [www.access.gpo.gov](http://www.access.gpo.gov)

International Air Transport Association Dangerous Goods Regulations (46<sup>th</sup> ed.) 2005. Available for purchase from IATA 800-716-6326 or [www.iata.org](http://www.iata.org)

International Air Transport Association Infectious Substances and Diagnostic Specimens Shipping Guidelines (5<sup>th</sup> ed.) 2004. Available for purchase from IATA 800-716-6326 or [www.iata.org](http://www.iata.org)

USPS regulations: Domestic Mail Manual C023: <http://pe.usps.gov>

U.S. Department of Transportation (DOT):

[www.dot.gov](http://www.dot.gov)

[www.tsi.dot.gov](http://www.tsi.dot.gov) (Transportation Safety Institute)

International Civil Aviation Organization (ICAO): [www.icao.int](http://www.icao.int)

### **51. Does your Mycobacteriology Laboratory use indicators, such as turn-around-time, to monitor the quality of performance of laboratories to which you refer specimens for nucleic acid amplification, culture, identification and/or drug susceptibility testing?**

Laboratories should monitor the turnaround times of the reference facility to which their specimens are sent. This can be accomplished by tracking the date of specimen shipment to date of receipt by the reference laboratory. In addition, the length of time that AFB smear, nucleic acid amplification, culture, identification, and susceptibility results are reported to the primary laboratory should be recorded<sup>10,42</sup>.

## **Public Health and Epidemiology**

### **52. Does your Mycobacteriology Laboratory receive and review a report of the prevalence and resistance patterns of *M. tuberculosis* complex isolated from your geographic area?**

It is important to maintain records of the institutional findings compared with the surrounding geographic area and region as a means to inform the physicians and/or house staff of local trends and findings. Regional and state information should be available from the local or state TB Control Program or public health laboratory.

By monitoring results from laboratory drug susceptibility tests, trends in resistance can be observed. This information will assist the healthcare providers in drug selection, permitting the medical community to deal more effectively with new patients or patient contacts. Implementing rapid technology that will reduce the turnaround time for reporting the drug susceptibilities of tuberculosis is critically needed to resolve the MDR/XDR-TB problem.

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

### **53. Does your Mycobacteriology Laboratory ensure universal genotyping by sending all initial *M. tuberculosis* complex isolates to the appropriate public health laboratory?**

Laboratories must support outbreak investigations by routinely sending all new *M. tuberculosis* complex isolates to their State Public Health Laboratory. Isolates will be sent to regional laboratories for genotyping (“DNA fingerprinting”) as part of the CDC Genotyping Network<sup>43</sup>. Genotyping is a powerful tool for tracking the spread of individual strains of *M. tuberculosis* complex<sup>44</sup> and has been used to:

- Study nosocomial transmission of MDR-TB among patients with HIV infections.
- Confirm re-infection in AIDS patients with a second strain of *M. tuberculosis* complex.
- Identify unrecognized sources of transmission through population surveys.
- Identify false-positive laboratory results such as cross-contamination or false positive smears.
- Trace hospital or nursing home acquired tuberculosis.
- Determine strain relatedness of tuberculosis within a community.
- Establish a background of prevalent strains within a community against which an outbreak or drug resistant pattern can be compared.

### **54. Does your Mycobacteriology Laboratory have access to laboratory training through your Public Health Laboratory?**

State Public Health Laboratories and State Tuberculosis Control Programs should take a lead role in improving proficiency in mycobacteriology methods. Training resources for even the most basic methods (such as fluorescent smear preparation and reading) are very limited. In order to maintain a proficient regional work force, states must offer training opportunities as needed<sup>42</sup>.

### **55. Does your Mycobacteriology Laboratory have access to a state or regional Mycobacteriology Laboratory network that can provide surveillance data, training and other resources?**

While participation in a Mycobacteriology Laboratory network is not a requirement, regional or state Mycobacteriology Laboratory Networks can:

- Provide the means for ongoing assessment of TB laboratory practices and capacity in the state and for the evaluation and implementation of testing algorithms on a state-wide basis.
- Serve as a conduit for transfer of information concerning technical issues and laboratory result reporting issues from national authorities and the state TB control program to laboratories.
- Serve as a forum to discuss and address relevant issues including laboratory safety practices, adherence to recommended testing methods, use of appropriate media, isolation rates, identifications, turnaround times, reporting processes, maintaining proficiency in small volume laboratories, and appropriate use of new technology such as nucleic acid amplification-TB testing, IGRA testing, gene sequencing for identification, and TB genotyping for identification of transmission links.
- Compile laboratory data and provide laboratory-based surveillance reports. Data can help monitor the incidence of mycobacteria isolation, *M. tuberculosis*

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

complex isolation, and TB drug resistance. Surveillance information can be shared with laboratories, public health departments, and health care providers.

- Develop and maintain state or regional TB isolate repositories.
- Ensure all cultured strains will be sent for universal genotyping.
- Provide training opportunities to maintain or improve proficiency in mycobacteriological methods.
- Collaborate in research.

### **Specimen Processing and Decontamination**

#### **56. Does your Mycobacteriology Laboratory process and culture at least 20 specimens per week?**

Proficiency in culture and identification of *M. tuberculosis* complex may be maintained by digestion and culture of a minimum of 20 specimens per week, along with the use of adequate controls. Application of state-of-the-art technology for the laboratory identification of *M. tuberculosis* complex is costly and requires considerable technical expertise that cannot be maintained in low volume laboratories. Other fiscal considerations include compliance with CLIA, safety, quality assurance, and proficiency testing regulations<sup>42</sup>.

It is recommended by the CDC, APHL and the American Thoracic Society that laboratories with insufficient specimen volume or those unable to provide accurate results in a timely fashion send specimens/cultures to qualified full service laboratories<sup>24, 42</sup>.

#### **57. Does your Mycobacteriology Laboratory take steps to eliminate cross-contamination between cultures?**

A 2000 review article authored by Burman and Reves<sup>45</sup> identified 14 studies which evaluated more than 100 patients and found a median false-positive rate of 3.1%. The mycobacteriology laboratory should have a plan for identification and review of possible false-positive cultures. Criteria that might prompt such a review include: patients having only a single culture-positive specimen; cultures with a low colony count on solid media or an extended time to detection for broth-based media; or isolates with unexpected drug resistance.

When processing specimens and inoculating media for mycobacterial culture, aseptic technique must be strictly observed to prevent cross-contamination<sup>4</sup>. Preventive measures are suggested below:

- Disinfect biological safety cabinet, centrifuge and tabletops with disinfectant after specimen and culture workup is completed.
- Perform all work on towels soaked with disinfectant in order to absorb any droplets or splatters that may inadvertently occur during culture manipulations.
- The supernatant from decontaminated specimens should be discarded into a splash-proof container with disinfectant via a funnel lined with gauze. Autoclave the discard container and contents **daily**.

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

- When possible leave an empty space in the rack between each specimen tube or media to be inoculated
- Add diluent to centrifuge tubes from individual tubes without the lip of the tube touching or creating an aerosol. Pouring from a common container is an opportunity to cross-contaminate specimens.
- To avoid droplet aerosol cross-contamination, open and remove caps from tubes **one at a time**. Opening several tubes at once creates an opportunity for crossover of droplet nuclei and might result in mixing tube caps.
- Only use individual disposable sterile pipettes for each transfer (i.e. pipette bovine serum albumin or sterile buffer with an individual sterile pipette and discard after each transfer).
- Use single delivery diluent tubes to transfer into centrifuged sediment container and do not pour from a bottle to avoid splashes. Discard immediately after use.
- After inoculation of sediment to all media using a sterile, dedicated pipette, add the last drop to a slide for AFB smear preparation to avoid contamination.

### **58. Does your Mycobacteriology Laboratory routinely process and culture specimens within 24 hours of receipt in the laboratory?**

Rapid specimen processing, AFB smear, nucleic acid amplification, culture and susceptibility reading and reporting of positive results are critical to the prompt diagnosis of TB, which is particularly urgent in this era of MDR and XDR MTB strains. Laboratories lacking the resources or specimen volume to meet this standard should utilize quality reference facilities that provide turnaround time to results within the recommended guidelines<sup>46</sup>.

### **59. Does your Mycobacteriology Laboratory use a refrigerated centrifuge(s) at a relative centrifugal force (RCF) of at least 3000 x g for 15 to 20 minutes to process mycobacterial specimens for culture?**

Heat buildup, which is generated during centrifugation, is extremely destructive to viable mycobacteria. Hence refrigerated centrifuges are highly recommended to prevent excess heat buildup when using a relative centrifugal force (RCF) of 3000 x g or higher<sup>47</sup>.

The centrifuge should be routinely monitored for centrifugal efficiency and the RCF posted and known by the operating staff. The staff must understand the difference between “revolutions per minute” (RPM) and “relative centrifugal force” (RCF or gravity force). At an RCF of at least 3800 for 15 minutes, there is an 82% correlation of positive AFB smears with culture as compared to 40% at 3000  $g^2$ . Old centrifuges that spin at 2300-3000 RPM (1500-2000 RCF) for 15 minutes attain theoretical sedimenting efficiencies ranging from 75-84% and this may be further reduced by lethal heat buildup<sup>24</sup>. Therefore, it is highly recommended that they be upgraded to refrigerated models.

In addition, angle head rotors are preferred for use in mycobacteriology to reduce frictional air resistance during centrifugation. To comply with safety regulations, sealed, aerosol-proof centrifuge cups must be used to prevent cracks or the collapse of centrifuge tubes.

### **60. Does your Mycobacteriology Laboratory prepare, stain, and examine acid-fast smears from all specimens (except blood) sent for mycobacterial culture?**

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

Microscopic examination of acid fast-stained smears is one of the first, easiest, least expensive, and most rapid methods for demonstrating the presence of acid-fast organisms in clinical specimens. Examination of smears can be helpful to provide a presumptive diagnosis of mycobacterial disease. Additionally, smears from respiratory specimens can help: assess infectiousness of patients with active tuberculosis disease; follow progress of antimicrobial therapy; and release non-infectious patients from isolation<sup>5</sup>.

### **Inoculation and Growth Detection**

#### **61. Does your Mycobacteriology Laboratory inoculate all specimens into a standardized broth system for the primary culture?**

Laboratories are continuously being challenged to reduce time to growth detection. This is accomplished through the use of a commercial liquid culture system as the primary method for isolating *M. tuberculosis* complex. Primary culture in a selective broth medium enhances the growth of *M. tuberculosis* complex and is preferred to culture on solid media alone. Once growth is detected, the organism can be identified by using specific procedures developed to rapidly identify *M. tuberculosis* complex.

##### **Advantages:**

- Average time from primary inoculation to detection of positive growth is 8-12 days.
- Identification of *M. tuberculosis* complex can be determined within 1 day after growth detection.

##### **Disadvantages:**

- Capital investment for instrumentation in laboratories culturing fewer than 20 cultures per week may be impractical

The N-acetyl L-cysteine-sodium hydroxide (NALC-NaOH) digestion-decontamination method is the procedure of choice for preparing specimen for inoculation into a broth system. Check the manufacture's guidelines for use of other decontamination methods in order to ensure that residual quantities of these substances in the inoculum will not inhibit mycobacterial growth in the system.

Specimens collected aseptically or from normally sterile sites can be inoculated directly to 7H9-based broth systems without being decontaminated. Aseptically collected specimens with volume greater than 10mL should be centrifuged at 3800 RCF before inoculation. Specimens that are thick or mucoid should be liquefied before centrifugation<sup>4,5</sup>.

#### **62. Does your Mycobacteriology Laboratory inoculate specimens other than blood to at least one solid medium?**

In addition to the inoculation of liquid culture medium good laboratory practice requires that at least one selective solid medium be inoculated at the same time. Growth on solid media can be used to detect mixed colony morphology and to provide a source for isolation of a pure culture.

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

If there is growth in the broth but no growth on the original solid medium, a second solid medium should be inoculated from the primary broth at the time the AFB smear(s) is prepared. Compare growth from the broth with results on solid medium and the original AFB smear for quality control purposes<sup>5</sup>.

If the specimen is from bone, joint tissue or a skin lesion, additional media is required. *M. haemophilum* may be present and an additional medium containing hemin, hemoglobin, or ferric ammonium citrate should be included. In addition, two sets of media should be set up for cultures from skin, joint, and soft tissue, one incubated at 35 to 37<sup>0</sup> C and the other at 25 to 30 <sup>0</sup>C. *M. marinum*, *M. ulcerans*, *M. chelonae* and *M. haemophilum* grow optimally at lower temperatures.

Conventional media should be incubated in 5-10% CO<sub>2</sub>. Egg based media should be incubated in CO<sub>2</sub> for a minimum of 7 to 10 days while Middlebrook media should remain in CO<sub>2</sub> for the length of incubation<sup>4</sup>.

Direct inoculation of blood onto solid media is not recommended due to the low numbers of mycobacteria that are present. Blood should be inoculated directly into a broth system following the manufactures' recommendations or, alternatively, concentrated using a system such as the Isolator lysis-centrifugation tube system prior to inoculation onto solid media<sup>24</sup>.

### **63. Does your Mycobacteriology Laboratory inoculate a negative control with each batch of cultures that are inoculated?**

Inoculating a negative control sample at the end of the run under processing conditions identical to those used for the patient specimens may help monitor whether contamination has been introduced during the decontamination and culture inoculation process. Record results as a part of the quality control record. Alternatively, processing solutions may be plated at the end of the run. Any growth of mycobacteria on the negative control is an alert for **immediate** investigation of positive cultures on that process run, including notification of health care provider(s) if questionable results have been reported<sup>5</sup>.

Conversely, positive controls are not recommended. This process has been shown to be a source of cross-contamination<sup>5</sup>.

### **64. Does your Mycobacteriology Laboratory use a continuously monitoring broth system or check broth cultures for evidence of growth every 2-3 days for weeks 1-3, and weekly thereafter for at least six weeks?**

Rapid detection of *M. tuberculosis* complex is critical to diagnosing pulmonary tuberculosis and to the detection of drug-resistance. Frequent monitoring of liquid cultures aids in detecting *M. tuberculosis* complex promptly. Broth systems are more useful for the earlier detection of *M. tuberculosis* complex than solid media. Average detection time for mycobacteria is approximately 8-12 days. The culture positivity rate in the broth medium is higher than it is on conventional solid medium<sup>4,5</sup>.

### **65. Does your Mycobacteriology Laboratory examine cultures on solid media for evidence of growth at least once weekly for 6-8 weeks?**

Standard practice requires frequent examination of cultures inoculated from the original specimen onto solid media. Rapid growers appear early during the examination and can be transferred or referred for identification. All cultures on solid medium should be held for at least six weeks, but

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

if the original smear was positive and the cultures are negative at six weeks, further incubation up to eight weeks is indicated<sup>4,5</sup>.

### **66. Does your Mycobacteriology Laboratory use a microscope or hand lens to examine solid media for earlier visualization of mycobacterial growth?**

In addition to broth culture, there are other techniques to facilitate a more rapid diagnosis of tuberculosis that should be considered. Microscopic examination of solid media is beneficial in detecting mixed mycobacterial infections and those rare strains that do not grow well in broth. Good results in isolating mycobacteria from clinical specimens have been achieved using Middlebrook 7H10 and/or 7H11 agar with microscopic examination.

Colony morphology directs the selection of the specific nucleic acid probes and other molecular tests for the rapid identification of *M. tuberculosis* complex and other mycobacteria.

The dissecting microscope is a valuable aid in examining young colonies, in determining the morphology of mature colonies, and in detecting the presence of minute colonies of the more slowly growing species of *Mycobacteria* on plated media. A 3-10x hand lens is useful to examine tubed media. Careful scrutiny using an inverted or dissecting microscope or hand lens is helpful for early observation of typical colonies of mycobacteria and facilitates early identification of *M. tuberculosis* complex using rapid methods<sup>4,5</sup>.

Colony morphology is best observed on isolated colonies. Colony variations on the transparent 7H10 or 7H11 agar media may be observed with the aid of a dissecting microscope. Under magnification young, developing colonies appear as clusters of bacilli that develop into typical colonies as they mature. Plates of a transparent, agar-based medium are inverted on the stage of a dissecting microscope. To examine the colonies, use 10-100x magnification and transmitted light, with the source below the stage so that it shines through the medium<sup>5</sup>.

Stain a smear from the growth on culture plate or L-J tube with the Ziehl-Neelsen or Kinyoun stain to determine the acid-fastness and purity of the growth. When in doubt about growth rate, make a subculture on liquid or solid medium using small inoculum and note the time required to visible growth:

- Rapid growers are fully matured within 7 days on subculture.
- Slow growers require more than 7 days<sup>5</sup>.

### **67. Does your Mycobacteriology Laboratory perform an acid-fast smear from:**

#### **67a. Broth cultures indicating growth?**

When growth is detected in the broth, prepare a smear, apply an acid-fast stain and examine the smear. Contamination may be increased in 7H9-based broth systems. Growth of AFB should be confirmed by a Ziehl-Neelsen or Kinyoun stain. *M. tuberculosis* complex should not be reported based on smear examination only. If contamination is detected, decontaminate the 7H9-based broth using one of the procedures for initial specimens or that is recommended by the manufacturer of the 7H9-based broth, streak on a selective solid medium and inoculate another 7H9-based broth.

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

Acid-fastness, cording and a slow growth rate in broth are suggestive of *M. tuberculosis* complex. However, an identification of *M. tuberculosis* complex from an initial diagnostic specimen must not be reported based on smear examination only<sup>4</sup>.

### **67b. Selected colonies on solid medium at an early stage of growth?**

When visual examination of the growth on transparent agar or Lowenstein-Jensen (L-J) medium reveals suspicious colonies, prepare and stain smears, using an acid-fast stain. Staining results and colony morphology guides the laboratorian in subsequent steps for confirmation and/or identification. (All plates should be resealed before removal from BSC).<sup>4</sup>

### **68. Does your Mycobacteriology Laboratory subculture all broth cultures exhibiting acid-fast growth to solid medium?**

Inoculating Middlebrook 7H10 or 7H11 agar allows development of colony morphology from growth in 7H9-based broth. Colonial types may be purified and further tested to identify other mycobacteria that may be present. A pure culture may be transferred for stock cultures, reference, or quality control. Transferring to an L-J slant also allows better discrimination of pigment for species identification of nontuberculous mycobacteria<sup>48</sup>.

### **69. Does your Mycobacteriology Laboratory use or ensure the use of a rapid method to confirm or rule out the presence of *M. tuberculosis* complex from positive cultures?**

Once growth is detected the organism can be specifically identified by a molecular method (e.g. DNA probe, PRA, DNA sequencing), line probes or High Pressure Liquid Chromatography (HPLC)<sup>1</sup>. This is especially important in cases where no NAAT result from the sediment is available.

Any acid-fast isolate that is not identified should be sent on the original media within one working day preferably with overnight delivery to a reference laboratory for identification. Biochemical identification (niacin, nitrate) is not a rapid method and is not recommended as the primary method to identify the initial patient isolate of *M. tuberculosis* complex.

Laboratories can use DNA probes for rapid and accurate identification of *M. tuberculosis* complex, *M. goodii*, *M. kansasii*, and *M. avium* complex. Colony morphology and acid-fast smear results direct the selection of the specific nucleic acid probe or other method for rapid identification of the mycobacterium. The probes have excellent sensitivity and specificity and allow for identification from liquid or solid culture media within two hours of detection of growth.

*M. bovis* and the vaccine strain, *M. bovis* BCG are members of the *M. tuberculosis* complex that should be differentiated from the other species in the complex, especially in initial isolates. *M. bovis* is universally resistant to pyrazinamide (PZA), one of the first-line drugs used to treat tuberculosis and has a unique epidemiology. If the isolate of *M. tuberculosis* complex shows PZA resistance upon susceptibility testing, the laboratory should perform further testing to determine whether the isolate is *M. bovis* or *M. bovis* BCG. HPLC, mycobacterial interspersed repetitive units (MIRU), and spacer oligonucleotide typing (spoligotyping) have proven useful in the identification of *M. bovis* and *M. bovis* BCG.

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

If necessary, identification of all species within the *M. tuberculosis* complex may be desirable and can be accomplished by specialized molecular testing (deletion analysis, line probes). Alternatively, the isolate can be sent to a reference laboratory for identification.<sup>49,50,51,52,53</sup>

Unusual or problem isolates should be sent to your state public health laboratory for resolution, including possible consultation with CDC.

### **70. Does your Mycobacteriology Laboratory monitor the turnaround time of test results to ensure that 80% of *M. tuberculosis* complex isolates from primary patient specimens are identified within 21 days of specimen collection?**

Primary culture in a 7H9-based broth medium (7-14 days), confirmation of positive growth by AFB smear (2-4 hours), and confirmation by the DNA probe (2-4 hours) or other rapid test will permit a report of *M. tuberculosis* complex within 14-21 days<sup>12</sup>.

When submitting mycobacterial isolates to a reference laboratory, contact this laboratory about submission criteria. Submit the isolate from original media whenever possible as subculturing will delay referral and identification.

A laboratory's decision to monitor, on a monthly or quarterly basis, the reporting of *M. tuberculosis* complex test results, as part of its QA program, represents a commitment to better patient care. If the laboratory program fails to meet a 21 day turnaround standard for identifying *M. tuberculosis* complex, management should focus on shortening turnaround time<sup>54,55,56</sup>.

### **71. Does your Mycobacteriology Laboratory retain positive *M. tuberculosis* complex cultures for at least one year and other *Mycobacterium spp.* for a minimum of three months?**

Epidemiologic investigations often use strains of *M. tuberculosis* complex that were isolated six to nine months previously. Therefore, it is good practice to keep isolates for at least one year. Outbreak investigations entail defining and identifying common patient exposures and the relatedness of *M. tuberculosis* complex isolates. Genotyping is the most accurate method to determine strain relatedness.

Many laboratories maintain a subculture of the first isolate in the refrigerator or freezer than can be retested if the patient fails to respond to therapy. The laboratory can re-examine the initial isolate and a later one to determine if the susceptibility pattern has changed during therapy<sup>4</sup>.

### **72. Does your Mycobacteriology Laboratory, as part of your laboratory QA program, correlate the smear positive and negative results with culture positive and negative results to evaluate smear/culture quality?**

Specimens that are smear positive are usually culture positive, unless the patient is on therapy. Smear positive, culture negative initial diagnostic specimens occur rarely. The occurrence of excessive numbers of smear positive, culture negative specimens could suggest acid-fast contaminants in the system. Check tap and distilled water first, then diluent, buffer, stain solutions, and other reagents. There is also the possibility that the decontamination system is too strong. The laboratory can monitor the percent of *M. gordonae* isolation to ensure against killing of *M. tuberculosis* complex during the digestion/concentration process.

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

The smear negative/culture positive specimen is usually associated with the number of organisms present. Culture with  $>10^4$  mycobacteria per mL of original specimen are usually smear positive<sup>4</sup>.

### **73. Does your Mycobacteriology Laboratory document the rate of contamination of culture media inoculated with digested/decontaminated sediment as a way of monitoring the specimen preparation and decontamination process?**

As a general rule, for L-J medium, approximately 2-5% of sputum specimens are contaminated. For liquid media, a slightly higher contamination rate is expected (The acceptable contamination rate for the Mycobacteria Growth Indicator Tube MGIT system is 7-8%)<sup>57</sup>.

If your laboratory is experiencing delays in delivery of specimens, the contamination rate may be greater than 5-8%<sup>5</sup>. Although increasing the concentration of NaOH will help reduce the contamination rate, it will also increase the possibility of die-off of *M. tuberculosis* complex during the digestion/concentration process. CDC guidelines should be followed for changes to the processing protocol.

To further reduce contamination of cultures, make sure that specimens are completely digested. Partially digested specimens may not be completely decontaminated. Thoroughly mix contents of the centrifuge tube to assure that the inside surface of the tube has been well decontaminated. Increase the N-Acetyl-L-Cysteine (NALC) concentration to digest thick, mucoid specimens.

Conversely, laboratory processing may kill too many mycobacteria, diminishing the overall recovery rate. An excellent discussion of centrifugal efficiency and digestant toxicity may be found in the *CDC Guide for Level III Laboratories*<sup>24</sup>.

Monitor the contamination rate periodically as a quality control for the decontamination process. Record, review, and maintain the value in your quality control record.

### **74. Does your Mycobacteriology Laboratory subculture colonies with differing morphology for use in species identification?**

Isolation of *Mycobacterium* species is not uncommon and may be clinically significant. If more than one colony type is observed, select colonies of each type, and prepare subcultures of each to establish purity of the cultures<sup>5</sup>. Send acid-fast isolates to a reference laboratory for identification if your laboratory cannot identify species of nontuberculous mycobacteria with certainty.

### **75. Does your Mycobacteriology Laboratory identify a broad range of nontuberculous mycobacteria?**

If your mycobacteriology laboratory has no or limited ability to identify nontuberculous mycobacteria to species, utilize reference laboratory resources for providing services to meet patient needs according to risk/disease prevalence<sup>4</sup>.

## **Susceptibility Testing**

### **76. Does your Mycobacteriology Laboratory perform or ensure susceptibility tests on all initial isolates of *M. tuberculosis* complex?**

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

CDC recommends that initial isolates from all patients be tested for drug susceptibility to confirm the anticipated effectiveness of chemotherapy using a first-line drug panel developed in consultation with your respective state and local TB Control Program. First line drugs include isoniazid, rifampin, ethambutol and pyrazinamide<sup>58</sup>. If a laboratory intends not to routinely test for this panel, it should seek approval from the TB Control Program.

The laboratory's results are essential for two distinct entities: primary care and public health practitioners. We must strive for a type of leadership that fuses all the different players involved into a synergistic network, making the whole more effective than the sum of its parts.

Susceptibility testing should be repeated if the patient continues to produce culture positive sputum after 3 months of treatment<sup>59</sup>. A laboratory's ability to maintain proficiency is absolutely dependent on the volume of tests performed. An APHL/CDC report on susceptibility testing suggests that laboratories processing fewer than 50 TB isolates per year refer specimens or cultures to laboratories that have demonstrated proficiency in DST.<sup>60</sup>

### **77. Does your Mycobacteriology Laboratory ship the initial positive culture (broth or solid media, whichever grows first) to a reference laboratory as soon as *M. tuberculosis* complex is detected if susceptibility testing is not performed in your laboratory?**

The role of the TB laboratory is critical in the diagnosis of active TB and even more so for drug-resistant TB. Definitive diagnosis of drug-resistant TB requires that *M. tuberculosis* complex be isolated and drug susceptibility results be completed and conveyed to the health care provider and local and/or state TB Control Program within 28 days from collection. Prompt turnaround time for laboratory results is of paramount importance in rapid diagnosis and appropriate treatment of multi drug-resistant TB (MDR-TB) and especially extensively drug-resistant TB (XDR-TB)<sup>61</sup>.

Growth detection and identification of *M. tuberculosis* complex may take a few weeks. Drug susceptibility testing of a TB isolate may require an additional two to three weeks. Slow growth of some mycobacteria (a common characteristic noted in many MDR-TB strains) further lengthens the time to identification and susceptibility testing. Delays in the return of culture confirmation and susceptibility results will delay identification of patients with drug-resistant TB and initiation of appropriate treatment<sup>61</sup>.

Antibiotic susceptibility results should be reported to the health care provider within 28 days of specimen collection. In order to meet this turnaround time, a portion of the primary positive culture (either an aliquot of broth or portion of solid media, whichever is available) needs to be shipped to a reference laboratory within one business day rather than waiting for a subculture on a solid medium which will delay testing.

### **78. Does your Mycobacteriology Laboratory determine or ensure susceptibility testing of *M. tuberculosis* complex isolates to primary drugs using a broth-based system?**

Broth methods are preferred for first-line testing as they are much faster than the proportion method using agar media (typically five to ten days versus 3 weeks).

In the mid-1980's, the first commercially broth-based drug susceptibility system became available<sup>62</sup>. Since then several other commercial broth systems have been developed to detect mycobacterial growth in a fully automated system (stand-alone)<sup>63, 64, 65</sup>.

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

### **79. Does your Mycobacteriology Laboratory use a strain of *M. tuberculosis* complex that is susceptible to all anti-mycobacterial agents being tested as a control once each week that patient isolates are tested?**

Quality control of *M. tuberculosis* complex susceptibility testing should include testing of an isolate that is completely susceptible to the antimicrobial agents being tested. The strain *M. tuberculosis* H37Rv (ATCC 27294) is well suited for use as a “pan-susceptible” control organism and its performance is well documented<sup>58</sup>. Alternately, *M. tuberculosis* H37Ra, which is believed to be avirulent and therefore is less likely to cause laboratory-acquired infections in the event of an accident, could be used if a laboratory has documentation that this strain performs as expected. An advantage of using this strain is that its unique HPLC pattern is easily detectable, should evidence of the possibility of cross-contamination become an issue. In addition, PCR-based spoligotyping and mycobacterial interspersed repetitive units (MIRU) provide a rapid and accurate way to identify H37 contamination<sup>66</sup>.

### **80. Does your Mycobacteriology Laboratory perform or ensure second-line drug susceptibility testing for *M. tuberculosis* complex when appropriate?**

Because successful treatment of drug-resistant TB depends on susceptibility test results of the *M. tuberculosis* complex isolate, second-line susceptibility tests should be initiated as soon as drug resistance is suspected or identified. In some jurisdictions, molecular methods are available to rapidly diagnose drug resistance to some antimicrobials.

Strong suspicion of drug resistance based on the patient’s prior treatment history or exposure to drug-resistant disease should be conveyed to the TB laboratory, which in turn should pursue the most rapid way to perform second-line drugs. The laboratory may consult with an expert TB health care provider and/or TB control. Second-line susceptibility tests should be ordered even before the first-line results have been returned in these circumstances. The laboratory should notify the health care provider of results as soon as possible.

When resistance to rifampin or any two primary drugs (isoniazid, ethambutol and pyrazinamide) is found, susceptibility tests should be requested for the full spectrum of second-line agents. Amikacin and/or kanamycin, capreomycin, quinolone (e.g., ofloxacin, levofloxacin), and ethionamide are the minimum second-line drugs to test.

Timely and frequent communication between the laboratory, health care provider, and TB control is essential<sup>61</sup>.

### **81. Does your Mycobacteriology Laboratory follow CLSI guidelines regarding the first-line and second-line drugs tested?**

The Clinical Laboratory Standards Institute (CLSI, formerly NCCLS) document M24-A (or most recent), entitled “Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard” describes antimycobacterial susceptibility testing for *M. tuberculosis* complex providing protocols, related quality control parameters, and interpretive criteria<sup>59</sup>.

See Appendix C for a list of 1<sup>st</sup> and 2<sup>nd</sup> line drugs.

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

### **82. Does your Mycobacteriology Laboratory repeat susceptibility testing if the patient is culture-positive after three months of therapy or shows clinical evidence of failure to respond to therapy?**

Susceptibility testing should be repeated if the patient continues to produce culture positive sputum after three months of treatment<sup>59</sup>. The detection of new resistance should be treated as a critical value and reported as soon as possible.

### **83. Does your Mycobacteriology Laboratory confirm or ensure confirmation of drug resistance?**

To ensure accurate administration of appropriate anti-TB drug therapy all resistant isolates should be confirmed by public health laboratories.

Discrepancies in test results can occur between different laboratories or using different methods. Reasons include:

- Some strains of *M. tuberculosis* complex have Minimum Inhibitory Concentrations (MICs) that are close to the critical concentration. Long experience has shown that the reproducibility for testing of these strains can be poor.
- The different laboratories may not have used the same specimen.
- If a subculture is tested, it may not represent the entire initial population.
- Errors can occur during drug susceptibility testing:
  - i. Failure to use a standardized inoculum (well-dispersed suspension)
  - ii. Failure to add a drug to a vial
  - iii. Adding the wrong drug or concentration
  - iv. Failure to recognize a mixed infection, *M. tuberculosis* complex and a non-tuberculous mycobacterium, which is more difficult to detect in broth-based systems
  - v. Failure to recognize contamination with a non-mycobacterial organism, which is more difficult to recognize in broth-based systems
- Changes in drug activity or support of mycobacterial metabolism can occur between different lots of culture media. Laboratories should check new batches of medium ingredients to verify that the medium they produce has the same drug activity as previously, validated lots of medium<sup>61</sup>.

### **84. Does your Mycobacteriology Laboratory follow a different algorithm if there is suspicion of drug resistance (any drug resistance, multi-drug resistance (MDR) or extensive drug resistance (XDR)) as indicated on the request form?**

Because successful treatment of drug-resistant TB depends on the susceptibility test results on the *M. tuberculosis* complex isolate, second-line susceptibility tests should be requested as soon as drug resistance is suspected or identified. The laboratory must have a mechanism whereby the health care provider can communicate this suspicion to the laboratory. A request form with the ability to check a box for suspicion of MDR-TB is one example. Second-line susceptibility tests should be ordered even before the first-line results have been completed in these circumstances.

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

Conventional agar proportion direct susceptibilities can sometimes be performed, which may hasten the results. However, if this assay is not available, susceptibility testing must be performed using a broth-based system.

When drug resistance to more than one first-line drug is suspected, susceptibility tests should include the full spectrum of second-line agents, and be setup at the same time as the first-line drugs.

Molecular drug resistance assays are available, though none are FDA cleared, for rapid testing from sputum sediment or a broth culture. Examples are line-probe assays, real time PCR, and Molecular Beacons. Line-probe assays are a family of novel DNA strip-based tests that use PCR and reverse hybridization methods for the rapid detection of mutations associated with rifampin and/or rifampin and isoniazid resistance. Line-probe assays are designed to identify *M. tuberculosis* complex and simultaneously detect mutations associated with drug resistance<sup>67, 68, 69</sup>.

### **85. Does your Mycobacteriology Laboratory telephone, fax, or electronically transmit a drug susceptibility report to the health care provider as soon as results are available and follow with a written report within 24 hours?**

It is important to report drug susceptibility results to the health care provider as soon as the information is available. Drug resistance to any first-line drug is considered a critical value and should be reported to infection control and/or public health, in addition to the health care provider. If the patient has drug resistant TB, the information will be used by the health care provider to modify the patient's drug regimen, especially if the patient is not improving on the assigned regimen<sup>10</sup>.

For more information on Critical Values refer to Appendix B.

### **86. Does your Mycobacteriology Laboratory telephone, fax, or electronically transmit a drug susceptibility report to the Tuberculosis Control Program in the state where the patient resides as soon as results are available?**

Drug susceptibility results must be communicated independently to the TB Control Program in the state where the patient resides. This practice will ensure prompt and focused attention on the patient with drug resistance and allow proper treatment<sup>42</sup>.

### **87. Does your Mycobacteriology Laboratory report at least 80% of initial *M. tuberculosis* complex drug susceptibility test results within 28 days of specimen collection?**

Laboratorians must accept the responsibility of their role in the proper management of patients infected with TB. Drug susceptibility testing is an essential link for patient management, especially in cases of drug resistance, MDR-TB and XDRTB.

All laboratories must focus on reducing turnaround time for drug susceptibility testing to less than 28 days from specimen collection to reporting of initial specimens or consider referring all *M. tuberculosis* complex testing to a reference laboratory<sup>10</sup>.

Reporting of at least 80% of primary *M. tuberculosis* complex drug susceptibility test results within 28 days accounts for the occurrence of contaminated samples, mixed with non-tuberculous mycobacteria, and fastidiously growing strains such as MDR-TB/XDRTB, *M. bovis*, and *M. africanum*.

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

### **88. Does your Mycobacteriology Laboratory monitor the turnaround time for reporting primary drug susceptibility test results on *M. tuberculosis* complex isolates to ensure reports are sent within 28 days of specimen collection?**

Completing reports on eighty percent of primary *M. tuberculosis* complex drug susceptibility test results on initial diagnostic specimens within 28 days of specimen receipt is reasonable, and a longer turnaround time in more than 20% indicates a need for improvement in identifying system weaknesses<sup>42</sup>.

The laboratory that recovers the initial *M. tuberculosis* complex isolate is responsible for assuring that susceptibility testing is performed. Whenever possible, the primary isolation media (i.e., a broth aliquot or slant) should be immediately submitted and a subculture should be retained in the originating laboratory. If a reference laboratory is used, the referring laboratory should periodically monitor the time period from date of collection to the receipt of susceptibility results performed by the reference laboratory<sup>10,12</sup>.

### **89. Does your Mycobacteriology Laboratory participate in a proficiency testing program for drug susceptibility testing?**

In order to satisfy CLIA requirements, the laboratory performing susceptibility testing needs to be enrolled with one of the proficiency testing providers<sup>1</sup> (i.e. CAP, New York State Department of Health, etc.)

To see the exact wording of the regulation, refer to CLIA 493.801-803,825.

The Centers for Disease Control and Prevention (CDC) is conducting a voluntary performance evaluation program to assess the laboratory's susceptibility testing process for drug-resistant strains of *M. tuberculosis* complex. Benefits of laboratory participation include the opportunity to conduct a free, anonymous self-assessment that will improve testing processes and will prepare laboratories to satisfy mandatory testing requirements.

**However, this is not a proficiency testing program.** Therefore, the testing components of the program are not intended for use by a laboratory to satisfy any regulatory requirement for participation in a proficiency testing program. Results will be reported solely on aggregate data of all participating laboratories. Other benefits of laboratory participation are:

- Analysis of characterized and referenced cultures with attributes closely resembling those of cultures encountered in routine clinical testing;
- Summary of aggregate methods and results reported by all participant laboratories for drug susceptibility testing;
- Provision of a mechanism for performing self-assessment for improvement of laboratory performance;
- Detection of problems with test systems and reagents;

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

- Receipt of reference strains of *M. tuberculosis* complex to be used for future quality control and quality assurance activities;
- Access to sources for technical consultations.

Program participants will conduct periodic testing of performance evaluation panels of isolates in the same manner that they evaluate patient isolates. Panels consist of *M. tuberculosis* complex strains exhibiting patterns of resistance to the primary anti-tuberculosis drugs. Laboratories submit testing results and provide CDC with information about the methods used. Shipment dates for the performance evaluation panels are announced. One month after CDC receives all responses, each participant laboratory will be provided with a preliminary report reflecting the susceptibility testing results for each strain. A detailed aggregate report of results and methods reported by all participants (without identification of individual laboratories) for each panel culture will be mailed before shipment of the next panel of *M. tuberculosis* complex isolates.

### **DIRECT DETECTION**

**90. Does your Mycobacteriology Laboratory perform or ensure access (via a reference laboratory) to nucleic acid amplification (NAA) testing for direct detection of *M. tuberculosis* complex in AFB smear-positive respiratory initial diagnostic specimens?**

Molecular methods have been developed for the detection of *M. tuberculosis* complex directly from clinical specimens. These methods can reduce the diagnostic time from weeks to days<sup>12</sup>. The sensitivity of these molecular methods is greater than 95% with smear positive respiratory specimens and specificity of greater than 96%. Predictive values may vary with laboratory methods, different prevalence of TB, and prevalence of other mycobacterial diseases. Therefore, it is important to collect information on the performance of the NAA in your setting and to provide this information to your health care providers.

At this time there are two FDA-cleared NAA assays available. One is approved for use with both smear positive and smear negative respiratory specimens, and the other is approved only for smear positive respiratory specimens. If the first smear-positive specimen is NAA negative, a second specimen should be tested. NAA should always be performed in conjunction with mycobacterial culture<sup>70</sup>.

**91. Does your Mycobacteriology Laboratory perform or ensure access (via a reference laboratory) to NAA testing for direct detection of *M. tuberculosis* complex in AFB smear-negative respiratory specimens from patients at high risk for TB?**

The guidelines state that “NAA testing should be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary tuberculosis for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management or TB control activities.” Culture remains the gold standard for the laboratory confirmation of TB and must be performed in conjunction with NAA testing.

There are two FDA-cleared tests for AFB smear positive respiratory specimens, one of which is also FDA cleared for use with AFB smear negative specimens. In

## ***Mycobacterium tuberculosis*: Assessing Your Laboratory**

addition, there are laboratory validated real-time PCR assays being offered. Laboratory validated assays must be validated in accordance with applicable FDA and Clinical Laboratory Improvement Amendments (CLIA) regulations.

It is not the role of the laboratory to determine if a patient is a TB suspect; therefore, it is the responsibility of the healthcare provider to determine the appropriate testing for the patient. If there is little suspicion of TB, but the healthcare provider is doing “rule-out” testing, NAA testing should not be performed. In this situation, testing should be limited to AFB smear and culture. The laboratory should work with the institutional quality assurance program to monitor the use of NAA testing in their patient population<sup>70</sup>.

### **92. Does your Mycobacteriology Laboratory perform or ensure access (via a reference laboratory) to a *M. tuberculosis* complex NAA test that allows detection of inhibitors?**

Specimens may contain inhibitors of NAA resulting in false negative results. Testing for inhibitors should be considered for specimens that are AFB-smear-positive and NAA test-negative. Each laboratory should establish the rate of inhibition to determine if routine testing for inhibitors is necessary. If inhibition testing is not performed on NAA test-negative specimens, it should be noted on the laboratory report. It may be desirable to note your laboratory’s inhibition rate on the negative laboratory report<sup>70</sup>.

### **93. Does your Mycobacteriology Laboratory telephone, fax, or electronically report result of *M. tuberculosis* complex NAA testing results within 48 hours of receipt for 75% of specimens tested in the laboratory?**

Rapid detection of *M. tuberculosis* complex infection plays a key role in TB control as outlined in Healthy People 2010<sup>12</sup>. Delay must be minimized so that the patient can be isolated and properly managed as soon as possible. In addition, manufacturer guidelines require that specimens be tested within a specified period from the time of collection and/or decontamination of the specimen in the laboratory. All NAA results should be immediately communicated to the health care provider, infection control, and public health officials.

### **94. Does your Mycobacteriology Laboratory perform or ensure access (via a reference laboratory) to molecular detection of drug resistance, especially to rifampin and isoniazid?**

Worldwide, an estimated 490,000 cases of multidrug-resistant tuberculosis (MDR TB) emerge each year (5.3% of all new and previously treated TB cases). If not adequately treated, patients harboring MDR TB continue to spread the disease.

Genetic studies have determined that in *M. tuberculosis* complex, resistance to antimycobacterial drugs is the consequence of spontaneous mutations in genes encoding either the target of the drug or the enzyme involved in drug activation. Recently, World Health Organization endorsed molecular line probe assays for rapid screening of patients at risk for MDR TB<sup>71</sup>.

The need for molecular detection of drug resistance, especially to rifampin and isoniazid, either in clinical specimens or in broth aliquots, will increase in this country and therefore, the laboratory should at least provide access to this novel technology via a reference laboratory if not able to perform in their own laboratory<sup>70</sup>.

## ***Mycobacterium tuberculosis: Assessing Your Laboratory***

At this time, there are no FDA approved methods. However, there are research use only (RUO) or laboratory developed tests available.

## BIBLIOGRAPHY

1. Federal Register. (2004) Final guidelines for Clinical Laboratory Improvement Act of 1988. [http://www.access.gpo.gov/nara/cfr/waisidx\\_04/42cfr493\\_04.html](http://www.access.gpo.gov/nara/cfr/waisidx_04/42cfr493_04.html)
2. Centers for Disease Control and Prevention. (2004). National plan for reliable tuberculosis laboratory services using a systems approach. Morbidity and Mortality Weekly Report (MMWR). 2005; 54 (No. RR-6)
3. American Thoracic Society; Centers for Disease Control and Prevention; Council of the Infectious Disease Society of America. (2000). Diagnostic standards and classification of tuberculosis in adults and children. American Journal of Respiratory and Critical Care Medicine. 161:1376--95.
4. Clinical and Laboratory Standards Institute. Laboratory detection and identification of mycobacteria; approved guideline. CLSI Document M48-A. Wayne, PA: CLSI; 2008.
5. Murray P.R. (Ed.). (2007). Manual of clinical microbiology 9<sup>th</sup> Edition. (Vol.1). Washington, DC: ASM Press.
6. Metchock, B. G., F. S. Nolte, and R. J. Wallace, Jr. (1999.) Mycobacterium. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover, editors. Manual of clinical microbiology, 7th ed. ASM Press, Washington, DC. 399-437.
7. Federal Register. 2006. Hazardous Materials: Infectious Substances; Harmonization with the United Nations Recommendations. 49 CFR: 172.174. Department of Transportation.
8. Centers for Disease Control and Prevention. (2005) Guidelines for preventing the transmission of mycobacterium tuberculosis in health-care settings. 2005. MMWR 2005; 54(No. RR-17).
9. Mase SR, Ramsay A, Ng V, Henry M, Hopewell PC, Cunningham J, Urbanczik R, Perkins MD, Aziz MA, Pai M. (2007) Yield of serial sputum specimen examinations in the diagnosis of pulmonary tuberculosis: a systematic review. International Journal of Tuberculosis and Lung Disease. 11:485-95.
10. Tenover, F.C., J.T. Crawford, R.E. Huebner, L.J. Geiter, C.R. Horsburg Jr., and R.C. Good. (1993). The resurgence of tuberculosis: is your laboratory ready? Journal of Clinical Microbiology. 31: 767-770.
11. Styr, BA, Shinnick TM, Ridderhof JC, Crawford JT, Tenover FC. (1997). Turnaround times for mycobacterial cultures. Journal of Clinical Microbiology. 35: 1041-1042.
12. U.S. Department of Health & Human Services. (2000). Healthy People 2010. (2<sup>nd</sup> ed.). Washington, D.C.: U.S. Government Printing Office.
13. National Committee for Clinical Laboratory Standards. (2004). A quality management system model; approved guideline. (2<sup>nd</sup> ed.). NCCLS Document HS1-A2. Wayne, PA: NCCLS.
14. National Committee for Clinical Laboratory Standards. (2004). Application of a quality system management model for laboratory services; approved guideline (3<sup>rd</sup> ed.). NCCLS Document GP26-A3. Wayne, PA: NCCLS.

15. Bennett A, Parks S. (2006). Microbial aerosol generation during laboratory accidents and subsequent risk assessment. Journal of Applied Microbiology. 100(4): 658-63.
16. Federal Register (1994). Occupational exposure to bloodborne pathogens; final rule. Part II, CFR 29: 1910.1030, Department of Labor, Occupational Safety and Health Administration.
17. Department of Health and Human Services. (2007). Biosafety in microbiological and biomedical laboratories. 5<sup>th</sup> edition. Government Printing Office.
18. Strong B.E, Kubica G.P. (1981). Isolation and identification of *Mycobacterium tuberculosis* a guide for the level II laboratory. PHS, HHS, Centers for Disease Control, Atlanta, GA.
19. Federal Register (1994). Respiratory protection; final rule. Part II, CFR 29: 1910.134, Department of Labor, Occupational Safety, and Health Administration.
20. Federal Register (1994). Hazard communication; final rule. Part II, CRF 29: 1910.1200, Department of Labor, Occupational Safety, and Health Administration.
21. Centers for Disease Control and Prevention. (2005). Controlling tuberculosis in the United States: recommendations from the American Thoracic Society, CDC, and the Infectious Diseases Society of America. MMWR 2005; 54(no. RR-12): 1-82.
22. Centers for Disease Control and Prevention. (2000). Targeted tuberculin testing and treatment of latent tuberculosis infection. MMWR. 2000; 49(No. RR-6): 1-51.
23. Centers for Disease Control and Prevention. (2005). Guidelines for using the Quantiferon TB-Gold test for detecting *Mycobacterium tuberculosis* infection. MMWR 2005; 54(No. RR-15): 49-55.
24. Kent, PT, Kubica GP. (1985). Public health mycobacteriology: a guide for the level III laboratory. Atlanta, GA: Centers for Disease Control and Prevention.
25. Mandell, G.L., J.E. Bennett, and R. Dolin. (2005). Mycobacterium tuberculosis. In Principles and Practice of Infectious Diseases. (6th ed.). Philadelphia; Elsevier: 2852-2886.
26. U.S. Department of Health and Human Services, Public Health Services, CDC and NIH. (2000). Primary containment for biohazards: selection, installation and use of biological safety cabinets. (2nd ed.). US Government Printing Office, Washington. September 2000.
27. Clinical and Laboratory Standards Institute. (2005). Protection of laboratory workers from occupationally acquired infections; Approved Guideline – Third Edition. CLSI document M29-A3. Wayne, PA: CLSI; 2005.
28. Health Canada. (2004). Laboratory biosafety guidelines. (3<sup>rd</sup> ed.). Ottawa, Canada: Health Canada.
29. Clinical and Laboratory Standards Institute. (2004). Training and competence assessment; approved guideline – second edition. CLSI Document GP21-A2. Wayne, PA: CLSI.
30. Clinical and Laboratory Standards Institute. (2007). Using proficiency testing to improve the clinical laboratory; approved guideline-second edition. CLSI Document GP27-A2. Wayne, PA: CLSI.

31. Clinical and Laboratory Standards Institute. (2006). Laboratory documents development and control; approved guideline – fifth edition. CLSI Document GP02-A5. Wayne, PA: CLSI.
32. Clinical and Laboratory Standards Institute. (2004). Quality control for commercially prepared microbiological culture media; approved standard-third edition. CLSI Document M22-A3. Wayne, PA: CLSI.
33. National Committee for Clinical Laboratory Standards. (1983). Labeling of laboratory prepared materials; proposed guideline. NCCLS Document GP04-P. Wayne, PA: NCCLS.
34. Health Insurance Portability and Accountability Act of 1996. Pub. L. No. 104-191. <http://aspe.hhs.gov/admsimp/pl104191.htm>
35. Smithwick RW. (1976). Laboratory manual for acid-fast microscopy (2<sup>nd</sup> ed.). Atlanta, GA: US Department of Health, Education, and Welfare; Centers for Disease Control.
36. Miörner H, Gebre N, Karlsson U, Jönsson G, Macaden R, Wolde A, Assefa A. (2004). Diagnosis of pulmonary tuberculosis. Lancet. 344:127.
37. Saceanu, C. A., Pfeiffer, N.C., McLean, T. (1993.) Evaluation of sputum smears concentrated by cyto centrifugation for detection of acid-fast bacilli. Journal of Clinical Microbiology. 31:2371-2374.
38. Koneman E.W. (2006) Koneman's color atlas and textbook of diagnostic microbiology, (6<sup>th</sup> ed.). New York: Lippincott Williams & Wilkins.
39. Isenberg, H.D. (2004). Clinical microbiology procedures handbook (2<sup>nd</sup> ed.). Washington, DC: ASM Press.
40. Forbes, B.A., Sahm, D.F., Weissfeld, A.S.(2007). Bailey & Scott's diagnostic microbiology (12<sup>th</sup> ed.). St. Louis, MO: Mosby.
41. Chedore, P, Th'ng C, Nolan D.H., Churchwell, G.M., Sieffer, D.E., Hale, Y.M., Jamieson F. (2002) Method for inactivating and fixing unstained smear preparations of mycobacterium tuberculosis for improved laboratory safety. Journal of Clinical Microbiology. 40: 4077-4080.
42. Behr MA, Warren SA, Salamon H, Hopewell PC, Ponce de Leon A, Daley CL, Small PM. (1999). Transmission of Mycobacterium tuberculosis from patient's smear-negative for acid-fast bacilli. Lancet. 353:444-449.
43. Centers for Disease Control and Prevention. (1995) Laboratory practices for diagnosis of tuberculosis – United States 1994. MMWR 44(31):587-90.
44. Association of Public Health Laboratories. (2004). The future of TB laboratory services: a framework for integration/collaboration/ leadership. Washington DC: APHL.
45. National TB Controllers Association/ CDC Advisory Group on Tuberculosis Genotyping. (2004). Guide to the application of genotyping to tuberculosis prevention and control. Atlanta, GA: US Department of Health and Human Services, CDC.
46. Kato-Maeda M, Bifani P.J., Kreiswirth B.N., Small P.M. (2001) The nature and consequence of genetic variability within Mycobacterium tuberculosis. Journal of Clinical Investigation. 107: 533-537.

47. Burman W.J. & Reves R.P. (2000). Review of false-positive cultures for *Mycobacterium tuberculosis* and recommendations for avoiding unnecessary treatment. Clinical Infectious Disease. 21:1390-1395.
48. Hale, Y.M. Pfyffer, G.E., Salfinger, M. (2001) Laboratory diagnosis of mycobacterial infections: new tools and lessons learned. Clinical Infectious Disease. 33: 834-846.
49. Rickman, T.W. & Moyer N.P. (1980). Proper centrifugal force can increase sensitivity of acid fast smears. Journal of Clinical Microbiology. 11: 618-620.
50. Isenberg, H.D. (2004). Clinical microbiology procedures handbook (2<sup>nd</sup> Ed). Washington, DC: ASM Press.
51. Floyd, M.M., Silcox, V.A., Jones, Jr., W.D., Butler, W.R., Kilburn, J.O. (1992). Separation of *Mycobacterium bovis* BCG from *Mycobacterium tuberculosis* and *Mycobacterium bovis* by using high-performance liquid chromatography of mycolic acids. Journal of Clinical Microbiology. 30:1327-1330.
52. Butler, W. R., Jost, K.C., Kilburn, J.O. (1991). Identification of mycobacteria by high-performance liquid chromatography. Journal of Clinical Microbiology. 29:2468-2472.
53. Butler, W. R. & Guthertz, L.S. (2001). Mycolic acid analysis by high-performance liquid chromatography for identification of *Mycobacterium* species. Clinical Microbiology Review. 14:704-726.
54. Kamerbeek J, Schouls L, Kolk A, van Agterveld M, van Soolingen D, Kuijper S, Bunschoten A, Molhuizen H, Shaw R, Goyal M, van Embden J. (1997). Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. Journal of Clinical Microbiology. 35:907-914.
55. Hlavsa, M. C., Moonan P.K., Cowan L.S., Navin TR, Kammerer JS, Morlock GP, Crawford JT, Lobue PA. (2008). Human tuberculosis due to *Mycobacterium bovis* in the United States, 1995-2005. Clinical Infectious Disease. 47:168-175.
56. Hinman, A. R., Hughes, J.M., Snider, Jr., D.E., Cohen, M.L. (1992). Meeting the challenge of multidrug-resistant tuberculosis: summary of a conference. MMWR. 41(no. RR-11):51-57.
57. Bird, B. R., Denniston, M.M., Huebner, R.E., and Good, R.C. (1996). Changing practices in mycobacteriology: a follow-up survey of state and territorial public health laboratories. Journal of Clinical Microbiology. 34:554-559.
58. Centers for Disease Control and Prevention. (1996). Prevention and control of tuberculosis in correctional facilities: Recommendations of the advisory council for the elimination of tuberculosis. MMWR. 45(RR-8):1-28.
59. Siddiqui S. & Rusch-Gerdes, S. (2006) FIND diagnostics MGIT procedure manual. FIND Diagnostics. [www.finddiagnostics.org](http://www.finddiagnostics.org)
60. National Committee for Clinical Laboratory Standards. (2003). Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes; approved standard. NCCLS document M24-A. Wayne, PA: NCCLS.
61. Centers for Disease Control and Prevention. (2003). Treatment of Tuberculosis, American Thoracic Society, CDC, and Infectious Disease Society of America. MMWR 52(No. RR-11).

62. Francis J. Curry National Tuberculosis Center. (2004). Drug-resistant tuberculosis: a survival guide for clinicians 2<sup>nd</sup> edition. California Department of Public Health.
63. Roberts, G.D. Goodman, N.L., Heifets L., Larsh H.W., Lindner T.H., McClatchy J.K., McGinnis M.R., Siddiqi S.H., Wright P. (1983). Evaluation of the BACTEC radiometric method for recovery of mycobacteria and drug susceptibility testing of *Mycobacterium tuberculosis* from acid-fast smear-positive specimens. Journal of Clinical Microbiology. 18: 689-696.
64. Woods, G.L., Fish, G., Paunt, M., Murphy, T. (1997). Clinical evaluation of Difco ESP culture system II for growth and detection of mycobacteria. Journal of Clinical Microbiology. 35: 121-124.
65. Whyte T., Hanahoe B., Collins T., Corbett-Feeney G., Cormican M. (2000). Evaluation of the BACTEC MGIT 960 and MB/BacT systems for routine detection of *Mycobacterium tuberculosis*. Journal of Clinical Microbiology. 38:3131-3132.
66. Pfyffer, G.E., Cieslak C., Welscher H.M., Kissling, P., Rusch-Gerdes, S. (1997). Rapid detection of mycobacteria in clinical specimens by using the automated BACTEC 9000 MB system and comparison with radiometric and solid culture systems. Journal of Clinical Microbiology. 35: 2229-2234.
67. Bifani P, Moghazeh S, Shopsin B, Driscoll J, Ravikovitch A, Kreiswirth BN. (2000). Molecular characterization of *Mycobacterium tuberculosis* H37Rv/Ra variants: distinguishing the mycobacterial laboratory strain. Journal of Clinical Microbiology. 38(9): 3200-3204.
68. Somoskovi A, Dormandy, J, Parsons, L.M, Kaswa M, Seng K, Rostogi N, Salfinger, M. (2007). Sequencing of the *pncA* gene in members of the *Mycobacterium tuberculosis* complex has important diagnostic applications: Identification of a species-specific *pncA* mutation in *Mycobacterium canettii*, and the reliable and rapid predictor of pyrazinamide resistance. Journal of Clinical Microbiology. 45(2):595-9.
69. Hillemann D, Rüsck-Gerdes S, Richter E. (2007). Evaluation of the GenoType MTBDRplus assay for rifampin and isoniazid susceptibility testing of *Mycobacterium tuberculosis* strains and clinical specimens. Journal of Clinical Microbiology. 45(8):2635-40.
70. Hale YM, Desmond EP, Jost KC Jr, Salfinger M. (2000). Access to newer laboratory procedures: a call for action. International Journal of Tuberculosis and Lung Disease. 4(12 Suppl 2):S171-5.
71. Centers for Disease Control and Prevention. (2009). Updated Guidelines for the Use of Nucleic Acid Amplification Tests in the Diagnosis of Tuberculosis. MMWR. 58(01); 7-10.
72. World Health Organization. (2008). Molecular line probe assays for rapid screening of patients at risk for multidrug-resistant tuberculosis (MDR-TB). (Policy Statement)

## **Appendix A: SPUTUM COLLECTION**

### **Indications for Sputum Collection**

- 1) To establish the initial diagnosis of tuberculosis
- 2) To monitor the infectiousness of the patient
- 3) To provide “proof of cure” or efficacy of treatment by negative smears and/or cultures
- 4) To re-evaluate potential infectiousness when treatment has been interrupted or inadequate.

### **Quality of Sputum Specimens**

A good sputum specimen consists of recently-discharged material from the bronchial tree, with minimum amounts of oral or nasal material. Satisfactory specimens are thick and contain mucoid or mucopurulent material. Ideally, a sputum specimen should have a volume of 3-5ml, although smaller quantities are acceptable if the quality is satisfactory. Poor quality sputum specimens are thin and watery and not suitable for testing. Saliva and nasal secretions are unacceptable.

If a patient finds it difficult to produce sputum, other methods, such as sputum induction may be used to obtain pulmonary secretions for diagnosis. Induced sputum resembles saliva; therefore, it is important that these specimens be marked “induced” in order not to be discarded as unsuitable.

### **Provider Instructions for Collection of Sputum Specimens**

1. Provide a sputum collection kit or sterile 50ml conical tube for sputum collection.
2. Follow infection control precautions and use personal protective equipment (PPE) as indicated by the clinical condition of the patient. The minimum standard for respiratory protection for tuberculosis or suspected tuberculosis is an N-95 or higher, fit-tested respirator.
3. Persons requiring sputum collection for TB smear and culture should have at least three consecutive sputum specimens obtained, each collected in 8-24 hour intervals, with at least one being an early morning specimen. (Reference MMWR 2005)
4. Instruct the patient on how to produce a sputum specimen versus saliva or nasopharyngeal discharge. Have the patient rinse mouth with water before collecting the specimen to minimize contamination with food particles, mouth wash, or oral drugs which might inhibit the growth of mycobacteria. Inhaling steam (hot shower or boiling water) may help sputum production.
  - a. If tap water in your area has abundant mycobacteria such as *M. gordonae* or *M. avium*, sterile water is indicated. If necessary, this can be carried out in

the home by boiling water in a heatproof container for ten minutes, then cooling before use.

- b. Tooth brushing with water is OK, but avoid antiseptic solutions such as mouthwash. Also consider potential water contamination as above and adjust accordingly.
5. Print the patient's name, specimen type and date/time of collection the tube label.
6. Refrigerate the specimen if it is not immediately being sent to the laboratory.
7. Provide supervised sputum collection for at least the first sputum specimen, until the patient demonstrates the ability to properly collect the specimen.
8. Persons who are suspected or confirmed as having TB can be so fearful of sputum specimen results that they will suppress a cough or even have another individual provide the specimen. When results do not fit the clinical picture, supervision of specimen collection should be done to ensure that the health of the public is protected.

**Patient Instructions for Collection of Sputum Specimens:**

1. The collection tube is very clean. Do not open it until you are ready to use it.
2. As soon as you wake up in the morning (before you eat or drink anything), brush your teeth and rinse your mouth with water. Do not use toothpaste or mouthwash.
3. If possible, go outside or open a window before collecting the sputum sample. This helps protect other people from TB germs when you cough.
4. Take a very deep breath and hold the air for 5 seconds. Slowly breathe out. Take another deep breath and cough hard until some sputum comes up into your mouth.
5. Spit the sputum into the plastic tube.
6. Take another deep breath and cough hard and spit the sputum into the plastic tube. Keep doing this until the sputum reaches the 5 mL line on the tube. (3mL is the minimum volume necessary for this test)
7. Screw the cap on tightly.
8. Wash and dry the outside of the tube.
9. Place the tube inside a biohazard bag, along with a small absorbent pad to offer protection from spills.

References:

[www.sahealthinfo.org/tb/culturecollection.htm](http://www.sahealthinfo.org/tb/culturecollection.htm)

[www.searo.who.int/en/Section10/Section17/Section53/Section482\\_1799.htm](http://www.searo.who.int/en/Section10/Section17/Section53/Section482_1799.htm)

<http://dhs.wisconsin.gov/tb/resources/guidelines/TBG-Sputum.doc>

## Appendix B: Critical Values

Critical Value	Recommended turn-around time
Positive AFB smear	Report within 24 hours of specimen receipt
Positive Nucleic Acid Amplification Test (NAAT)	Report within 48 hours of specimen receipt
New <i>M. tuberculosis</i> complex positive culture results from any specimen source	Report identification of <i>M. tuberculosis</i> complex in $\leq 21$ days
<i>M. tuberculosis</i> complex first-line drug susceptibility testing results on isolates which demonstrate resistance to any of the first-line drugs.	Report first line drug susceptibility results $\leq 28$ days
New mycobacteria (all species) positive culture results from <u>blood, CSF, or other normally sterile body sites</u>	report within 24 hours of positive culture detection
New positive primary cultures where the original AFB smear on the specimen was <u>negative</u>	report within 24 hours of positive culture detection

### References:

1. Tenover, F.C., J.T. Crawford, R.E. Huebner, L.J. Geiter, C.R. Horsburg Jr., and R.C. Good. (1993). The resurgence of tuberculosis: is your laboratory ready? Journal of Clinical Microbiology. 31: 767-770.
2. Styrt, BA, Shinnick TM, Ridderhof JC, Crawford JT, Tenover FC. (1997). Turnaround times for mycobacterial cultures. Journal of Clinical Microbiology. 35: 1041-1042.
3. U.S. Department of Health & Human Services. (2000). Healthy People 2010. (2<sup>nd</sup> ed.). Washington, D.C.: U.S. Government Printing Office.

**Appendix C**  
**Anti-Tuberculosis Drugs and Their Recommended Critical Concentrations in**  
**Middlebrook 7H10 and 7H11 Agar Medium**

		Critical Concentrations (µg/ml)	
		7H10 Agar	7H11 Agar
<b>Primary Drugs</b>	Isoniazid (INH) Low	0.2	0.2
	Isoniazid (INH) High	1.0	1.0
	Rifampin*	1.0	1.0
	Ethambutol Hydrochloride	5.0	7.5 <sup>§</sup>
	Pyrazinamide (PZA) ‡	NR	NR
<b>Secondary Drugs<sup>£</sup></b>			
	Capreomycin	10.0	10.0
	Ethionamide	5.0	10.0
	Ethambutol Hydrochloride <sup>¶</sup>	10.0	10.0
	Kanamycin <sup>#</sup>	5.0	6.0
	Ofloxacin**	2.0	2.0
	p-Aminosalicylic Acid (PAS)	2.0	8.0
	Rifabutin <sup>‡‡</sup>	0.5	0.5
	Streptomycin Low	2.0	2.0
	Streptomycin High	10.0	10.0

\* Rifampin is the class representative for rifapentine.

§ Data supporting equivalency with 7H10 (5.0µg/ml) are limited.

‡ NR= not recommended. For pyrazinamide testing, the manufacturer's directions for the BACTEC 460TB procedure should be followed.

£ All secondary drugs should be tested on isolates that are resistant to rifampin or resistant to any two primary drugs. Testing to cycloserine, which is an option therapeutically, is not recommended due to technical problems with the test (Pfyffer et al.)

¶ Ethambutol should be tested at this higher concentration as a secondary drug.

# Kanamycin is the class representative for amikacin.

\*\* Ofloxacin is the class representative for the fluoroquinolones.

‡‡ Some investigators have included a higher concentration, usually 1.0 to 2.0 µg/ml. The clinical significance of these concentrations, especially in the setting of rifampin resistance is unknown.

**References:**

1. National Committee for Clinical Laboratory Standards. (2003). Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes; approved standard. NCCLS document M24-A. Wayne, PA: NCCLS.
2. Heifets, LB. Drug susceptibility tests and clinical outcome of chemotherapy. *In: Heifets LB, ed. Drug Susceptibility in Chemotherapy of Mycobacterial Infections.* Boca Raton, FL. CRC Press; 1991.
3. Pfyffer GE, Bonato DA, Ebrahimzadeh A, et al. Multicenter laboratory validation of susceptibility testing of *Mycobacterium tuberculosis* against classical second-line and new antimicrobial drugs by using the radiometric BACTEC 400TB technique and the proportion method with solid media. *J Clin Microbiol.* 1999; 37:3179-3186.