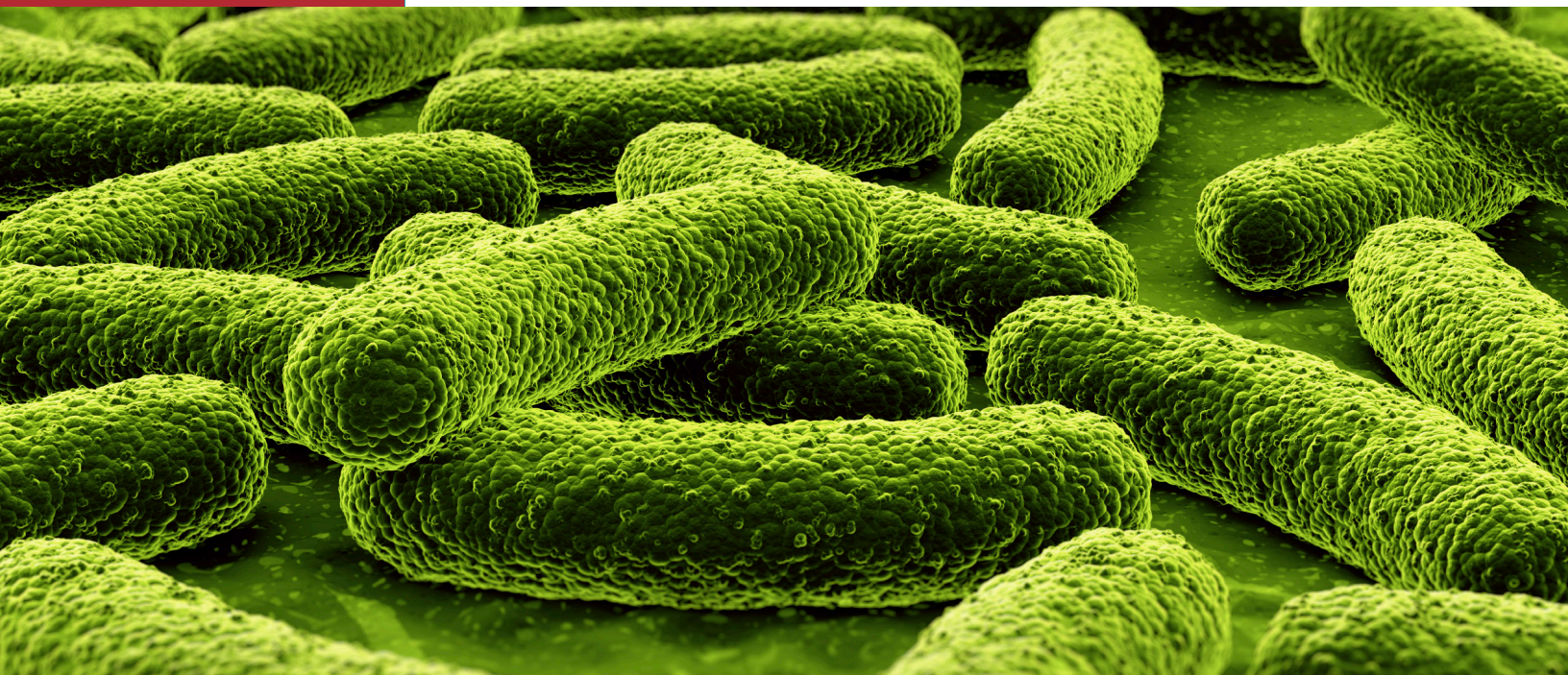


# *Mycobacterium tuberculosis:* Assessing Your Laboratory



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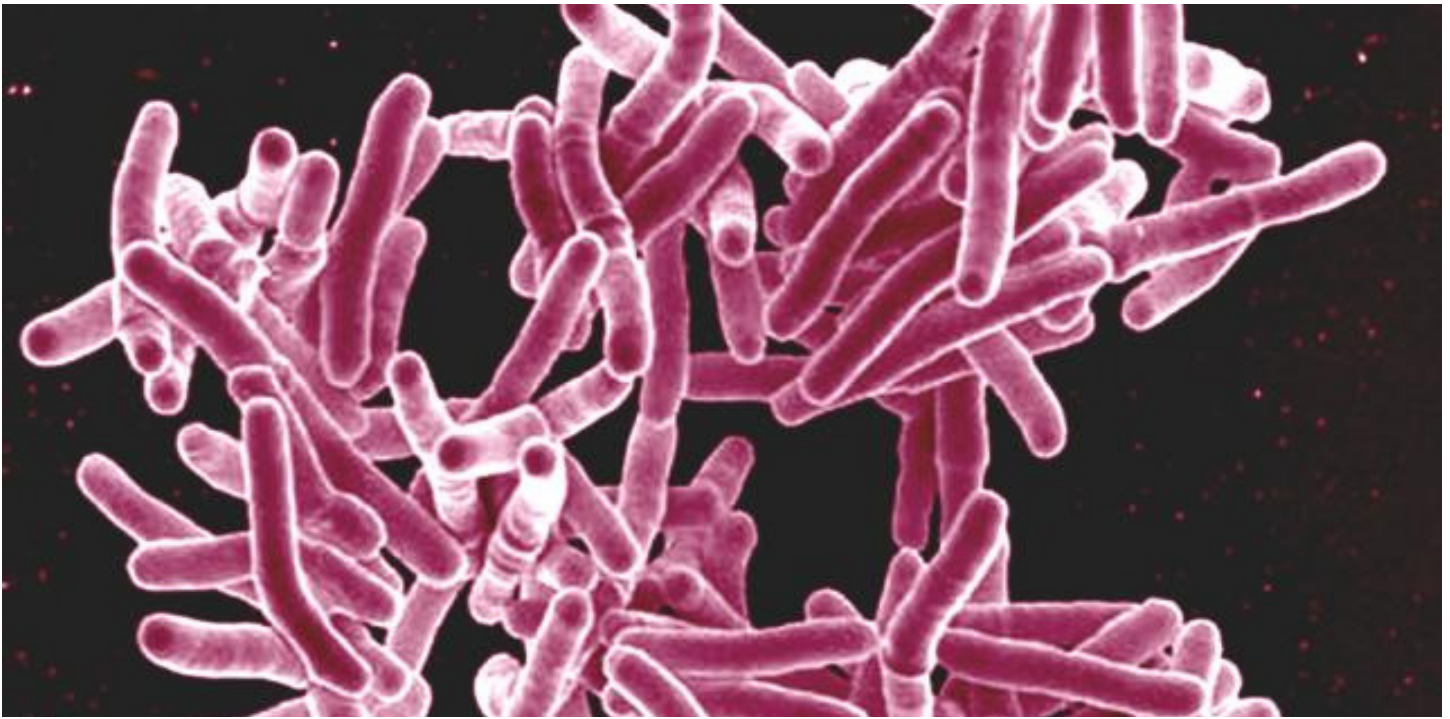
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## List of Acronyms

APHL	Association of Public Health Laboratories
AFB	Acid Fast Bacilli
AII	Airborne Infection Isolation
APHL	Association of Public Health Laboratories
ATS	American Thoracic Society
BCG	Bacillus Calmette–Guérin
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BSC	Biosafety Cabinet
BSL-2	Biosafety Level 2
BSL-3	Biosafety Level 3
CAP	College of American Pathologists
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulation
CLIA	Clinical Laboratory Improvement Amendments
CLSI	Clinical and Laboratory Standards Institute
CSF	Cerebrospinal Fluid
Ct	Cycle Threshold
DNA	Deoxyribonucleic acid
DOT	Department of Transportation
DST	Drug Susceptibility Testing
ETH	Ethambutol
FDA	Food and Drug Administration
HCFA	Health Care Financing Administration
HEPA	High Efficiency Particular Air Filter
HIPAA	Health Insurance Portability and Accountability Act
HPLC	High Pressure Liquid Chromatography
IGRA	Interferon Gamma Release Assay
INH	Isoniazid
IQCP	Individualized Quality Control Plan
JCAHO	Joint Commission on Accreditation of Healthcare Organizations
L-J	Lowenstein-Jensen
LPA	Line Probe Assay
MALDI-TOF	Matrix-Assisted Laser Desorption Ionization Time-of-Flight
MDDR	Molecular Detection of Drug Resistance
MDR	Multidrug Resistant
MGIT	Mycobacteria Growth Indicator Tube
MIC	Minimum Inhibitory Concentration
MIRU	Mycobacterial Interspersed Repetitive Units
MMWR	Morbidity and Mortality Weekly Report
MPEP	Model Performance Evaluation Program
MSDS	Material Safety Data Sheets
MTB	Mycobacterium tuberculosis
MTBC	Mycobacterium tuberculosis complex
NAA	Nucleic Acid Amplification
NALC	N-Acetyl-L-Cysteine

NALC-NaOH	N-acetyl L-cysteine-sodium hydroxide
NIH	National Institutes of Health
NIOSH	National Institute for Occupational Safety and Health
NPO	Nothing Per Mouth
NTCA	National Tuberculosis Controller's Association
NTGS	National Tuberculosis Genotyping Service
NTM	Nontuberculous Mycobacteria
OSHA	Occupational Safety and Health Administration
PAPR	Powered Air Purification Respirators
PAS	p-Aminosalicylic Acid
PCR	Polymerase Chain Reaction
PT	Proficiency Testing
PZA	Pyrazinamide
QA	Quality Assurance
QC	Quality Control
QI	Quality Improvement
RCF	Relative Centrifugal Force
RIF	Rifampin
RPM	Revolutions Per Minute
RUO	Research Use Only
TAT	Turnaround Time
TB	Tuberculosis
TST	Tuberculin Skin Test
UV	Ultraviolet
XDR	Extensively Drug Resistant



## Background

Tuberculosis (TB) is a serious infectious disease. An estimated 10-15 million United States citizens have latent tuberculosis infections and without detection and treatment, approximately 10% of these individuals will develop tuberculosis at some point in their lives. In 2017, 9,093 new TB cases were reported in the US, a decrease of 1.6% from 2016.<sup>1</sup> The growing threat of multidrug resistant (MDR) and extensively-drug resistant (XDR) TB not only leads to increased cases of tuberculosis but also increased costs. TB-related healthcare costs approach \$1 billion each year in the US and costly outbreaks still occur.

Quality laboratory testing is essential in order to reach the goal of TB elimination in the US. Continued improvements in laboratory testing programs are key and directly translate into expedited and improved treatment and control. The laboratory community must provide healthcare providers and TB controllers with accurate results within acceptable turnaround 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 mycobacteriology laboratory and an opportunity to thoroughly review your procedures, assign priorities and adopt a plan to update and improve your laboratory practices.

## Intended Use

*Mycobacterium tuberculosis: Assessing Your Laboratory* is intended for any public health, clinical or commercial laboratory that performs TB/mycobacteriology testing in the US. It is designed to be a self-assessment tool and information submitted to the tool from different laboratories is not compiled and data is not shared in any way.

The tool consists of a series of 96 questions divided into 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 Clinical Laboratory Improvement Amendments (CLIA), College of American Pathologist (CAP), Joint Commission on Accreditation of Healthcare Organizations (JCAHO), state-specific health codes or public health reporting requirements or any other accrediting agency or regulatory body.

## 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 one of these questions 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 until the deficiency can be remedied.

**Yellow Questions** – A negative answer to one of these questions 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 turnaround 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 six months to one year to determine your laboratory’s progress.

## 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. Specimen source
  - g. Date and time of collection
  - h. Clinical information
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 and transport container?
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, clear and leak-proof 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 its QA program?
12. Does your mycobacteriology laboratory monitor delivery time to ensure a minimal amount of time has elapsed (ideally less than 24 hours) between specimen collection and its arrival at the laboratory?
13. Does your mycobacteriology laboratory verify that each specimen container has the patient's name and a secondary identifier (e.g. date of birth, identification number) and that it matches 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 its QA program?
17. Does your mycobacteriology laboratory include the following information on the specimen report form:
  - a. 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. Test report date
  - e. Test performed
  - f. Specimen source
  - g. 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 fit testing at least annually if N95 or N100 respirators are used, as part of the occupational health program?
21. Does your mycobacteriology laboratory decontaminate all personal protective equipment (PPE) 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 when a new test is added or there is a change in protocol or there is a change in personnel?
25. Does your mycobacteriology laboratory provide the following for new employees as part of the occupational health program:
  - a. A two-step Mantoux TST or IGRA
  - b. A medical evaluation, including a chest radiograph, if the TST or IGRA is positive?
26. Does your mycobacteriology laboratory provide or ensure for all employees as part of the occupational health program:
  - a. An annual 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 TST or IGRA results
  - e. A periodic (at least annual) symptom review for people with a history of latent TB infection (LTBI) or prior tuberculosis
27. Does your mycobacteriology laboratory use a Class I or II, Biological Safety Cabinet (BSC) that has been certified at least annually?
28. Does your mycobacteriology laboratory work with clinical specimens in at least a Biosafety Level 2 (BSL-2) laboratory?
29. Does your mycobacteriology laboratory work with cultures suspected or confirmed to contain *M. tuberculosis* complex (MTBC) in a BSL-3 laboratory or a BSL-2 laboratory with BSL-3 practices?

30. Does your mycobacteriology laboratory provide safety training on aerosol prevention techniques for all employees before assigning work with mycobacterial specimens or cultures?
31. Does your mycobacteriology laboratory perform all manipulations on mycobacterial specimens and cultures that may generate aerosols only in a BSC?
32. Does your mycobacteriology laboratory use a centrifuge equipped with aerosol-free carriers with O-rings?
33. Does your mycobacteriology laboratory limit access into the laboratory when clinical specimens are being processed or when working with mycobacterial cultures?
34. Does your mycobacteriology laboratory have a one-pass (non-recirculating) ventilation system?
35. 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?
36. Does your mycobacteriology laboratory provide personal protective equipment that includes cuffed laboratory coats or gowns, gloves, respiratory and face protection?
37. Does your mycobacteriology laboratory utilize MALDI-TOF mass spectrometry for identification and have an instrument located in a BSL-2 or BSL-3 space? If located in a BSL-2 space has your laboratory performed an inactivation study on the MALDI-TOF extracts to ensure that no live organism is removed from BSL-3 space?
38. Does your mycobacteriology laboratory utilize PCR for identification and have an instrument located in a BSL-2 or BSL-3 space? If located in a BSL-2 space has your laboratory performed an inactivation study on the DNA extracts to ensure that no live organism is utilized in this testing?

## General Laboratory Practice

39. Does your mycobacteriology laboratory monitor and evaluate the overall quality of the general laboratory systems?
40. Does your mycobacteriology laboratory participate in an approved proficiency testing program?
41. Does your mycobacteriology laboratory follow standard operating procedures and maintain the results of quality control for each test procedure for at least two years?
42. Does your mycobacteriology laboratory monitor and evaluate the overall quality of analytical systems?
43. Does your mycobacteriology laboratory evaluate and document employees' competency before they are allowed to work independently?
44. Does your mycobacteriology laboratory evaluate and document employees' competency at least annually?
45. 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?
46. Does your mycobacteriology laboratory record the date and time positive results are telephoned, faxed or electronically reported to healthcare provider(s) and public health officials?

## Smears from Clinical Specimens

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

50. 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?
51. 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?
52. Does your mycobacteriology laboratory telephone, fax or electronically report all positive acid-fast smear results to both the patient's healthcare provider and the public health department as soon as results are known and within 24 hours from the specimen receipt?
53. 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?
54. Does your mycobacteriology laboratory use indicators, such as turnaround 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

55. Does your mycobacteriology laboratory receive and review a report of the prevalence and resistance patterns of MTBC isolated from your geographic area?
56. Does your mycobacteriology laboratory ensure universal genotyping by sending all initial MTBC isolates to the appropriate public health laboratory?
57. Does your mycobacteriology laboratory have access to laboratory training through your public health laboratory?
58. 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

59. Does your mycobacteriology laboratory process and culture at least 20 specimens per week?
60. Does your mycobacteriology laboratory take steps to eliminate cross-contamination between cultures?
61. Does your mycobacteriology laboratory routinely process and culture specimens within 24 hours of receipt in the laboratory?
62. Does your mycobacteriology laboratory use a refrigerated centrifuge(s) at a relative centrifugal force (RCF) of at least 3,000 x g for 15-20 minutes to process mycobacterial specimens for culture?
63. Does your mycobacteriology laboratory prepare, stain and examine acid-fast smears from all specimens (except blood) sent for mycobacterial culture?

## Inoculation and Growth Detection

64. Does your mycobacteriology laboratory inoculate into a selective broth system for the primary culture of mycobacteria?
65. 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?
66. Does your mycobacteriology laboratory inoculate specimens other than blood to at least one solid medium?
67. Does your mycobacteriology laboratory inoculate a negative control with each batch of cultures that are inoculated?
68. Does your mycobacteriology laboratory examine cultures on solid media for evidence of growth weekly for 6-8 weeks?
69. Does your mycobacteriology laboratory use a microscope or hand lens to examine solid media for earlier visualization of mycobacterial growth?

70. Does your mycobacteriology laboratory perform an acid-fast smear from:
- Broth cultures indicating growth
  - Selected colonies on solid medium at an early stage growth
71. Does your mycobacteriology laboratory subculture all broth cultures exhibiting acid-fast growth to solid medium?
72. Does your mycobacteriology laboratory use or ensure the use of a rapid method to confirm or rule out the presence of MTBC from positive cultures?
73. Does your mycobacteriology laboratory monitor the turnaround time of test results to ensure that 80% of MTBC isolates from primary patient specimens are identified within 21 days of specimen collection?
74. Does your mycobacteriology laboratory retain positive MTBC cultures for at least one year and other *Mycobacterium* spp. for a minimum of three months?
75. 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?
76. 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?
77. Does your mycobacteriology laboratory identify a broad range of nontuberculous mycobacteria?
78. If growth on solid medium is suggestive of a possible mixed culture, does your mycobacteriology laboratory subculture colonies with differing morphology for use in species identification?

## Susceptibility Testing

79. Does your mycobacteriology laboratory perform or ensure susceptibility tests on all initial isolates of MTBC according to CLSI guidelines?
80. 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?
81. Does your mycobacteriology laboratory determine or ensure susceptibility testing MTBC isolates to primary drugs using a broth-based system?
82. Does your mycobacteriology laboratory use a strain of MTBC that is susceptible to all anti-mycobacterial agents being tested as a control once each week that patient isolates are tested?
83. Does your mycobacteriology laboratory perform or ensure second-line drug susceptibility testing for MTBC when appropriate?
84. 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?
85. Does your mycobacteriology laboratory confirm or ensure confirmation of drug resistance?
86. Does your mycobacteriology laboratory follow a different algorithm if there is suspicion of drug resistance (any drug resistance, MDR or XDR) as indicated on the request form?
87. Does your mycobacteriology laboratory have a policy to transmit a molecular or growth-based drug susceptibility report to the healthcare provider as soon as results are available?
88. Does your mycobacteriology laboratory telephone, fax or electronically transmit a drug susceptibility report to the TB Control Program in the state or jurisdiction where the patient resides as soon as results are available?
89. Does your mycobacteriology laboratory report at least 69% of initial *M. tuberculosis* complex drug susceptibility test results within 17 calendar days from identification of *M. tuberculosis* complex from culture?

90. Does your mycobacteriology laboratory monitor the turnaround time for reporting primary drug susceptibility test results on MTBC isolates to ensure reports are sent within 17 calendar days from identification of MTBC from culture?
91. Does your mycobacteriology laboratory participate in a proficiency testing program for drug susceptibility testing?

## Direct Detection

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

## 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>2</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 hard-copy) 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 *M. tuberculosis complex* (MTBC) isolated, first-line drug susceptibility testing automatically performed)<sup>3</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 healthcare 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>2</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 healthcare 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 healthcare professional. Because of the infectious nature of MTBC and the danger to the healthcare 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 one sputum specimen monthly to monitor treatment until at least two sequential monthly specimens are culture-negative.<sup>4</sup>

The efficacy of the laboratory procedure used to perform NAA 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 one hour. Specimens delayed longer than 30 minutes before transport should be refrigerated. See Clinical and Laboratory Standards Institute (CLSI), *M48: Laboratory Detection and Identification of Mycobacteria, 2nd Edition* (Table 3) for a comprehensive list of specimen types and recommendations for collection and transportation.<sup>5</sup>

To see the exact wording of the regulation, refer to the Code of Federal Regulations: [Title 42, Part 493](#). For additional details you may refer to the accompanying section in the [Interpretive Guidelines for Laboratories](#).

## **3. As part of your 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>6</sup>

Personal communication with providers underscores the importance of an acceptable specimen and helps to educate the healthcare professional about the laboratory specimen requirements and test parameters (e.g. specificity, sensitivity, positive predictive values and negative predictive values). Communication enhances the healthcare team approach and reinforces the value of each individual's contribution to total patient care.<sup>3</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. **Specimen Source**
- g. **Date and time of collection**
- h. **Clinical information**

The information listed is required by CLIA and must be present or solicited from the requestor when not included.<sup>2</sup> [QA 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 or jurisdictional TB 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>2</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 CLIA regulation, refer to the Code of Federal Regulations: [Title 42, Part 493](#). For additional details you may refer to the accompanying section in the [Interpretive Guidelines for Laboratories](#).

#### 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>2</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 CLIA regulation, refer to the Code of Federal Regulations: [Title 42, Part 493](#). For additional details you may refer to the accompanying section in the [Interpretive Guidelines for Laboratories](#).

#### 6. Does your mycobacteriology laboratory include instructions to the provider for the volume and type of specimen required and transport container?

CLIA regulations state that a laboratory should define and include policies on specimen acceptability within provider instructions.<sup>2</sup> Provider instructions should include a request for individual sputum volume of not less than 3 mL.<sup>6</sup> However,

the optimal volume is 5-10 mL.<sup>6</sup> Less than 3 mL 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 healthcare provider instructions.

To see the exact wording of the CLIA regulation, refer to the Code of Federal Regulations: [Title 42, Part 493](#). For additional details you may refer to the accompanying section in the [Interpretive Guidelines for Laboratories](#).

## **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>2</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 3,000 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 CLIA regulation, refer to the Code of Federal Regulations: [Title 42, Part 493](#). For additional details you may refer to the accompanying section in the [Interpretive Guidelines for Laboratories](#).

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 specimens 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>5</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 US Government Printing Office, [www.gpo.gov](http://www.gpo.gov)
- International Air Transport Association Dangerous Goods Regulations (59<sup>th</sup> Ed.) 2018. 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 (7<sup>th</sup> Ed.) 2006. Available for purchase from IATA 800-716-6326 or [www.iata.org](http://www.iata.org)
- US Postal Service regulations. Domestic Mail Manual C023: <https://pe.usps.com/archive/html/dmmarchive20030810/C023.htm>
- US Department of Transportation (DOT): [www.transportation.gov](http://www.transportation.gov)  
Transportation Safety Institute: [www.tsi.dot.gov](http://www.tsi.dot.gov)
- 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, clear and leak-proof 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 3,000 x g are recommended. They are optimal for collection and processing specimens because:

- 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>7</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. CLSI, *M48: Laboratory Detection and Identification of Mycobacteria, 2<sup>nd</sup> Edition*<sup>5</sup> recommends refrigeration if transportation to the laboratory is delayed more than one hour. Laboratories may include a cold pack with specimen transport materials. The cold pack can be frozen and packed with specimens to keep them cool during shipment. Every effort should be made to get specimens to the laboratories as soon as possible. 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?**

The US Department of Transportation (DOT) requires training for all persons involved in the transportation of hazardous materials and infectious substances in commerce. To see the exact wording of the DOT regulation, refer to the Code of Federal Regulations: [Title 49, Part 172](#).

Category A “infectious substances” specifically include MTBC 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>8</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 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>9,10</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>7</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 to 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 a minimal amount of time has elapsed (ideally less than 24 hours) 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 QA guidelines require “accurate, reliable and prompt reporting of test results.”<sup>2</sup> Patient treatment can be significantly enhanced by a timely acid-fast smear report. In many jurisdictions specimens have to be shipped by mail or private courier and cannot

arrive at the laboratory within 24 hours. Laboratories should ensure that specimens are received as rapidly as possible from their submitters.

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 healthcare 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>11</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 each specimen container has the patient’s name and a secondary identifier (e.g. date of birth, identification number) and that it matches the submission form?**

Your laboratory must have available and follow a documented policy for proper labeling of specimens.<sup>2</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.

The exact wording of the regulation is available in the Code of Federal Regulations: [Title 42, Part 493.1232](#). For additional details you may refer to the accompanying section in the [Interpretive Guidelines for Laboratories](#).

**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>2</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

The exact wording of the regulation is available in the Code of Federal Regulations: [Title 42, Part 493.1242](#). For additional details you may refer to the accompanying section in the [Interpretive Guidelines for Laboratories](#).

**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>2</sup>

The exact wording of the regulation is available in the Code of Federal Regulations: [Title 42, Part 493.1291](#). For additional details you may refer to the accompanying section in the [Interpretive Guidelines for Laboratories](#).

Initial positive critical results, as defined by your laboratory, should be telephoned, faxed or electronically reported to the healthcare 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 TB from infected individuals to his or her contacts in the community or healthcare setting. A single, undiagnosed 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>11</sup> and TB culture results<sup>12</sup> is recommended:

- a. Report positive microscopy results within 24 hours of specimen receipt
- b. Report results confirming the identification of MTBC 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 healthcare 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 healthcare 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 its QA 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 one 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</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**
- 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>2</sup> Your laboratory may seek input from healthcare providers and TB Control Program for additional relevant information to be included on your report form.

The exact wording of the regulation is available in the Code of Federal Regulations: [Title 42, Part 493.1291](#). For additional details you may refer to the accompanying section in the [Interpretive Guidelines for Laboratories](#).

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. MTBC 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>14</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.

### **18a. Does your mycobacteriology laboratory follow a written biosafety plan that defines safe laboratory practices?**

Federal “Bloodborne Pathogens” Regulations<sup>15</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>16,17</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:

- 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 be found in the CDC Laboratory Manual, [Isolation and Identification of \*Mycobacterium tuberculosis\*: A Guide for the Level II Laboratory](#).<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. The exact wording of the Occupational Safety and Health Administration (OSHA) regulation can be found in the Code of Federal Regulations; [Title 29, Part 1910.134\(c\)](#) which 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 fit testing at least annually if N-95 or N-100 respirators are used, as part of the occupational health program?**

The Occupational Safety and Health Standards require employers ensure annual fit testing if N-95 or N-100 respirators are used. The exact wording of the OSHA regulation can be found in the Code of Federal Regulations: [Title 29, Part 1910.134\(f\)](#) which 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 (PPE) 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>16</sup>

The laboratory must have a method for decontaminating all laboratory wastes, preferably within the laboratory. Autoclave indicators (e.g. 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 [Biosafety in Microbiological and Biomedical Laboratories](#) (BMBL).<sup>16</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 when a new test is added, there is a change in protocol or there is a change in personnel?**

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 [Guidelines for Preventing the Transmission of Tuberculosis in Health Care Facilities \(2005\)](#)<sup>9</sup> and in [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 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 MDR-TB and XDR-TB in the patient population should also be taken into account. The frequency of TST or 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 MTBC, they should be evaluated for infection periodically using TST or IGRA.<sup>9</sup>

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 for Occupational Safety and Health (NIOSH) Type C (>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>9</sup>

**25. Does your mycobacteriology laboratory provide or ensure the following for new employees as part of the occupational health program:**

**25a. A two-step Mantoux 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 *Mycobacterium 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 TB, should be exempt from TST screening. Employees with a positive initial skin test should be referred for medical evaluation.<sup>22</sup>

**25b. 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>9</sup>

**26. Does your mycobacteriology laboratory provide or ensure for all employees as part of the occupational health program:**

**26a. An annual TST or IGRA on tuberculin or IGRA-negative employees?**

For laboratories with careful documentation of TSTs for a 3-5 year period with no conversions, annual skin testing for employees is appropriate. In a laboratory where transmission of TB 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>9</sup>

**26b. Medical evaluation if the TST or IGRA converts or if symptoms of TB are exhibited?**

If medical evaluation suggests TB, immediate investigation and follow-up should be initiated. TST or IGRA testing of other employees should also be initiated. Employees with symptoms of tuberculosis should be examined immediately by their healthcare provider.<sup>9</sup>

**26c. Medical evaluation, follow-up and counseling for any known exposure event or TST or 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>23,24</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 MTBC should be determined to implement appropriate preventive therapy for the worker.

In the event of the TST or 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>25</sup>
- Review and re-evaluate the adequacy of the safety plan.

**26d. 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>9</sup>

**26e. A periodic (at least annual) symptom review for people with a history of latent TB infection (LTBI) or prior 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 TB 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>9</sup>

**27. Does your mycobacteriology laboratory use a Class I or II 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 risk 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 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>16</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.

**Table 1: Comparison of Biological Safety Cabinets<sup>16</sup>**

BSC Class	Face velocity (lfpm)	Airflow Pattern	Nonvolatile Radionuclides and Toxic Chemicals	Volatile adionuclides and Toxic Chemicals	Biosafety Level	Product Protection
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

BSC Class	Face velocity (lfpm)	Airflow Pattern	Nonvolatile Radionuclides and Toxic Chemicals	Volatile Radionuclides and Toxic Chemicals	Biosafety Level	Product Protection
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 plenum under negative pressure to room	YES	When exhausted outdoors (minute amounts)	2,3	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, Centers for Disease Control and Prevention.<sup>16</sup>

**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 MTBC (Biosafety Level 2/3, depending on whether pure cultures are 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 MTBC (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 MTBC; B2 is designed for “total (100%) exhaust” and is not required for the mycobacteriology laboratory.

**28. 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 MTBC, clinical specimens from suspected or known cases of TB must be considered potentially infectious and handled with appropriate precautions. The [Biosafety in Microbiological and Biomedical Laboratories \(BMBL\)](#)<sup>16</sup> recommends that BSL-2 practices and procedures, containment equipment and facilities be used for the manipulation of these clinical specimens.<sup>16</sup> Newer CDC recommendations published in 2012 state that TB clinical specimen processing and decontamination be performed in BSL-3 facilities.<sup>17</sup> If your laboratory processes and decontaminates clinical specimens in a BSL-2 facility, a risk assessment must be performed to determine if this is a safe practice in your facility. All aerosol-generating activities must be performed in a biosafety cabinet.<sup>17</sup>

**29. Does your mycobacteriology laboratory work with cultures suspected or confirmed to contain MTBC in a BSL-3 laboratory or a BSL-2 laboratory with BSL-3 practices?**

The [Biosafety in Microbiological and Biomedical Laboratories \(BMBL\)](#)<sup>16</sup> 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 MTBC.<sup>17</sup>

Engineering controls include:

- Separate room or suite specifically designed for mycobacterial culture

- One-pass (non-recirculating) ventilation system
- At least annual certification of Class I, II or BSC
- Exhaust air from BSC discharged through HEPA filters or recirculated only after passing through filters certified to remove 99.97% of particulates 0.3µm or larger
- Negative pressure in culture area and a means to monitor negative pressure (Smoke tubes or differential pressure-sending 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 12 room air exchanges per hour provide removal of 99% of airborne particulates within 30-45 minutes).

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

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. 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. 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 MTBC 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>9</sup>

### **31. 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 5µm 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>26</sup>

All procedures that create aerosols should be performed only in a fully functioning, certified 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 an 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 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 linear feet of air per minute, 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>27</sup>**

The activity of UV lights for sterilization/decontamination is limited by a number of factors including penetration, relative 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.

**32. 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.](#)<sup>25</sup>

**33. 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.

**34. 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 12 air changes per hour are acceptable and provide removal of 99% of airborne particulate within 45 minutes.<sup>16</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>9</sup>

**35. 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?**

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>28</sup>

**36. Does your mycobacteriology laboratory provide personal protective equipment that includes cuffed laboratory 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. 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>16</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 healthcare 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 N95 or N100 masks are used. Fit testing is not required for PAPRs.<sup>29</sup>

**37. Does your mycobacteriology laboratory utilize MALDI-TOF mass spectrometry for identification and have an instrument located in a BSL-2 or BSL-3 space? If located in a BSL-2 space has your laboratory performed an inactivation study on the MALDI-TOF extracts to ensure that no live organism is removed from BSL-3 space?**

An inactivation study needs to be performed to confirm a laboratory's inactivation method is effective and compatible with MALDI-TOF MS testing. Various methods have been utilized including heat inactivation and chemical treatment dependent on the downstream testing. Published heat treatment methods include temperatures ranging from 80 °C -100 °C with various incubation times, bead beating and ethanol treatments.

CLSI has published a new guideline, *M58 Methods for the Identification of Cultured Microorganisms Using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry*,<sup>30</sup> and the safety and accuracy of MALDI-TOF MS testing has been evaluated for highly pathogenic organisms including spore formers.<sup>31</sup> The vendors for these available instruments also provide inactivation protocols for mycobacteria. Bruker has developed a combination of a secure inactivation protocol and subsequent reliable cell disruption, using a silica-bead protocol to disrupt the cell wall and extract the proteins. Biomerieux indicates that the VITEK® MS Mycobacterium/ Nocardia kits contain all reagents necessary for simple inactivation and extraction for identification of these organisms on the VITEK® MS. Whichever method is selected the testing laboratory needs to design and perform an inactivation study before any testing is implemented. A method utilized should be validated with a protocol that tests a high level of organism ( $>10^6$  per mL). A typical protocol would include two sets of dilutions prepared of *M. tuberculosis* (10 fold dilutions straight to  $10^{-7}$  or  $10^{-8}$ ). One set is treated by the selected inactivation method and one set is not then both sets are plated on culture plates in triplicate and incubated for eight weeks at 37 °C and observed for colony forming units at each dilution. Inactivation protocols typically must demonstrate a minimum of a 6 log to 8 log reduction with zero growth. A laboratory should repeat this work when new staff are hired, new equipment or reagents are acquired and when standard operating procedures change as part of a routine quality assessment.<sup>31-35</sup>

**38. Does your mycobacteriology laboratory utilize PCR for identification and have an instrument located in a BSL-2 or BSL-3 space? If located in a BSL-2 space, has your laboratory performed an inactivation study on the DNA extracts to ensure that no live organism is utilized in this testing?**

An inactivation study needs to be performed to confirm a laboratory's inactivation method is effective and compatible with molecular testing. Various methods have been utilized which include heat inactivation at temperatures ranging from 80 °C -100 °C with incubation times ranging from 5-60 minutes.<sup>35-38</sup> An appropriate method should be selected and an inactivation study should be designed and performed before any testing is implemented. The method utilized should be validated with a protocol that tests a high level of organism ( $>10^6$  per ml). A typical protocol would include two sets of dilutions prepared of *M. tuberculosis* (10 fold dilutions straight to  $10^{-7}$  or  $10^{-8}$ ). One set is treated by the selected inactivation method and one set is not then both sets are plated on culture plates in triplicate and incubated for eight weeks at 37 °C and observed for colony forming units at each dilution. Inactivation protocols typically must demonstrate a minimum of a 6 log to 8 log reduction with zero growth. A laboratory should repeat this work when new staff are hired, new equipment or reagents are acquired and when standard operating procedures change as part of a routine quality assessment.

## General Laboratory Practice

### 39. Does your mycobacteriology laboratory monitor and evaluate the overall quality of the general laboratory systems?

The routine implementation and review of quality management systems enables laboratories to produce optimal testing outcomes. Clinical microbiologists perform quality control (QC) and QA procedures in an effort to achieve overall quality improvement (QI) in their laboratories. Both QC and QA are essential to maintaining high quality laboratory operations. QC ensures laboratory equipment and supplies are meeting performance standards, while QA involves analyzing the processes and procedures conducted during pre and post analytical phases of testing.

QI has been practiced in the clinical microbiology field for many years prior to its conceptual inception. QI encompasses employee training and laboratory error prevention practices and provides a holistic approach to harmonizing individual QC and QA practices.

For more information about implementing quality management systems in your laboratory, please review the following resources:

- CLSI, *QMS01-A4: Quality Management System: A Model for Laboratory Services; Approved Guideline; 4th Edition*<sup>13</sup>
- CLSI, *EP-23A: Laboratory Quality Control Based on Risk Management, 1st Edition*<sup>39</sup>
- *Implementing a Quality Management System in the Medical Microbiology Laboratory*<sup>40</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>2</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.

The exact wording of the regulation, is available in the Code of Federal Regulations: [Title 42, Part 493.1231](#). For additional details refer to the accompanying section in the [Interpretive Guidelines for Laboratories](#).

Laboratories must also have the following policies in place in each of the following areas to ensure the quality of general laboratory systems from specimen collection or receipt in the laboratory to reporting of results:

- Positive specimen identification and optimal integrity: Code of Federal Regulations: [Title 42, Part 493.1232](#)
- Documentation and investigation of problems and complaints in the laboratory (Code of Federal Regulations: [Title 42, Part 493.1233](#)) and documentation of corrective actions (Code of Federal Regulations: [Title 42, Part 493.1282](#))
- Documentation of communication efforts and investigation of communication problems between the laboratory and authorized individuals who order or receive test results: Code of Federal Regulations: [Title 42, Part 493.1234](#)
- Quality of pre-analytic systems such as test requesting, specimen submission, handling and referral testing: Code of Federal Regulations: [Title 42, Part 493.1242](#).
- Quality of post-analytic systems such as assessment of reporting accurate and reliable manual or electronic test results (Code of Federal Regulations: [Title 42, Part 493.1291](#)) and mechanisms for monitoring and for and correcting inaccuracies (Code of Federal Regulations: [Title 42, Part 493.1299](#)).

### 40. 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.

PT isolates 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 specimens and the staff may not consult with personnel from other laboratories concerning the isolates. The isolates may not be referred to another laboratory for testing. The individual performing the testing and the laboratory director must certify that the PT isolates were integrated into the daily workload and treated the same way as patient specimens. PT records must be retained for two years.<sup>41,42</sup>

The exact wording of the regulations are available in the Code of Federal Regulations: [Title 42, Part 493.825](#) and [Title 42, Part 493.913](#).

For more guidance on proficiency testing, see CLSI, *QMS03: Training and Competence Assessment, 4th Edition*<sup>41</sup> and *QMS24: Using Proficiency Testing and Alternative Assessment to Improve Medical Laboratory Quality, 3rd Edition*.<sup>42</sup>

#### **41. 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 current director must approve, sign and date all modifications to procedures, indicating approval prior to use. 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>2</sup>

The exact wording of the regulations are available in the Code of Federal Regulations: [Title 42, Part 493.1105](#) and [Title 42, Part 493.1251](#).

##### **Quality control**

The laboratory must follow an individualized quality control plan (IQCP) to monitor and evaluate the quality of the analytical testing process.<sup>2</sup> All quality control readings must be documented and the records maintained for a minimum of two years.

The exact wording of the regulations are available in the Code of Federal Regulations: [Title 42, Part 493.1253-54](#) and [Title 42, Part 493.1255-56](#).

For more information on establishing quality control protocols see CLSI, *QMS02-A6: Development and Management of Laboratory Documents, 6th Edition*.<sup>43</sup>

#### **42. Does your mycobacteriology laboratory monitor and evaluate the overall quality of analytical systems?**

When introducing new test methods or modifying an FDA-approved test, 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 responsible for having an IQCP to monitor the accuracy and precision of the complete analytical process.<sup>2</sup>

The exact wording of the regulations are available in the Code of Federal Regulations: [Title 42, Part 493.1253-54](#) and [Title 42, Part 493.1255-56](#).

For additional guidance on quality control policies see CLSI, *QMS01-A4: Quality Management System: A Model for Laboratory Services; Approved Guideline, 4th Edition*.<sup>13</sup>

#### **43. Does your mycobacteriology laboratory evaluate and document employees' competency before they are allowed to work independently?**

The knowledge, skills and abilities that are needed for employees performing mycobacteriology testing must be defined. Training of new employees must address all of the competencies needed to perform quality testing and to work safely in the laboratory. In 2015 CDC and APHL published competency guidelines for public health laboratory professionals.<sup>44</sup> This document describes core competencies needed to deliver the services of public health laboratories efficiently and effectively. It is the responsibility of the laboratory leadership to ensure that employees are competent prior to being allowed to work independently.<sup>45</sup>

Competency assessment includes, but is not limited to the following elements:

- Direct observation of routine test performance.
- Direct observation of performance of routine instrument maintenance and function checks
- Monitoring the recording and reporting of test results
- Reviewing intermediate test results, worksheets and quality control, proficiency testing and preventive maintenance records
- Assessment of test performance through testing of previously analyzed specimens, internal blind testing samples or external proficiency testing samples
- Assessment of problem solving skills

#### **44. Does your mycobacteriology laboratory evaluate and document employees' competency at least annually?**

Competency assessment must be performed periodically to verify employees continued demonstration of their knowledge, skills and abilities to perform their work. During the first year of an individual's duties, competency must be assessed at least semiannually. Competency assessments must be performed for all employees when there are organizational or technological changes that affect work processes, such as test method or instrumentation changes. When problems are identified with an individual's performance, retraining and competency assessment must be performed. CLSI, *QMS01-A4: Quality Management System: A Model for Laboratory Services; Approved Guideline, 4<sup>th</sup> Edition*<sup>13</sup> provides guidance in the development of training and competence assessment programs that can be used by laboratories and healthcare organizations.<sup>13</sup> CLIA requires competency assessment of employees at least annually.<sup>2</sup>

#### **45. 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 and written procedures to capture missing information to ensure test results are accurately and reliably sent to the final report destination in a timely manner.<sup>2</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.

The exact wording of the regulation is available in the Code of Federal Regulations: [Title 42, Part 493.1291](#). For additional details you may refer to the accompanying section in the [Interpretive Guidelines for Laboratories](#).

#### **46. Does your mycobacteriology laboratory record the date and time positive results are telephoned, faxed or electronically reported to healthcare provider(s) and public health officials?**

Written procedures for reporting and documenting life-threatening results and/or critical values—such as AFB positive smears—to healthcare 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>2</sup>

- a. Person making the call should identify him/herself

- b. Name of the healthcare provider being called
- c. Patient's name and date of birth
- d. Type of specimen source
- e. Date specimen collected
- f. Laboratory results
- g. Verify and read-back by healthcare provider
- h. Date and time of call

The exact wording of the regulation is available in the Code of Federal Regulations: [Title 42, Part 493.1291](#). For additional details you may refer to the accompanying section in the [Interpretive Guidelines for Laboratories](#).

To assure patient confidentiality, all test results must be protected.<sup>46</sup> Laboratories must abide by the Health Insurance Portability and Accountability Act (HIPAA) of 1996.

## Smears from Clinical Specimens

### 47. Does your mycobacteriology laboratory process at least 15 acid-fast smears per week?

Laboratories processing less than 15 specimens per week should consider referring mycobacteriology specimens to another laboratory with established quality of performance. It is recognized by the American Thoracic Society (ATS), CDC and CLSI that to maintain technical proficiency in acid-fast microscopy and smear examination, each microscopist should prepare and read a minimum of 10-15 smears per week.<sup>5</sup> 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 microscopists process 15 or less specimens per week then the laboratory should engage in other proficiency activities that could include: participation in multiple proficiency programs, develop an internal proficiency testing program and at least 20% of AFB-stained smears be reviewed blindly by a supervisor or another employee.

A QA program should be established to review AFB smear results periodically.<sup>5</sup> Per CLSI M48 Section 4.4.1.6<sup>5</sup>, the following indicators should be reviewed to detect:

- overall elevated numbers of positive or negative smears, relative to the expected patient population
- consecutive positive AFB smears from different patients
- AFB smear-positive specimens that fail to grow mycobacteria
- AFB smear-negative specimens that grow two or more colonies of mycobacteria on solid culture
- whether a second reader has randomly checked a certain percentage of smear-negative slides.

MTBC 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.

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

Direct smears are not encouraged. If rapid results are necessary utilization of NAA are recommended. If direct smears are used for rapid results, 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>47-49</sup>

### 49. 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 AFB in clinical specimens because of increased sensitivity and the ease of reading the smears. Fluorescent stains are 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.

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:

- the brightfield microscope is easier to use and maintain than the fluorescent one
- the presence of numerous microorganisms in culture negates the need for high sensitivity afforded by the fluorochrome-stain
- rapidly growing nontuberculous mycobacteria can fail to fluoresce in fluorochrome-stained smears
- they allow for the visualization of non-AFB contaminants.<sup>47</sup>

Detailed information concerning acid-fast stain procedures has been published. Examples include *Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 7<sup>th</sup> edition*,<sup>50</sup> the *Manual of Clinical Microbiology-12<sup>th</sup> edition*<sup>6</sup> and the *Clinical Microbiology Procedures Handbook-4<sup>th</sup> edition*.<sup>51</sup>

**50. 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>47</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
	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:

- Ensure that the wavelength of your lamp is appropriate for your stain and that it has optimal brightness. Excitation and emission (barrier) filters are necessary for visualization of the fluorescently-stained smear (specific to the staining method used, check package insert) Note usage and frequency of bulb changes.
- 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°C to 75°C for at least two hours in a BSC and maintains a uniform temperature across its surface.<sup>52</sup> Several alternative methods include: heat fixing slides at 80° C for 15 minutes or using a method using phenol and ethanol<sup>52</sup>.
- Examine stain reagents for signs of precipitation, debris or deterioration.

- Re-stain control slides with a new lot number of stain reagents.
- Ensure that staining racks are being used for smear preparation and not staining jars or dishes.
- Water quality is a key factor in staining. Use filtered distilled or deionized water is recommended. If tap water is being used, consider that disinfecting chemicals in the water could interfere with your fluorescence.

**51. 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 fluorochrome and carbol fuchsin stains varies. The former are usually read at 250-400X and the latter under oil immersion at 1,000X.<sup>54</sup>

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

AFB Number per view fields (1,000X oil immersion)	AFB Number per view fields (250X)	Interpretation
None per 300 fields	None per 30 fields	No AFB seen
1-2 per 300 fields	1-2 per 30 fields	Doubtful <sup>5</sup> , 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>47</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>55</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 TB is high.

**52. Does your mycobacteriology laboratory telephone, fax or electronically report all positive acid-fast smear results to both the patient’s healthcare 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 MTBC 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>56</sup>

It is the responsibility of laboratories to monitor the turnaround time (TAT) 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 time frames listed in Appendix B as recommended by CDC, APHL and ATS and mandated by many city departments of health.<sup>21</sup> The healthcare provider may identify patients for whom negative smear results should be communicated as a critical value (e.g. a patient in respiratory isolation).

**53. 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 TAT 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>11</sup>

Specimens that may contain MTBC, 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 US Government Printing Office: [www.gpo.gov](http://www.gpo.gov)
- International Air Transport Association Dangerous Goods Regulations (59<sup>th</sup> ed.) 2018) 2005. Available for purchase from [www.iata.org](http://www.iata.org)
- International Air Transport Association Infectious Substances and Diagnostic Specimens Shipping Guidelines (7<sup>th</sup> Ed.) 2006. Available for purchase from [www.iata.org](http://www.iata.org)
- USPS regulations: Domestic Mail Manual C023
- U.S. Department of Transportation (DOT): [www.dot.gov](http://www.dot.gov) and [www.tsi.dot.gov](http://www.tsi.dot.gov) (Transportation Safety Institute)
- International Civil Aviation Organization (ICAO): [www.icao.int](http://www.icao.int)

**54. Does your mycobacteriology laboratory use indicators, such as turnaround-time (TAT), 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 TAT 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>11,57</sup>

## Public Health and Epidemiology

**55. Does your mycobacteriology laboratory receive and review a report of the prevalence and resistance patterns of MTBC 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 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 TAT for reporting the drug susceptibilities of TB is critically needed to resolve the MDR/XDR-TB problem.

**56. Does your mycobacteriology laboratory ensure universal genotyping by sending all initial MTBC isolates to the appropriate public health laboratory?**

Laboratories must support outbreak investigations by routinely sending all initial MTBC isolates to their state public health laboratory. Isolates will be sent to regional laboratories for genotyping as part of the [National TB Genotyping Service \(NTGS\)](#).<sup>57</sup> Genotyping is a powerful tool for tracking the spread of individual strains of MTBC<sup>58</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 MTBC.
- 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 TB.
- Determine strain relatedness of TB within a community.
- Establish a background of prevalent strains within a community against which an outbreak or drug resistant pattern can be compared.

**57. Does your mycobacteriology laboratory have access to laboratory training through your public health laboratory?**

Public health laboratories and TB 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, public health laboratories must offer training opportunities as needed.<sup>59</sup>

**58. 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, MTBC isolation and TB drug resistance. Surveillance information can be shared with laboratories, public health departments and healthcare providers
- Develop and maintain state or regional TB isolate repositories
- Ensure all initial MTBC isolates will be sent for universal genotyping
- Provide training opportunities to maintain or improve proficiency in mycobacteriological methods
- Collaborate in research

## Specimen Processing and Decontamination

**59. Does your mycobacteriology laboratory process and culture at least 20 specimens per week?**

Proficiency in culture and identification of MTBC 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 MTBC 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>59</sup>

It is recommended by CDC, APHL and ATS 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>25,59</sup>

**60. Does your mycobacteriology laboratory take steps to eliminate cross-contamination between cultures?**

A review article authored by Burman and Reves<sup>60</sup> in 2000 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.
- 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.

### **61. 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-TB 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>61</sup>

### **62. Does your mycobacteriology laboratory use a refrigerated centrifuge(s) at a relative centrifugal force (RCF) of at least 3,000 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 an RCF of 3,000 x g or higher.<sup>62</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 RCF or gravity force. At an RCF of at least 3,800 x g for 15 minutes, there is an 82% correlation of positive AFB smears with culture as compared to 40% at 3,000 x g.<sup>63</sup> Old centrifuges that spin at 2,300-3,000 RPM (1,500-2,000 RCF) for 15 minutes attain theoretical sedimenting efficiencies ranging from 75-84% and this may be further reduced by lethal heat buildup.<sup>62</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. Ensure that the rating for the centrifuge tube matches the RPM or RCF that are being utilized.

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

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 TB disease; follow progress of antimicrobial therapy; and release non-infectious patients from isolation.<sup>6</sup>

## Inoculation and Growth Detection

### 64. Does your mycobacteriology laboratory inoculate into a selective broth system for the primary culture of mycobacteria?

Laboratories are continuously being challenged to reduce time to growth detection because rapid detection of MTBC is critical to diagnosing pulmonary TB and to the detection of drug-resistance. This is accomplished through the use of a commercial broth system as the primary method for isolating MTBC.

Primary culture in a selective broth medium enhances the growth of MTBC and is preferred to culture on solid media alone because broth systems are more useful for the earlier detection of MTBC than solid media. The culture positivity rate in the broth medium is higher than it is on conventional solid medium and average detection time is approximately 8-12 days.<sup>5,6</sup>

Once growth is detected, the organism can be identified by using specific procedures developed to rapidly identify MTBC. DNA sequencing (i.e. Sanger, pyrosequencing) or line probe assay (LPA) can be performed to identify suspected MDR-TB.

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 manufacturer'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 3,800 x g before inoculation. Specimens that are thick or mucoid should be digested before centrifugation.<sup>5,6</sup>

### 65. 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 MTBC is critical to diagnosing pulmonary TB and to the detection of drug-resistance. Frequent monitoring of broth cultures aids in detecting MTBC promptly.

Advantages of a continuously monitoring broth system:

- Average time from primary inoculation to detection of positive growth is 8-12 days.
- Identification of MTBC can be determined within one day after growth detection.

Disadvantages of a continuously monitoring broth system: Capital investment for instrumentation in laboratories culturing fewer than 20 cultures per week may be impractical

### 66. Does your mycobacteriology laboratory inoculate specimens other than blood to at least one solid medium?

In addition to the inoculation of broth medium, good laboratory practice generally includes 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. However, some laboratories have observed that for their patients and with the conditions of specimen transport and culture used in their laboratory, inoculation of solid media does not improve sensitivity of detection of mycobacteria. For these laboratories, following a thorough validation study, it may be acceptable to discontinue the routine inoculation of solid media, except for the types of specimens which require a specialized medium or incubation at more than one temperature (see below). 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>6</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 °C and the other at 25

to 30°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 7-10 days while Middlebrook media should remain in CO<sub>2</sub> for the length of incubation.<sup>5</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 manufacturers' recommendations or, alternatively, concentrated using a system such as the Isolator lysis-centrifugation tube system prior to inoculation onto solid media.<sup>62</sup>

**67. Does your mycobacteriology laboratory inoculate a negative control with each batch of cultures that are inoculated?**

Inoculating a negative control 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 healthcare provider(s) if questionable results have been reported.<sup>6</sup>

Conversely, positive controls are not recommended. This process has been shown to be a source of cross-contamination.<sup>6</sup>

**68. 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 if the original smear was positive and the cultures are negative at six weeks, further incubation up to eight weeks is indicated.<sup>5,6</sup>

**69. 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 MTBC 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 MTBC using rapid methods.<sup>5,6</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>6</sup>

Stain a smear from the growth on culture plate or Lowenstein-Jensen (LJ) 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 broth or solid medium using small inoculum and note the time required to visible growth:

- Rapidly growing mycobacteria are fully matured within seven days on subculture.
- Slowly growing mycobacteria require more than seven days.<sup>6</sup>

## 70. Does your mycobacteriology laboratory perform an acid-fast smear from:

### 70a. 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. MTBC should not be reported based on smear examination only. If contamination is detected, it may be helpful to 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. But if other specimens from the same patient have yielded pure growth of a mycobacterium, these steps may not be necessary.

Acid-fastness, cording and a slow growth rate in broth are suggestive of MTBC. However, an identification of MTBC from an initial diagnostic specimen must not be reported based on smear examination only.<sup>5</sup> Microscopic morphology may assist, however, in the choice of DNA probes if these are the primary identification method in use.

### 70b. Selected colonies on solid medium at an early stage of growth

When visual examination of the growth on transparent agar or LJ 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>5</sup>

## 71. 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 LJ slant also allows better discrimination of pigment for species identification of nontuberculous mycobacteria.<sup>51,64</sup>

## 72. Does your mycobacteriology laboratory use or ensure the use of a rapid method to confirm or rule out the presence of MTBC from positive cultures?

Once growth is detected the organism can be specifically identified by a molecular method (e.g. DNA probe, (PCR) Restriction enzyme analysis, DNA sequencing), line probes, matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry or High Pressure Liquid Chromatography (HPLC).<sup>65-67</sup> This is especially important in cases where no nucleic acid amplification test (NAA) 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 MTBC.

Laboratories can use DNA probes for rapid and accurate identification of MTBC, *M. gordonae*, *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 MTBC 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 TB and has a unique epidemiology. If the isolate of MTBC shows PZA resistance upon susceptibility testing, the laboratory should perform further testing to determine whether the isolate is *M. bovis* or *M. bovis* BCG. HPLC68, mycobacterial interspersed repetitive units (MIRU) and spacer oligonucleotide typing (spoligotyping) have proven useful in the identification of *M. bovis* and *M. bovis* BCG.<sup>69</sup> Identification of *M. bovis* can also be accomplished by DNA sequencing methods, looking for the *pnca* 169 C→G polymorphism which is characteristic of this species.<sup>70</sup>

If necessary, identification of all species within the MTBC may be desirable and can be accomplished by specialized molecular testing (deletion analysis, line probes).<sup>69</sup> Alternatively, the isolate can be sent to a reference laboratory for identification.<sup>65,66,68,71,72</sup>

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

**73. Does your mycobacteriology laboratory monitor the turnaround time of test results to ensure that 80% of MTBC 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 MTBC 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 MTBC 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 MTBC, management should focus on shortening turnaround time.<sup>73-75</sup>

**74. Does your mycobacteriology laboratory retain positive MTBC cultures for at least one year and other *Mycobacterium* spp. for a minimum of three months?**

It should be standard of practice to submit cultures to the appropriate public health laboratory for genotyping. 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 MTBC 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>5</sup>

**75. 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 MTBC during the digestion/concentration process.

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>5</sup>

**76. 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 LJ medium, approximately 2-5% of sputum specimens are contaminated. For broth media, a slightly higher contamination rate is expected (the acceptable contamination rate for the Mycobacteria Growth Indicator Tube (MGIT) system is 7-8%).<sup>76</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 MTBC during the digestion/concentration process. Standardized guidelines should be followed for changes to the processing protocol.<sup>77</sup>

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. A discussion of centrifugation and digestant toxicity may be found in the *Manual of Clinical Microbiology, 12<sup>th</sup> Edition*.<sup>6</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.

### **77. Does your mycobacteriology laboratory identify a broad range of nontuberculous mycobacteria?**

If your mycobacteriology laboratory has no or limited ability to identify nontuberculous mycobacterial species, utilize reference laboratory resources for providing services to meet patient needs according to risk/disease prevalence.<sup>4</sup> Routine identification of nontuberculous mycobacterial species can also serve as a quality control practice when implemented. Identification of nontuberculous mycobacterial species can aid in both the investigation of MTBC outbreaks and the detection of contaminated MTBC samples.

Clinicians use a combination of clinical and microbiological criteria for the diagnosis of nontuberculous mycobacterial disease.<sup>7</sup>

### **78. If growth on solid medium is suggestive of a possible mixed culture, does your mycobacteriology laboratory subculture colonies with differing morphology for use in species identification?**

Isolation of one or more *Mycobacterium* species is not uncommon and may represent clinically significant disease or environmental contamination. 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>6</sup> Send acid-fast isolates to a reference laboratory for identification if your laboratory cannot identify species of nontuberculous mycobacteria with certainty.

The diagnostic criteria and treatment regimen for nontuberculous mycobacterial infection may be more difficult for a clinician to apply because of underlying disease and concomitant infections.<sup>7</sup>

## **Susceptibility Testing**

### **79. Does your mycobacteriology laboratory perform or ensure susceptibility tests on all initial isolates of MTBC according to CLSI guidelines?**

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>78</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 three months of treatment.<sup>79</sup> A laboratory's ability to maintain proficiency is highly 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 drug susceptibility testing (DST).<sup>80</sup>

CLSI, *M24: Susceptibility Testing of Mycobacteria, Nocardia spp. and Other Aerobic Actinomycetes; Approved Standard, 3rd Edition* describes antimycobacterial susceptibility testing for MTBC providing protocols, related quality control parameters and interpretive criteria, specifically Appendices B and C.<sup>78</sup>

For a summary of information on First and Second-line drugs and their critical concentrations see [Appendix C](#).

### **80. Does your mycobacteriology laboratory ship the initial positive culture (broth or solid media, whichever grows first) to a reference laboratory as soon as MTBC 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 MTBC be isolated and drug susceptibility results be completed and conveyed to the healthcare provider and local and/or state TB Control Program within 28 days from collection. Prompt TAT for laboratory

results is of paramount importance in rapid diagnosis and appropriate treatment of MDR-TB; and especially XDR-TB.<sup>81</sup>

- Growth detection and identification of MTBC 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>81</sup>

Antibiotic susceptibility results should be reported to the healthcare provider within 28 days of specimen collection. In order to meet this TAT, 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.

### **81. Does your mycobacteriology laboratory determine or ensure susceptibility testing of MTBC isolates to primary drugs using a broth-based system?**

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

In the mid-1980's, the first commercially broth-based drug susceptibility system became available.<sup>67</sup> Since then several other commercial broth systems have been developed to detect mycobacterial growth in a fully automated system (stand-alone).<sup>82-84</sup>

### **82. Does your mycobacteriology laboratory use a strain of MTBC that is susceptible to all anti-mycobacterial agents being tested as a control once each week that patient isolates are tested?**

Quality control of MTBC 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>85</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 MIRU provide a rapid and accurate way to identify H37 contamination.<sup>85</sup>

### **83. Does your mycobacteriology laboratory perform or ensure second-line drug susceptibility testing for MTBC when appropriate?**

Because successful treatment of drug-resistant TB depends on susceptibility test results of the MTBC 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 healthcare 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 healthcare 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., levofloxacin) and ethionamide are the minimum second-line drugs to test. Additionally, in accordance with CLSI M24; “Isolates with monoresistance to the INH critical concentration should also be tested for susceptibility to second-line agents if the clinician plans to include a fluoroquinolone in the treatment regimen.”<sup>78</sup>

Please note that when primary drug resistance is found, this finding should be reported and second-line drug susceptibility testing should be initiated immediately, without waiting for repeat testing to confirm the primary drug resistance. Confirmation of primary drug resistance and second-line drug susceptibility testing can be done concurrently. Timely and frequent communication between the laboratory, healthcare provider and TB control is essential.<sup>81</sup>

**84. 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>79</sup> The detection of new resistance should be treated as a critical value and reported as soon as possible.

**85. 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. If your laboratory needs to refer drug susceptibility testing there are two options available in the US. All US PHLs are eligible to send samples to the [Molecular Detection of Drug Resistance Service \(MDDR\)](#)<sup>86</sup> at CDC. Laboratories with low volumes (<50 TB isolates per year) may submit to the [National Public Health Laboratory Drug Susceptibility Reference Center for \*Mycobacterium tuberculosis\*](#) or MDDR.

Confirmation of drug resistance should also include:

- Examine MGIT tube to ensure broth looks like expected MTBC growth, not turbid.
- Perform AFB smear of the drug containing tube which had growth to confirm MTBC
- Examine purity plate to ensure there is no contamination from NTM

However, as described in question 83, detection of drug resistance should be reported immediately, preferably by phone, to the healthcare provider and the TB Control Program, with consultation regarding ongoing confirmation of results. Confirmation of resistance may be expedited if molecular methods are used. Check with your local or state public health laboratory about the availability of molecular detection of drug resistance.

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

- Some strains of MTBC have Minimum Inhibitory Concentrations (MICs) that are close to the critical concentration. Experience has long shown that the reproducibility for testing of these strains can be poor.<sup>87</sup>
- 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:
  - Failure to use a standardized inoculum (well-dispersed suspension)
  - Failure to add a drug to a vial
  - Adding the wrong drug or concentration
  - Failure to recognize a mixed infection, MTBC and a nontuberculous mycobacterium, which is more difficult to detect in broth-based systems
  - 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>81</sup>

**86. Does your mycobacteriology laboratory follow a different algorithm if there is suspicion of drug resistance (any drug resistance, MDR or XDR) as indicated on the request form?**

Because successful treatment of drug-resistant TB depends on the susceptibility test results of the MTBC isolate, rapid molecular tests that identify DNA mutations associated with drug resistance should be requested as soon as drug resistance is suspected or identified to provide more timely guidance for clinical management. The laboratory should have a mechanism whereby the healthcare provider can communicate this suspicion to the laboratory. A request form with the ability to check a box for suspicion of MDR-TB is recommended.

Rapid molecular testing, directly on sediments or as soon as growth is available in culture by assays such as GeneXpert line probe assay (LPA) and DNA sequencing, should be performed before or concurrent to growth-based drug susceptibility

testing (DST). If molecular testing is not available in-house, the laboratory should have a referral algorithm in place. Rapid molecular assays reduce turnaround time for results to hours or days with some results being more accurate than growth-based assay results. Growth-based DST, first-line and second-line, can be performed using agar proportion or commercial broth systems, however, these assays require growth of the organism with results available weeks later.<sup>88</sup>

Since September 2009, CDC has offered [MDDR](#) service to domestic TB control programs and healthcare providers when rifampin resistance and/or MDR-TB is suspected on broth aliquots/isolates<sup>89</sup> or NAA-positive sediments. For more information, visit the [CDC DTBE, Laboratory Information](#) webpage. Fixed tissue specimens are also accepted with prior approval by the CDC Infectious Diseases Pathology Branch for tuberculosis testing. More information on this service can be obtained on the [CDC Infectious Disease Pathology Branch, Requirements for Submitting Diagnostic Specimens/Cases to IDPB](#) webpage.

**87. Does your mycobacteriology laboratory have a policy to transmit a molecular or growth-based drug susceptibility report to the healthcare provider as soon as results are available?**

It is important to report drug susceptibility results to the healthcare 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 healthcare provider. If the patient has drug-resistant TB, the information will be used by the healthcare provider to modify the patient's drug regimen, especially if the patient is not improving on the assigned regimen.<sup>11</sup>

For more information on Critical Values refer to Appendix B.

**88. Does your mycobacteriology laboratory telephone, fax or electronically transmit a drug susceptibility report to the TB 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>59</sup>

**89. Does your mycobacteriology laboratory report at least 69% of initial MTBC drug susceptibility test results for initial diagnostic specimens within 17 calendar days from identification of MTBC from culture?**

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

All laboratories must focus on reducing TAT for drug susceptibility testing to less than 17 calendar days from identification of MTBC testing from culture or refer to a reference laboratory.<sup>11</sup>

Reporting of at least 69% of primary MTBC drug susceptibility test results within 17 calendar days from identification of MTBC from culture takes into account the occurrence of contaminated specimens, mixed with nontuberculous mycobacteria and fastidiously growing strains such as MDR-TB/XDR-TB, *M. bovis* and *M. africanum*.<sup>90</sup>

**90. Does your mycobacteriology laboratory monitor the turnaround time for reporting primary drug susceptibility test results on MTBC isolates to ensure reports are sent within 17 calendar days from identification of MTBC from culture?**

Completing reports on 69% of primary MTBC drug susceptibility test results on initial diagnostic specimens within 17 calendar days from identification of MTBC from culture is reasonable and a longer TAT of more than 30% indicates a need for improvement in identifying system challenges or weaknesses.<sup>56</sup>

The laboratory that recovers the initial MTBC 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 for drug susceptibility testing 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 the date the isolate was received to the date the susceptibility results are received from the reference laboratory.<sup>11,12</sup> Additionally, the reference laboratory should be monitoring that isolates are received in a timely manner and communicate with the referring laboratory if that is not case.

## 91. 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>2</sup> (i.e. CAP, New York State Department of Health, etc.).

The exact wording of the regulation is available in the Code of Federal Regulations: [Title 42, Part 493.801](#), [Title 42, Part 493.803](#) and [Title 42, Part 493.825](#). For additional details you may refer to the accompanying section in the [Interpretive Guidelines for Laboratories](#).

The CDC conducts a voluntary performance evaluation program, the [Model Performance Evaluation Program \(MPEP\)](#), to assess the laboratory's susceptibility testing process for drug-resistant strains of MTBC. Benefits of laboratory participation include the opportunity to conduct an anonymous self-assessment at no charge 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
- Opportunity to test drug-resistant isolates
- 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
- Receipt of reference strains of MTBC 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 MTBC 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 MTBC isolates.

## Direct Detection

### 92. Does your mycobacteriology laboratory perform or ensure access (via a reference laboratory) to nucleic acid amplification (NAA) testing for direct detection of MTBC in [AFB smear-positive initial respiratory diagnostic specimens](#)?

Molecular methods have been developed for the detection of MTBC 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 healthcare providers.

There are two FDA-cleared NAA assays available for AFB smear positive and smear negative sputum specimens. If the first smear-positive specimen is NAA negative, a second specimen should be tested. If the second specimen is also NAA negative the specimen may be AFB smear positive due to a NTM rather than MTBC. NAA should always be performed in conjunction with mycobacterial culture.<sup>24</sup>

It has been shown for one of the FDA cleared NAA assays that one or two specimens tested by NAA is comparable to or has greater sensitivity than three serial AFB smears for the prediction of cultures positive for MTBC.<sup>91,92,93</sup>

**93. Does your mycobacteriology laboratory perform or ensure access (via a reference laboratory) to nucleic acid amplification NAA testing for direct detection of MTBC in AFB smear-negative respiratory specimens from patients at high risk for TB?**

The 2009 guidelines state that “NAA testing should be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB 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.”<sup>24</sup> Culture remains the gold standard for the laboratory confirmation of TB and must be performed in conjunction with NAA testing. Laboratorians should keep in mind that many TB patients are not started on therapy until there is a positive laboratory result indicating the presence of *M. tuberculosis*. For smear-negative patients, NAA testing may lead to appropriate isolation of patients and initiation of timely therapy, which could be significantly delayed if NAA testing is not performed.

There are two FDA-cleared NAA assays available for respiratory specimens. In addition, there are laboratory developed real-time PCR assays being offered. Laboratory developed assays must be validated in accordance with applicable FDA and CLIA regulations.

In 2015, the FDA approved an addition to the Intended Use section of the Xpert MTB/RIF<sup>®</sup> assay package insert. This allowed for use of the result MTB NOT DETECTED, as an alternative to examination of serial acid-fast stained sputum smears, to aid in the decision of whether continued airborne infection isolation (AII) is warranted. This change was based on published data that a negative Xpert MTB/RIF<sup>®</sup> assay result from one or two sputum specimens is highly predictive of the results of two or three negative acid-fast sputum smears.<sup>91-94</sup>

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>24</sup>

**94. Does your mycobacteriology laboratory perform or ensure access (via a reference laboratory) to a MTBC NAA test that includes detection of inhibitors or contains internal controls?**

Specimens, when tested using NAA, may contain inhibitors resulting in false-negative results. Testing for inhibitors or use of internal or specimen processing controls (dependent on direct detection assay) may help detect possible false-negative results and should be considered for specimens that are AFB-smear-positive and NAA test-negative.

Additional interpretative comments appropriate to the type of molecular detection assay may reflect the use of an inhibition, a control, the possibility of high cycle threshold (Ct) values from particular real-time PCR assays reflecting assay interference or insufficient DNA or internal controls specific to the test manufacturer indicating the sample was not properly processed or PCR was inhibited.<sup>95</sup>

Each laboratory should establish the rate of inhibition, especially for laboratory developed tests as well as any FDA-cleared assay to determine if routine testing for inhibitors is necessary. CLSI states that if inhibition is a significant cause of false-negatives, an inhibition control should be included. While significant is not described, a suggestion would be that if >1% of specimens demonstrate inhibition control should be included in the assay. 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>88,96,97</sup>

**95. Does your mycobacteriology laboratory telephone, fax or electronically report result of MTBC NAA testing results within 48 hours of receipt for 75% of specimens tested in the laboratory?**

Rapid detection of MTBC infection plays a key role in TB control as outlined in Healthy People 2020.<sup>98</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 positive results should be considered critical values and communicated immediately to the appropriate healthcare provider, infection control and public health officials.

**96. 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 MDR-TB emerge each year (5.3% of all new and previously treated TB cases).<sup>99</sup> If not adequately treated, patients harboring MDR-TB continue to spread the disease.

Genetic studies have determined that in MTBC, 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. The World Health Organization has endorsed molecular line probe assays and Xpert MTB/RIF<sup>®</sup> for rapid screening of patients at risk for MDR-TB.<sup>100</sup> In the United States, an automated molecular beacon-based test system is FDA-cleared (Xpert MTB/RIF<sup>®</sup>) for detection of mutations associated with resistance to rifampin.<sup>101</sup>

The need for molecular detection of drug resistance, especially to rifampin and isoniazid, either in clinical specimens or in broth aliquots, will increase 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>24</sup> In addition to [MDDR](#), which is available at CDC (see Question 86 above and Reference 82), similar services may be available at state public health laboratories.

At this time, the only FDA approved method is the molecular beacon assay described in Reference 82. Research use only (RUO) or laboratory developed tests are available including DNA sequencing. These assays detect mutations associated with drug resistance and are available at CDC and the Microbial Diseases Laboratory, California Department of Public Health. When suspected rifampin resistance is detected using the molecular beacon assay, confirmation by a sequencing-based method is strongly recommended.

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# Appendix A: Guidelines for Submission of Sputum Specimens for Tuberculosis Testing

(originally published by APHL, April 2018. Available at <https://www.aphl.org/aboutAPHL/publications/Documents/ID-2018Apr-Guidelines-TB-Sputum-Submission.pdf>)

A [key role of the public health laboratory](#) is to provide specialized testing for low incidence, high risk diseases such as tuberculosis (TB). This includes testing of patient samples to identify *Mycobacterium tuberculosis*, perform drug susceptibilities for treatment guidance and identify clusters of active disease transmission through genotyping.<sup>1</sup>

## Background

Public health laboratories throughout the US perform diagnostic and reference services in support of the [National Plan for Elimination of TB](#). The level of service provided and frequency these services are provided vary among states. This guidance may be used by laboratories to assist in the development of institutional policies to ensure proper collection and submission of sputum specimens for TB testing.

In 2015, the Association of Public Health Laboratories (APHL) and the [US Centers for Disease Control and Prevention](#) (CDC) described [models of network collaboration](#) in six states.<sup>2</sup> The article recommended that laboratorians and TB control officials work together to design a system to prioritize testing and maximize resources to obtain prompt, reliable test results. Recommended benchmarks were offered to improve laboratory TB services and TB control. The article discussed strategic planning to help jurisdictions select appropriate resources and testing algorithms to serve their population and public health system. Capacity, capability and cost analysis were factors which must be evaluated for the maintenance and improvement of TB services.

## Sputum Specimen Collection

The diagnosis of TB, management of patients with the disease, and public health TB control services rely on accurate and timely laboratory test results. Laboratory services are an essential component of effective TB control, providing key information to clinicians and public health agencies.

Quality specimens are vital for the laboratory diagnosis of TB. Sputum, a respiratory secretion originating from deep within the lungs, is the most frequent specimen collected for TB testing. Patients should be instructed on the difference between sputum, saliva or nasopharyngeal secretions and the necessity for a deep, productive cough. Sputum specimens are preferably collected under the direction of a trained health care professional. Sputum induction with hypertonic saline may be necessary to obtain quality specimens when a patient is unable to produce sputum spontaneously and bronchoscopy may be considered for patients who are unable to produce sputum at all.

Specimens should be collected in containers that are sterile, clear, plastic and leak-proof such as a 50-ml screw-cap centrifuge tube. Sputum collection devices and wide-mouth sterile collection containers are commercially available. It is recommended that specimens be delivered to the public health laboratory within 24 hours of collection. Samples that cannot immediately be transported to the laboratory should be refrigerated to reduce growth of contaminating endogenous respiratory organisms. More detailed instructions for collection of spontaneous and induced sputum specimens are included in Appendix A.

## TB Testing for Initial Diagnosis

For [initial diagnosis of pulmonary TB](#), collect a series of three sputum specimens 8-24 hours apart, with at least one obtained as an early morning specimen.<sup>3</sup> Optimally, specimens should be collected before drug therapy is started, since a few days of treatment may inhibit growth and prevent isolation of *M. tuberculosis* complex. Certain commercial nucleic acid amplification (NAA) tests cannot be performed if patients have been on anti-tuberculous therapy for three days or more when using the Cepheid Xpert MTB/RIF<sup>®</sup> nucleic acid amplification test (Xpert MTB/RIF<sup>®</sup>) or seven days or more with the [Hologic Amplified MTD Test](#).<sup>4,5</sup> Samples submitted for the initial diagnosis of TB should be tested by both concentrated smear and culture. Reports of AFB smear results should be made to the submitting agency within 24 hours. Cultures should be held for a period of at least six weeks before being reported as negative. It is recommended that NAA testing be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB where a diagnosis of TB is being considered but has not yet been established, and where the test result would alter case management and TB control activities.<sup>6</sup>

## TB Testing for Release from Airborne Isolation Infection (All)

[Several criteria](#) have been used to determine when a patient with suspected TB may be released from hospitalized airborne isolation:

- 1) an alternative diagnosis is established that explains the clinical presentation,
- 2) a multi-drug course of chemotherapy has been administered for a minimum of two weeks and there is clinical evidence of improvement such as a decrease in symptom severity, radiographic findings indicating improvement, or other medical determination of improvement, and
- 3) the patient's sputum or bronchial secretions are free of acid fast bacilli as determined by three consecutive negative smear results.<sup>3</sup>

Specimens to be tested for patient release from hospitalized airborne isolation should be collected at 8-24 hour intervals, with at least one obtained as an early morning specimen. It is not necessary to perform cultures on these specimens unless multi-drug resistant *M. tuberculosis* (MDR-TB) is present.

In February 2015, the [US Food and Drug Administration](#) approved the use of the Xpert MTB/RIF<sup>®</sup> as an alternative to the examination of three initial sputum smears to aid in the decision to discontinue All for patients with a high suspicion of pulmonary TB.<sup>7</sup> However, Xpert MTB/RIF<sup>®</sup> should not be used as an alternative for the three negative smears following two weeks of treatment as it can detect dead and live bacteria. According to this change, negative results using the Xpert MTB/RIF<sup>®</sup> on either one or two sputum specimens may be used in making decisions to discontinue All. The National Tuberculosis Controller's Association (NTCA) and APHL have created a [consensus statement](#) on the use of Xpert MTB/RIF<sup>®</sup> for this purpose.<sup>8</sup> A decision to remove a patient with a negative Xpert MTB/RIF<sup>®</sup> result from All must consider the patient's clinical presentation and the possible risk of transmission of TB from an infectious patient to others. Such a decision should not be based on sputum test results alone. It has been suggested that use of Xpert MTB/RIF<sup>®</sup> could provide cost savings to the health care system by reducing the time a patient is in isolation.<sup>9</sup>

## TB Testing to Monitor the Course of Treatment

For patients whose sputum cultures are positive before treatment, the best method to measure the effectiveness of therapy is to obtain specimens for culture at least monthly until cultures convert to negative.<sup>10</sup> If a sputum culture becomes contaminated with non-acid fast bacteria, laboratories should request submission of a new sputum specimen to avoid gaps in patient monitoring. Patients with MDR-TB should have cultures performed monthly for the entire course of treatment. Patients whose cultures have not become negative or whose symptoms do not resolve despite three months of therapy should be re-evaluated for potential drug-resistant disease, as well as for potential failure to adhere to the treatment regimen. Laboratories should consider consultation with their [TB Centers of Excellence for Training, Education and Medical Consultation](#).

## TB Testing and Follow-up for Drug Resistance

Patients with MDR-TB should have cultures performed monthly for the entire course of treatment. Second-line drug susceptibility testing should be considered for patients who:

- have had prior therapy
- are contacts of patients with drug resistant TB
- have demonstrated resistance to rifampin or to other first-line drugs, or
- have positive cultures after three or more months of treatment.<sup>10</sup>

If drug susceptibility test results show resistance to rifampin or any other first line drugs, or if the patient remains symptomatic or smear/culture positive after three months, a tuberculosis medical expert should be consulted. Consider consultation with the [TB Centers of Excellence for Training, Education and Medical Consultation](#).

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## APPENDIX A: SPUTUM COLLECTION FOR TUBERCULOSIS\*

### Purpose

To obtain sputum specimens for AFB smear microscopy and culture from a patient with suspected pulmonary tuberculosis.

### Materials and Equipment Required

1. Sterile, filtered water or normal saline (150-250 mL)
2. N95 mask (particulate respirator) for AFB
3. Gloves
4. Box of tissues
5. Sterile specimen container approved by the laboratory for sputum collection and transport
6. ALSO, for Nebulized Sputum Induction:
  - a. A hand-held nebulizer with mouthpiece and 15 mL vial of 3% saline. Note: A mask may be used if a patient absolutely cannot use the mouthpiece.
  - b. Patients who are unable to protect their airway or are at risk for aspiration should be NPO for 3 hours prior to the induction procedure to reduce the risk of vomiting and aspiration.

## Procedure

Ensure that the patient is outdoors or placed in an airborne isolation room or negative-pressure sputum collection booth with the door shut. The air in the negative-pressure room or booth should be drawn out of the space and vented outside of the building.

### **PREPARATION (FOR SPONTANEOUS OR INDUCED SPUTUM)**

1. Instruct the patient to gently brush his/her teeth, gingival margins, tongue and buccal surfaces using sterile, filtered water or normal saline to rinse
2. Do not use toothpaste, commercial mouth wash preparations, nose drops, or any medications containing alcohol, or oil. Instruct the patient to avoid taking oral antibiotics immediately before the sputum collection procedure.
3. Instruct the patient to gargle several times with sterile, filtered water or normal saline after brushing. Do not use tap water or bottled water, as it may contain non-tuberculous mycobacteria that may alter findings.

### **SPONTANEOUSLY PRODUCED SPUTUM COLLECTION**

1. Observe standard precautions at all times.  
**Note:** *N95 masks must be worn by healthcare personnel for AFB cough-producing procedures.*
2. Coach the patient and supervise the first sputum collection, at a minimum, in order to obtain a good quality sputum sample that represents secretions from the lower respiratory tract.  
**Note:** *The patient should understand that sputum is material that is brought up from the lungs and that nasal secretions and saliva or spit are not acceptable.*
3. Instruct the patient to inhale deeply, as far as possible, and then exhale slowly three times.
4. After the third breath, direct the patient to inhale completely and try to cough hard to produce sputum from deep in the lungs. The patient may feel a rattle or tickle as the sputum moves up from the lungs into the throat.
5. Instruct the patient to expectorate the sputum into a sterile specimen container.
6. When there is at least 5 mL (1 teaspoon) of sputum, replace the lid on the container and tighten it so it does not leak. Apply Parafilm to the outer edges of the sterile specimen container to further protect the container from leaking during shipment and processing.  
**Note:** *High-quality sputum is required for smear, culture and NAA testing. For AFB NAA testing alone, a minimum of 1 mL of raw sputum (or 0.5 mL of sputum sediment) is needed. It is preferred to collect 5-10 mL of raw sputum.*
7. If the patient is in a negative air pressure room or booth, ask the patient remain in the booth or room until cleared to leave.
8. Label the specimen with time and date of its collection and place it in a specimen bag. Attach a laboratory request form, if applicable.
9. Document the procedure in the appropriate flow sheet or medical record.  
**Note:** *Documentation also is required for unsuccessful procedures.*

### **NEBULIZED SPUTUM INDUCTION AND COLLECTION**

1. Observe standard precautions at all times.  
**Note:** *N95 masks must be worn by healthcare personnel for AFB cough-producing procedures.*
2. Place approximately 5 mL of 3% saline into the hand-held nebulizer. Set the flow at 6-8 L/min and nebulize saline for 7-10 minutes or until sputum is expectorated. The maximum nebulization time is 20 minutes.  
**Note:** *More saline may be added to the nebulizer if more than 10 minutes is needed to produce an adequate cough.*
3. Ask the patient to inhale the nebulized 3% saline deeply 2-3 times followed by a vigorous cough. This will assist in expectorating quality sputum. Collect the sputum into a sterile specimen container.  
**Note:** *Coaching the patient is very important in order to get quality results in a timely manner.*  
**Note:** *High-quality sputum is required for smear, culture and NAA testing. For AFB NAA testing alone, a minimum of 1 mL of raw sputum (or 0.5 mL of sputum sediment) is needed. It is preferred to collect 5-10 mL of raw sputum.*

4. Label the specimen with time and date of its collection and place it in a specimen bag. Attach a laboratory request form, if applicable.
5. Document the procedure in the appropriate flow sheet or medical record.  
**Note:** *Documentation also is required for unsuccessful procedures.*

\*Adapted from [Controlling TB in Correctional Facilities](#). Atlanta, GA, 1995.

## 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 (NAA)	Report within 48 hours of specimen receipt
New MTBC positive culture results from any specimen source	Report identification of MTBC in ≤ 21 days
MTBC first-line drug susceptibility testing results on isolates which demonstrate resistance to any of the first-line drugs.	Report first line drug susceptibility results ≤ 28 days
Detection of mutations associated with drug resistance. Note that probe-based results should be confirmed by sequencing where possible.	Report within 48 hours of specimen receipt.
New mycobacteria (all species) positive culture results from blood, CSF or other normally sterile body sites	Report within 24 hours of positive culture detection
New positive primary cultures where the original AFB smear on the specimen was negative	Report within 24 hours of positive culture detection

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## Appendix C: Anti-tuberculosis Drugs and Their Recommended Critical Concentrations in Middlebrook 7H10,7H11 Agar Medium and MGIT

		Critical Concentrations (µg/ml)		
		7H10 Agar	7H11 Agar	MGIT <sup>d</sup>
Primary Drugs	Isoniazid (INH) Low	0.2	0.2	0.1
	Isoniazid (INH) High	1.0	1.0	0.4
	Rifampin*	1.0	1.0	1.0
	Ethambutol	5.0 10.0	7.5§ NR	5.0
	Pyrazinamide (PZA) <sup>‡</sup>	NR	NR	100
Secondary Drugs <sup>£</sup>	Amikacin <sup>+</sup>	4.0	-	1.0
	Capreomycin	10.0	10.0	2.5
	Ethionamide	5.0	10.0	5.0
	Kanamycin <sup>#</sup>	5.0	6.0	2.5
	Levofloxacin <sup>**</sup>	1.0	-. <sup>‡‡</sup>	1.5
	Moxifloxacin <sup>a</sup>	0.5 and 2.0	0.5	0.25 <sup>f</sup>
	Ofloxacin <sup>b</sup>	See Footnote		
	p-Aminosalicylic Acid (PAS)	2.0	8.0	
	Rifabutin <sup>c</sup>	0.5	0.5	0.5
	Streptomycin Low Streptomycin High <sup>e</sup>	2.0 10.0	2.0 10.0	1.0 4.0

\* 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.)

+ Amikacin and kanamycin are aminoglycosides, but resistance to kanamycin may not indicate resistance to amikacin. It may be desirable to test both aminoglycosides.

# Kanamycin is the class representative for amikacin.

\*\* Testing levofloxacin at 1.0 µg/µl is equivalent to testing ofloxacin at 2.0 µg/µl.

‡‡ Breakpoints for establishing susceptibility have not been determined.

a The number of studies for moxifloxacin susceptibility testing by agar proportion method is limited; further studies are required.

b Ofloxacin is the class representative for the fluoroquinolones. Ofloxacin may still be included in some second-line drug panels (e.g. Sensititre MYCOTB) but is not used frequently in clinical practice and is not commonly used for treatment. Laboratories should consider testing for other Fluoroquinolones such as levofloxacin and/or moxifloxacin.

c 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.

d Woods GL, Lin S-YG, Desmond E. 2019. Susceptibility test methods: Mycobacteria, Nocardia and other actinomycetes. In: Manual of Clinical Microbiology, 12th ed., Carroll KC, et al., eds. Amer. Soc. Microbiol. Washington D.C.

e Higher test concentrations are listed for isoniazid and streptomycin because some clinicians may use these results to guide treatment. The higher concentrations are not considered to be critical concentrations.

f If resistant at 0.25 µg/mL, the clinician may want to consider treating the patient with a higher dose of moxifloxacin. To support this decision, testing at 2.0 µg/ml may be considered. Farhat MR, Mitnick CD, Franke MF, et al. 2014. Concordance of Mycobacterium tuberculosis fluoroquinolone resistance testing: implications for treatment. Int J Tuberc Lung Dis 19(3):339-41.

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