

# **MYCOBACTERIUM TUBERCULOSIS: ASSESSING YOUR LABORATORY**

Developed by  
The Association of State and Territorial Public  
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and  
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**AND**  
U.S. DEPARTMENT OF HEALTH & HUMAN SERVICES  
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# **MYCOBACTERIUM TUBERCULOSIS:**

## **ASSESSING YOUR LABORATORY**

### Preface

The number of people with active tuberculosis in the United States steadily increased from 1985 until 1992. The population groups in the United States that are at increased risk for infection with *M. tuberculosis* include the medically underserved, low-income populations, immigrants from countries with a high prevalence of tuberculosis, and residents of long-term-care facilities. Those at increased risk for developing disease following infection include individuals with human immunodeficiency virus (HIV) infection; close contacts of infectious cases; children less than 5 years old; patients with renal failure, silicosis, and diabetes mellitus; and individuals receiving treatment with immunosuppressive medications. If the diagnosis of tuberculosis is delayed, subsequent steps to confine contagious patients are likewise delayed and nosocomial infections may result.

As multidrug resistant tuberculosis (MDR-TB) increasingly becomes a public health problem, the impact on the medical community can be alarming. Investigations of four MDR-TB outbreaks in hospitals in Florida and New York City demonstrated that most cases of MDR-TB occurred among individuals known to be infected with HIV. The case fatality rate was high (72-89%) and the median interval between diagnosis and death was short (4-16 weeks).

Laboratory methods to promote growth and reduce the turnaround time for reporting test results on mycobacterial specimens are now available. It is the responsibility of the laboratory to respond by implementing these methods. This self-assessment will provide encouragement and information to assist you in this effort. The commitment is yours.

*Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.*

This laboratory self-assessment is part of the National action plan to combat multidrug-resistant tuberculosis (MMWR, 1992; 41 [RR-11]:1-30 ). This document was produced by ASTPHLD through cooperative agreement #U60-CCU303019 with the Centers for Disease Control and Prevention, Public Health Practice Program Office. This document was developed by a contractor based on the contributions, and technical and scientific review of a panel of Mycobacteriology experts convened by ASTPHLD and CDC.

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General Resources

CDC: Division of Tuberculosis Elimination Educational and Training Materials

National Laboratory Training Network

Commercial Resources

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RFLP Reference Laboratories, DNA Fingerprinting for *M. Tuberculosis* Strains

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## INTRODUCTION

After three decades of steady decline, reported cases of TB have increased in the United States by 20 percent, from a low of 22,201 cases in 1985 to a high of 26,673 cases in 1992. In 1993, 25,313 cases were reported, representing a 5% decline in numbers of cases. The increases were due primarily to five factors:

- (1) The deterioration of the public health infrastructure
- (2) Immigration of persons from countries with a high prevalence of TB
- (3) Occurrence of TB in persons infected with the human immunodeficiency virus (HIV)
- (4) Outbreaks and transmission of TB in congregative setting such as hospitals, correctional, and residential care facilities
- (5) Outbreaks of multi-drug resistant TB (MDR-TB)

The control of tuberculosis requires the active support of the entire laboratory community and coordination of the appropriate levels of service for smears, cultures, and drug susceptibility testing. The late diagnosis of TB and delayed recognition of drug resistance have contributed to the dissemination of MDR-TB.

It is imperative that we in the laboratory community prepare now to assist in detecting of tuberculosis early by reducing the laboratory turnaround time for reporting positive smear, culture, identification, and susceptibility results. The laboratory has been challenged to respond in four ways:

- Report results of acid-fast stains within 24 hours of receipt in the laboratory.
- Detect growth of mycobacteria in liquid medium within 10 days of specimen receipt.
- Identify *Mycobacterium tuberculosis* isolates by mycolic acid pattern, the AccuProbe or the BACTEC NAP test within two to three weeks of specimen receipt.
- Determine the susceptibility of new *M. tuberculosis* isolates to primary drugs and report results within three to four weeks of receiving the specimen.

Equipment, space, and airflow must create a safe environment within the laboratory for handling and manipulating infectious materials. An immediate assessment of laboratory design, airflow, and equipment will help you decide if changes are necessary. If your laboratory receives fewer than 20 mycobacteriology specimens per week, you may decide that providing a safe work environment requires a greater investment than your institution can make. Also, ATS recommends a minimum of 20 mycobacteriology specimens per week to remain proficient. It will be important to find a good reference laboratory that can provide the needed service quickly and accurately.

Although speed is important, safety and accuracy must first be considered when implementing a plan of action to reduce laboratory turnaround time. Reports of laboratory cross-contamination underscore the need for laboratories to review and adapt to methods that will minimize the opportunity for false-positive reports.

This self-assessment will use the American Thoracic Society (ATS) recommendation for voluntary classification of laboratories into Level I, II, and III(1). This classification system was devised by the ATS with the cooperation of the Centers Disease Control and Prevention (CDC). A complete description of levels is appended for your review (Appendix 1) and briefly summarized as follows:

### LEVELS OF MYCOBACTERIOLOGY LABORATORY SERVICE (AMERICAN THORACIC SOCIETY)

<u>Level I Laboratory:</u> (CAP/HCFA Level 1&2)	<p>Collect good specimens; ship to Level II or III Laboratory for culture and susceptibility tests.</p> <p>May prepare and examine smears for presumptive diagnosis of tuberculosis or for patient follow-up. Participate in proficiency testing program for acid-fast smears, if applicable.</p> <p><b>(NOTE: This self-assessment instrument only applies to Level I laboratories that prepare and examine AFB smears. This self-assessment is not designed for laboratories, facilities, or clinics that only collect and transport specimens.)</b></p>
<u>Level II Laboratory:</u> (CAP/HCFA Level 2&3)	<p>Perform all procedures performed by Level I Laboratory. Perform microscopic examination. Isolate organisms in pure culture. Identify <i>Mycobacterium tuberculosis</i> complex. Perform susceptibility tests on <i>M. tuberculosis</i> complex*. Refer mycobacterial isolates other than <i>M. tuberculosis</i> to Level III Laboratory for identification and susceptibility testing.</p> <p>Proficiency testing for a Level II Laboratory should include acid-fast smears, isolation of mycobacteria, identification of <i>M. tuberculosis</i> complex, and susceptibility of <i>M. tuberculosis</i> complex.</p>
<u>Level III Laboratory:</u> (CAP/HCFA Level 4&5)	<p>Perform all procedures of Level I and II Laboratories. Identify all mycobacteria. Perform susceptibility tests* on other mycobacteria.</p> <p>Proficiency testing for a Level III Laboratory should include acid-fast smears, isolation of mycobacteria, and identification and susceptibility testing of other mycobacteria.</p>

\* Laboratory personnel should not perform susceptibility tests unless they:

- (a) can identify the organism they are testing and
- (b) perform a sufficient number of susceptibility tests to be aware of the many problems associated with the procedure.



**This assessment will provide you the information, encouragement and opportunity to thoroughly review your procedures, assign priorities, and adopt a plan to update your laboratory practices if needed. Use the questionnaire to identify areas that can make a difference in your mycobacteriology program. Decide what prevents you from creating an environment for change. Whether it be equipment, personnel, or space, no change can occur until you have a plan. Create a mood and a climate for introducing newer methods and newer techniques; then work to make it happen. YOU CAN MAKE THE DIFFERENCE AND YOU CAN EFFECT CHANGE!**



## SCORING THE SELF-EVALUATION QUESTIONS

1. The questions have been rated taking the following criteria into account:
  - Safety
  - Good Laboratory Practices
  - CDC guidelines and initiatives (Appendix 5)
  - A ranking provided by a committee of experts in the field (see Technical Advisory Committee listing in introductory pages)
2. Allow several people in your organization to participate in the self-assessment process. Score sheets may be copied as necessary. Separate scoring sheets have been developed for the Level I (pp. 9-12), Level II (pp. 13-20), and Level III (pp. 21-27) laboratories.
3. Answer questions defined by your level of service, at the level you are working. You may be exceeding the ordinary Level I practice by adding tests ordinarily rated for Level II laboratories.

**(Note: This self-assessment instrument is designed for laboratories that prepare and examine AFB smears, not laboratories or facilities that only collect and transport specimens.)**

Level I laboratories that prepare smears and perform AFB microscopy should only answer questions 1-41.

4. All questions can be answered with a yes or no response.
  - a. If the answer is **yes**, record the maximum point value (in column labeled "**VALUE**") in the "**SCORE**" column.  
**EXAMPLE:** The answer to question #1 is affirmative--record a point value of "3" in the "**SCORE**" column.

QUEST	VALUE	SCORE	COL/ HAND	SAFETY	LAB PRAC	QA/QC	CDC RECOM
1	3	3					

- b. If the answer is **no** record a "0" in the "**SCORE**" column.  
**EXAMPLE:** The answer to question #2 is negative--record a point value of "0" in the "**SCORE**" column.

QUEST	VALUE	SCORE	COL/ HAND	SAFETY	LAB PRAC	QA/QC	CDC RECOM
1	3	3					
2	3	0					



**Example:** Your Laboratory exceeds the basic Level I Laboratory activity by inoculating cultures, but refers growth. This activity will require that you answer all questions on concentration, safety, and culture examination, including QC and QA. Answer those questions, and include the total in your VALUE (denominator) and SCORE (numerator) totals.

9. To determine your percent score, divide the SCORE by the VALUE.

Total of Affirmative responses (SCORE Column)

---

Total of Affirmative and Negative Responses (VALUE Column)

The same method should be used to determine the percentage score in the categories listed in columns 4-8. Use the total values listed on chart for your Level (I-III) of service as the denominator and your total value of affirmative responses as the numerator.

10. Convert the scores to percent by dividing your score in each column by the total number of points possible for that column.

## INTERPRETING YOUR SCORE

1. After the key players in your organization have had an opportunity to score the self-assessment, meet to compare and analyze the results.
2. Objectively evaluate the results. This self-assessment will not be useful unless you act upon identified needs.
3. Reference scores of laboratories that are deemed to be performing at an "excellent" level are given in the appendix: Reference Scores, Appendix 11. Use these scores to assess where your laboratory stands in comparison with other organizations.
4. **The questions marked with an \* and appear in red throughout the self assessment are "key questions." These are considered of such significance that a negative answer to them should be a flag to evaluate whether or not your laboratory should be offering testing for mycobacteria.** If your answer to any one of these 10 questions is "no", you should immediately take steps to implement change, or begin referring your mycobacteriology specimens to a full-service laboratory in your area.
5. Develop a plan of action that includes the newer, rapid methods to facilitate early recognition of M. tuberculosis. Consult with the medical staff, the local or state health department, and the local or state TB control office. The Guidelines have been developed to provide advice as you formulate a plan.
6. Follow the plan--the results can be dramatic (see Appendix 2: "Success Story: New York State Laboratory").
7. We suggest that you repeat the self assessment periodically to track your progress.

**REMEMBER THAT ANY PLAN BEGINS WITH YOU AND YOUR LABORATORY! WITH YOUR ACTION MAYBE WE CAN STILL ELIMINATE TB IN THE UNITED STATES BY THE YEAR 2010!**

# NOTES

**MYCOBACTERIUM TUBERCULOSIS SELF-ASSESSMENT SURVEY  
SCORE SHEET FOR LEVEL I LABORATORIES  
(ANSWER QUESTIONS 1 - 41)**



**Affirmative Response:** Enter question VALUE in SCORE column and in unshaded category column.

**Negative Response:** Enter "0" in SCORE column and in unshaded category column.

QUEST	VALUE	SCORE	COL/ HAND	SAFETY	LAB PRAC	QA/QC	CDC RECOM
1	3						
2	3						
3	3						
4	2						
5	2						
6	2						
7	6						
8	3						
9	6						
10	3						
11	3						
12	2						
13	6						
14	4						
15	3						
16	3						
17	3						
18	4						
19	4						
20	4						
21	8						
*22	9						
Page Scores							



## MYCOBACTERIUM TUBERCULOSIS SELF ASSESSMENT/LEVEL I

QUEST	VALUE	SCORE	COL/ HAND	SAFETY	LAB PRAC	QA/QC	CDC RECOM
Previous page scores							
23	8						
24	3						
25	3						
26	6						
27	6						
28	3						
*29	8						
30	7						
31	5						
32	8						
33	7						
*34	8						
35	3						
36	8						
37	8						
38	6						
39	3						
40	3						
*41	8						
Your Total Scores							
Possible Scores	197		63	59	98	63	123

To determine your percent score, divide your total score by the possible score.

If your laboratory exceeds the basic Level I laboratory activity, but is not a true Level II laboratory, you can correct for this by answering the questions in Level II that apply to your operations and adjusting the possible score (denominator) based on the additional questions answered in the survey.

The same method can be used to determine the percent score in the categories listed in columns 4-8. Use the possible score listed on chart as the denominator and your total score as the numerator.

Percent scores may be compared with the reference scores from "excellent" laboratories located in Appendix 12.

### **Work Space**

$$\text{Your Total Score} \quad \underline{\quad\quad} \div \text{Possible Score} \quad \underline{\quad\quad} \times 100 = \underline{\quad\quad}\%$$

$$\text{Your Col/Hand Score} \quad \underline{\quad\quad} \div \text{Possible Col/Hand Score} \quad \underline{\quad\quad} \times 100 = \underline{\quad\quad}\%$$

$$\text{Your Safety Score} \quad \underline{\quad\quad} \div \text{Possible Safety Score} \quad \underline{\quad\quad} \times 100 = \underline{\quad\quad}\%$$

$$\text{Your Lab Prac Score} \quad \underline{\quad\quad} \div \text{Possible Lab Prac Score} \quad \underline{\quad\quad} \times 100 = \underline{\quad\quad}\%$$

$$\text{Your QC/QA Score} \quad \underline{\quad\quad} \div \text{Possible QC/QA Score} \quad \underline{\quad\quad} \times 100 = \underline{\quad\quad}\%$$

$$\text{Your CDC Recom Score} \quad \underline{\quad\quad} \div \text{Possible CDC Recom Score} \quad \underline{\quad\quad} \times 100 = \underline{\quad\quad}\%$$

# NOTES

**MYCOBACTERIUM TUBERCULOSIS SELF-ASSESSMENT SURVEY  
SCORE SHEET FOR LEVEL II LABORATORIES  
(ANSWER QUESTIONS 1-39; 42-80)**

**Affirmative Response:** Enter question VALUE in SCORE column and in unshaded category column.

**Negative Response:** Enter "0" in SCORE column and in unshaded category column.

QUEST	VALUE	SCORE	COL/ HAND	SAFETY	LAB PRAC	QA/QC	CDC RECOM
1	3						
2	3						
3	3						
4	2						
5	2						
6	2						
7	6						
8	3						
9	6						
10	3						
11	3						
12	2						
13	6						
14	4						
15	3						
16	3						
17	3						
18	4						
19	4						
20	4						
21	8						
*22	9						
Page Scores							

**MYCOBACTERIUM TUBERCULOSIS SELF ASSESSMENT/LEVEL II**

QUEST	VALUE	SCORE	COL/ HAND	SAFETY	LAB PRAC	QA/QC	CDC RECOM
Previous Page Scores							
23	8						
24	3						
25	3						
26	6						
27	6						
28	3						
*29	8						
30	7						
31	5						
32	8						
33	7						
*34	8						
35	3						
36	8						
37	8						
38	6						
39	3						
*42	8						
43	9						
44	4						
45	6						
46	5						
47	5						
48	4						
49	3						
*50	9						
Page Scores							

**MYCOBACTERIUM TUBERCULOSIS SELF ASSESSMENT/LEVEL II**

QUEST	VALUE	SCORE	COL/ HAND	SAFETY	LAB PRAC	QA/QC	CDC RECOM
Previous Page Scores							
51	4						
52	4						
*53	8						
54	7						
55	8						
56	3						
57	7						
58	3						
59	8						
60	6						
61	4						
62	8						
63	8						
*64	8						
65	8						
*66	8						
67	5						
68	2						
69	4						
70	2						
71	5						
72	2						
73	8						
74	8						
75	8						
*76	8						
Page Scores							

QUEST	VALUE	SCORE	COL/ HAND	SAFETY	LAB PRAC	QA/QC	CDC RECOM
Previous Page Scores							
77	8						
78	5						
79	5						
80	3						
Your Total Scores							
Possible Scores	414		60	93	269	115	290

To determine your overall (column 2) percent score, divide your total score (column 3) by the possible score.

The same method can be used to determine the percent score in the categories listed in columns 4-8. Use the possible score listed on chart as the denominator and your total score as the numerator.

Percent scores may be compared with the reference scores from "excellent" laboratories located in Appendix 12.

### Work Space

Your Total Score \_\_\_\_\_ ÷ Possible Score \_\_\_\_\_ x 100 = \_\_\_\_\_%

Your Col/Hand Score \_\_\_\_\_ ÷ Possible Col/Hand Score \_\_\_\_\_ x 100 = \_\_\_\_\_%

Your Safety Score \_\_\_\_\_ ÷ Possible Safety Score \_\_\_\_\_ x 100 = \_\_\_\_\_%

Your Lab Prac Score \_\_\_\_\_ ÷ Possible Lab Prac Score \_\_\_\_\_ x 100 = \_\_\_\_\_%

Your QC/QA Score \_\_\_\_\_ ÷ Possible QC/QA Score \_\_\_\_\_ x 100 = \_\_\_\_\_%

Your CDC Recom Score \_\_\_\_\_ ÷ Possible CDC Recom Score \_\_\_\_\_ x 100 = \_\_\_\_\_%

# NOTES



# NOTES

# NOTES

# NOTES

**MYCOBACTERIUM TUBERCULOSIS SELF-ASSESSMENT SURVEY  
SCORE SHEET FOR LEVEL III LABORATORIES  
(ANSWER QUESTIONS 1-39; 42-84)**

**Affirmative Response:** Enter question VALUE in SCORE column and in unshaded category column.

**Negative Response:** Enter "0" in SCORE column and in unshaded category column.

QUEST	VALUE	SCORE	COL/ HAND	SAFETY	LAB PRAC	QA/QC	CDC RECOM
1	3						
2	3						
3	3						
4	2						
5	2						
6	2						
7	6						
8	3						
9	6						
10	3						
11	3						
12	2						
13	6						
14	4						
15	3						
16	3						
17	3						
18	4						
19	4						
20	4						
21	8						
*22	9						
Page Scores							

**MYCOBACTERIM TUBERCULOSIS SELF ASSESSMENT/LEVEL III**

QUEST	VALUE	SCORE	COL/ HAND	SAFETY	LAB PRAC	QA/QC	CDC RECOM
Previous Page Scores							
23	8						
24	3						
25	3						
26	6						
27	6						
28	3						
*29	8						
30	7						
31	5						
32	8						
33	7						
*34	8						
35	3						
36	8						
37	8						
38	6						
39	3						
*42	8						
43	9						
44	4						
45	6						
46	5						
47	5						
48	4						
49	3						
*50	9						
Page Scores							

\*Signifies **Key** Question

**MYCOBACTERIUM TUBERCULOSIS SELF ASSESSMENT/LEVEL III**

QUEST	VALUE	SCORE	COL/ HAND	SAFETY	LAB PRAC	QA/QC	CDC RECOM
Previous Page Scores							
51	4						
52	4						
*53	8						
54	7						
55	8						
56	3						
57	7						
58	3						
59	8						
60	6						
61	4						
62	8						
63	8						
*64	8						
65	8						
*66	8						
67	5						
68	2						
69	4						
70	2						
71	5						
72	2						
73	8						
74	8						
75	8						
*76	8						
Page Scores							

QUEST	VALUE	SCORE	COL/ HAND	SAFETY	LAB PRAC	QA/QC	CDC RECOM
Previous Page Scores							
77	8						
78	5						
79	5						
80	3						
81	5						
82	4						
83	7						
84	2						
Your Scores							
Possible Scores	432		60	93	285	117	297

To determine your overall (column 2) percent score, divide your total score (column 3) by the possible score.

The same method can be used to determine the percent score in the categories listed in columns 4-8. Use the possible score listed on chart as the denominator and your total score as the numerator.

Percent scores may be compared with the reference scores from "excellent" laboratories located in Appendix 12.

### Work Space

Your Total Score \_\_\_\_\_ ÷ Possible Score \_\_\_\_\_ x 100 = \_\_\_\_\_%

Your Col/Hand Score \_\_\_\_\_ ÷ Possible Col/Hand Score \_\_\_\_\_ x 100 = \_\_\_\_\_%

Your Safety Score \_\_\_\_\_ ÷ Possible Safety Score \_\_\_\_\_ x 100 = \_\_\_\_\_%

Your Lab Prac Score \_\_\_\_\_ ÷ Possible Lab Prac Score \_\_\_\_\_ x 100 = \_\_\_\_\_%

Your QC/QA Score \_\_\_\_\_ ÷ Possible QC/QA Score \_\_\_\_\_ x 100 = \_\_\_\_\_%

Your CDC Recom Score \_\_\_\_\_ ÷ Possible CDC Recom Score \_\_\_\_\_ x 100 = \_\_\_\_\_%

## NOTES





# NOTES

# NOTES

# **MYCOBACTERIUM TUBERCULOSIS: ASSESSING YOUR LABORATORY**

## **PART I: SELF ASSESSMENT QUESTIONS**

**ALL LABORATORIES ANSWER QUESTIONS 1-39**

### **SPECIMEN COLLECTION AND HANDLING**

**Does your Mycobacteriology Laboratory:**

- 1. Provide written instructions that are easily understood by the person collecting the patient specimen?**
- 2. Include instructions to the providers for:**
  - a. The submission form and its use?**
  - b. Specimen labeling?**
  - c. The volume of specimen required?**
  - d. Packaging the specimen for delivery or transport?**
- 3. Obtain the following information when not included on the submission form:**
  - a. Patient's name?**
  - b. Physician's name, address, telephone number?**
  - c. Test(s) to be performed?**
  - d. Date and time of collection?**
  - e. Pertinent specimen information?**
- 4. Provide transport containers and specimen submission forms to health care providers upon request?**
- 5. Supply new, sterile, 50ml plastic conical centrifuge tubes with screw-cap closures for collecting respiratory specimens?**
- 6. Monitor the number of specimens collected per patient (3-6) as part of the quality assurance program?**
- 7. Monitor the delivery time to assure that less than 24 hours have elapsed between specimen collection and its arrival at the laboratory?**
- 8. Communicate regularly with providers to promote understanding and cooperation?**
- 9. Verify that the patient's name and/or identification number on each specimen container matches that on the submission form?**

10. Furnish the health care provider with a copy of the laboratory's:
  - a. Criteria for rejecting specimens?
  - b. Reporting policy?
11. Include the following information on the specimen report form:
  - a. Name and address of your laboratory?
  - b. Date and time the specimen was received in your laboratory?
  - c. Name and address of testing laboratory, if different from "a"?
  - d. Test results?
  - e. Drug susceptibility results, when performed?
12. Record the number of specimens rejected and the reason for rejection as part of the quality assurance program?
13. Report unsatisfactory specimens to the provider within 24 hours of receipt?

## **SAFETY**

Does your Mycobacteriology Laboratory:

14. Follow a written biosafety plan that:
  - a. Defines safe laboratory practice?
  - b. Includes procedures for handling spills and other emergencies?
15. Require employees to review the biosafety plan annually?
16. Follow a written chemical hygiene plan that defines safe laboratory practice?
17. Evaluate the risk associated with the procedures performed in your laboratory?
18. Monitor the Mantoux tuberculin skin test (TST) conversion rate of your personnel as a part of a risk assessment plan?
19. Provide for *new* employees:
  - a. A two step Mantoux tuberculin skin test (TST)?
  - b. A medical evaluation, including a chest radiograph, if TST is positive?
20. Provide for *all* employees:
  - a. An annual Mantoux test on TST negative employees?

- b. A chest radiograph and medical evaluation if the skin test converts to positive or symptoms of tuberculosis are exhibited?
  - c. Medical evaluation, counseling, and follow-up for any known exposure event or TST conversion?
  - d. A permanent record of skin testing results?
21. Provide safety training on aerosol prevention techniques for all employees before assigning work with TB specimens or cultures?
  - \*22. Use a Class I, II, or III biological safety cabinet (BSC) that has been certified annually?
  23. Perform all manipulations on mycobacterial specimens and cultures that may generate aerosols *only* in a BSC?
  24. Provide personal protection equipment that includes laboratory coats or gowns, gloves, and face protection?
  25. Decontaminate all personal protection equipment before it leaves the laboratory area?

## LABORATORY PRACTICE

Does your Mycobacteriology Laboratory:

26. Participate in an approved proficiency testing program?
27. Follow standard operating procedures and maintain the results of quality control for each test procedure for two years?
28. Label all reagents to indicate identity, strength or concentration, storage requirements, preparation and expiration dates?
- \*29. Prepare and examine >10 acid-fast smears per week?
30. Use the fluorochrome stain as the primary acid-fast stain for smears made from the patient's specimen?
31. Check positive and negative reactivity of fluorochrome acid-fast stain each day of use by staining and examining known acid-fast and non-acid-fast organisms?
32. Examine acid-fast smears the day they are stained?
33. Report an approximation of the number of acid-fast organisms viewed on the slide?

\* Key question: A negative response to any key question should cause you to reconsider whether your laboratory should offer TB testing!

- \*34. Telephone, fax, or electronically report all positive acid-fast smear results to the health care provider as soon as results are known, but within 24 hours from specimen receipt?
- 35. Record the date and time positive smear results were telephoned, faxed, or electronically reported to the health care provider?
- 36. Telephone, fax or electronically report all positive acid-fast smear results to the public health department no longer than 24 hours from specimen receipt and follow with a written report?
- 37. Deliver or mail a written microscopy report to the health care provider within 48 hours of specimen receipt in the laboratory?
- 38. Maintain all patient reports and test records for two years?
- 39. Assure confidentiality of all patient information?

**ONLY LEVEL I LABORATORIES ANSWER QUESTIONS 40 & 41**

Does your Mycobacteriology Laboratory:

- 40. Limit access into the laboratory when specimens are being processed?
- \*41. Send all specimens to a full service laboratory for culture within 24 hours of receipt?

**LEVEL II AND III LABORATORIES ANSWER QUESTIONS 1-39; 42-80**

Does your Mycobacteriology Laboratory:

- \*42. Process and culture >20 specimens per week?
- 43. Take steps to eliminate cross-contamination between cultures?
- 44. Routinely process and culture specimens seven days a week?
- 45. Use a refrigerated centrifuge(s) at a relative centrifugal force (RCF) of at least 3000 for 15 minutes to process mycobacterial specimens for culture?
- 46. Use *only* safety carriers equipped with O-ring closures when centrifuging specimens?

\* Key question: A negative response to any key question should cause you to reconsider whether your laboratory should offer TB testing!

47. Prepare, stain, and examine acid-fast smears from *all* specimens sent to the laboratory for mycobacterial culture?
48. Have an isolation room for mycobacteriology that is separate from the rest of the laboratory?
49. Keep laboratory doors closed when mycobacterial specimens are being processed?
- \*50. Have a one-pass (non-recirculating) ventilation system that establishes an airflow pattern, moving from clean (e.g., corridor) to least clean area (e.g., the isolation laboratory)?
51. Monitor the environmental conditions in the isolation room annually to determine the number of air exchanges and the negative pressure status?
52. Control access to the laboratory when working with mycobacterial specimens?
- \*53. Inoculate all digested/decontaminated, concentrated sediments into a selective broth, e.g., the BACTEC System, for the primary culture?
54. Inoculate all specimens not requiring decontamination into a broth, e.g., the BACTEC System, for the primary culture?
55. Inoculate digested/decontaminated, concentrated sediments of mycobacterial specimens to at least one solid medium?
56. Inoculate a negative control each day cultures are inoculated?
57. Perform direct drug susceptibility testing with the primary drugs using the concentrated sediment of smear positive specimens?
58. Inoculate a control with a strain of *M. tuberculosis* susceptible to all antimycobacterial agents being tested, each time a drug susceptibility test is performed?
59. Examine broth cultures for evidence of growth every 2-3 days for weeks 1-3, and weekly thereafter for a total of 6 weeks?
60. Examine cultures on solid media for evidence of growth twice weekly for 1-4 weeks, and weekly thereafter for a total of 8 weeks?
61. Use a microscope or hand lens to examine agar plates and/or tubes for earlier visualization of mycobacterial growth?

\* Key question:           A negative response to any key question should cause you to reconsider whether your laboratory should offer TB testing!

62. Perform an acid-fast smear from:
  - a. BACTEC vials exhibiting growth?
  - b. Selected colonies at an early stage of growth on solid medium?
63. Subculture all BACTEC vials exhibiting acid-fast growth to solid medium?
- \*64. Use a rapid method to presumptively or specifically confirm the presence of *M. tuberculosis* complex:
  - a. From BACTEC vials?
  - b. From growth on solid media?
65. Telephone, fax, or electronically transmit a confirmed report of *M. tuberculosis* complex to provider as soon as the results are available and follow with a written report within 24 hours?
- \*66. Average 14-21 days between receiving specimen and reporting *M. tuberculosis* complex on positive specimens?
67. Monitor the turnaround time of test results to ensure that the majority of *M. tuberculosis* complex are identified within 21 days of specimen receipt?
68. Retain positive mycobacterial cultures for 1 year?
69. Correlate the smear positive and negative results with culture positive and negative results to evaluate the smear/culture quality?
70. Document the percent of specimens producing contaminating growth on culture media inoculated with digested/decontaminated sediment as a way of monitoring the specimen preparation process?
71. Subculture colonies with differing morphology for use in species identification?
72. Compare the prevalence of *M. tuberculosis* complex isolated in your laboratory to the prevalence in your geographic area?
73. Perform susceptibility tests on all initial isolates of *M. tuberculosis*?
74. Determine the susceptibility of *M. tuberculosis* complex to primary drugs using a liquid system such as BACTEC?

\* Key question: A negative response to any key question should cause you to reconsider whether your laboratory should offer TB testing!



75. Telephone, fax, or electronically transmit a drug susceptibility report to provider as soon as results are available and follow with a written report within 24 hours?
- \*76. Average 15-30 days between receiving specimen and reporting primary drug susceptibility test results on *M. tuberculosis* complex?
77. Telephone, fax, or electronically report results of confirmed *M. tuberculosis* complex and resistant strains of *M. tuberculosis* to the Tuberculosis Control Program in the state where the patient resides, as soon as the results are available?
78. Monitor the turnaround time for reporting primary drug susceptibility test results on *M. tuberculosis* isolates to ensure that reports are sent within 30 days of specimen receipt?
79. Monitor the resistance patterns of *M. tuberculosis* and make information available to house staff, local and state Tuberculosis Control Program?
80. Submit outbreak-associated strains of *M. tuberculosis* through the state laboratory to a restriction fragment length polymorphism (RFLP) reference laboratory for fingerprinting?

### LEVEL III LABORATORIES ANSWER QUESTIONS 1-39; 42-84

Does your Mycobacteriology Laboratory:

81. Identify a broad range of *Mycobacterium* species?
82. Perform drug susceptibility studies against other mycobacteria in addition to *M. tuberculosis*?
83. Use one of the following rapid methods to identify mycobacteria:
  - a. DNA probe?
  - b. High performance liquid chromatography (HPLC)?
84. Not report the identification of *M. tuberculosis* based solely on polymerase chain reaction (PCR) results?

\* Key question: A negative response to any key question should cause you to reconsider whether your laboratory should offer TB testing!

# **MYCOBACTERIUM TUBERCULOSIS: ASSESSING YOUR LABORATORY**

## **PART II: GUIDELINES**

### **SPECIMEN COLLECTION AND HANDLING**

Regulations implementing the Clinical Laboratory Improvement Amendments of 1988 (CLIA) specifically addresses the laboratory responsibility in the area of patient test management (19). (Appendix 3, Interpretive Guidelines).

**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 assure optimum patient specimen integrity and positive identification throughout the pretesting, testing, and post-testing processes.

The efficacy of the laboratory smear examination, culture procedures and susceptibility testing from clinical specimens depends upon the collection and transport of quality specimens. A poor specimen collected and transported haphazardly will probably yield useless or misleading results. Specimens should be collected in sterile containers and transported without delay. The laboratory staff can provide information that will promote high quality specimens.

#### **Procedures for Specimen Submission and Handling**

The laboratory must have available and follow written policies and procedures for:

- Education of the patient to properly produce specimens
- Specimen collection
- Specimen labeling
- Specimen preservation, when appropriate (i.e., urine or gastric specimens)
- Specimen processing
- Specimen transport

*The laboratory must follow a written procedure for specimen submission and handling.*

These policies and procedures must assure positive identification and optimal integrity of the specimen from collection to reporting.

**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 not revise results or information directly related to the interpretation of results provided by the testing laboratory.

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

## ALL LABORATORIES ANSWER QUESTIONS 1-39

### 1. Does your Mycobacteriology Laboratory provide written instructions that are easily understood by the person collecting the patient specimen?

The health care professional responsible for collecting mycobacteriology specimens should be informed by the laboratory of the necessity for utmost care in collection and handling the specimens. The results of tests, as they affect patient diagnosis and care, can be directly related to the quality of specimen collected and delivered to the laboratory. It is advisable for the laboratory to develop a working relationship with the health care professionals who collect the specimens for mycobacteriology 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.

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 specimen needed and on ways to minimize contamination of early morning, mid-stream urine specimens.

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.

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

Written laboratory instruction to providers should include the following:

- Collect a series of three to six single, early morning deep cough sputum specimens on consecutive days. If two of the first three sputum smears are positive, three specimens are enough to confirm the diagnosis. If none or only one of the first three sputum smears is positive, then additional (usually three) specimens are needed for culture confirmation of disease. A few patients shed mycobacteria in small numbers and only irregularly; for these patients, the greater the number of specimens cultured, the greater the likelihood of obtaining a positive culture.
- Collect specimens before chemotherapy is started; even a few days of drug therapy may kill or inhibit sufficient numbers of mycobacteria to prevent isolation.
- Multiple specimens collected on the same patient on the same day do not represent separate specimens.

*The accuracy of a laboratory test results can be directly related to the quality of the specimen collected and delivered to the laboratory.*

The efficacy of the laboratory procedure used to culture mycobacteria from clinical specimens depends on the manner in which the specimen is obtained and handled. Therefore, following collection, specimens should be transported as quickly as possible to the laboratory, preferably within 30 minutes. Specimens delayed longer than 30 minutes before transport should be refrigerated.

Other lung secretion specimens include induced sputum, gastric lavage, bronchial washings, and laryngeal exudates. Non-sputum specimens include urine, body fluids, tissue, and wound swabs. Each type specimen requires special handling and transport.

**2. Does your Mycobacteriology Laboratory include instructions to the providers for:**

- a. The submission form and its use?**
- b. Specimen labeling?**
- c. The volume of specimen required?**
- d. Packaging the specimen for delivery or transport?**

**2a.** Instruction to the provider should encourage completing the specimen submission form as a part of the institutional or hospital information system. The provider should be encouraged to separate the specimen from the form during shipment by placing it between the outer and inner container, or in a pocket provided for the specimen form. CLIA regulation 493.1105 requires that the specimen form include the following information:

- Patient name or other unique identifier
- Name and address or suitable identifiers of an authorized person requesting test and, if appropriate, the person using the test results
- Test(s) to be performed
- Date of specimen collection

This information must become a part of the patient's record and be retained for at least two years. Other information that is of value includes:

- Hospital or clinic number
- Social security or Medicaid/Medicare number
- Patient's age or birth date
- Record of antituberculosis drugs
- Specimen number in series of samples on a single individual
- Specimen type
- Time of collection
- Relevant clinical history
- Name of contact person to notify if the results are positive
- If the specimen is received from another laboratory

It is important to seek the assistance of the state Tuberculosis Control Officer and infectious disease physician when designing a new 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.

**2b. Does your Mycobacteriology Laboratory include instructions to provider for specimen labeling?**

The labeling must assure positive identification and optimum integrity of the patient specimen from collection to reporting. Laboratory written policy, in keeping with the above CLIA regulation, can define an acceptable labeling requirement and should be included in provider instructions. Providers should be expected to comply with this reasonable request, and any deviation from the laboratory requirement for proper labeling is reason to reject the specimen.

**2c. Does your Mycobacteriology Laboratory include instructions to the provider for the volume of specimen required?**

Provider instructions should include a request for individual sputum volume of not less than 5ml, nor more than 10ml. This specimen volume provides adequate space in the 50ml conical centrifuge tube for adding the decontaminant, diluent, and subsequently centrifuging without compromising the quality of the specimen. Less than 5ml does not provide the optimal opportunity for recovery of mycobacteria; however, the decision to refuse to process a sample <5ml in volume is controversial. Low quality or inadequate quantity of sputum specimen should be called to the attention of attending medical personnel. The microbiologist should be prepared to process any intact specimen received. The requirement for submitting acceptable volumes of different specimens should be included in the client/physician instructions.

**2d. Does your Mycobacteriology Laboratory include instructions to the provider for packaging the specimen for delivery or transport?**

In-house specimens should be collected in appropriate tubes (50ml plastic screw-capped centrifuge tubes), and 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.

Specimen forms are handled by staff who are not protected by personal protection equipment. It is very important to keep the form separated from the specimen itself, keep it uncontaminated for the safe handling by personnel not wearing protective gear. Any form that may have been contaminated by the specimen should be sterilized by autoclaving.

Federal Postal Regulations must be met when using the postal service to send samples containing etiologic agents. The same safety precautions should apply when samples are being transported by any other third party carriers (Appendix 4). Double transport cartridges suitable for specimen transport by a third party carrier or by the U.S. Postal Service are available. These containers are rugged, reusable, and designed for safety. Proper packaging reduces the number of broken specimens, contains and absorbs leaking specimens, and helps ensure the safety of personnel handling them.

*The transport system must:*

- *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.*

**3. Does your Mycobacteriology Laboratory obtain the following information when not included on the submission form:**

- a. Patient's name?**
- b. Physician's name, address, telephone number?**
- c. Test(s) to be performed?**
- d. Date of collection?**
- e. Pertinent specimen information?**

*CLIA regulations require that certain information is included on specimen form:*  
*a. Patient's name.*  
*b. Physician's name (or other authorized person's), address, telephone number.*  
*c. Test to be performed.*  
*d. Date of collection.*  
*3. Pertinent specimen information.*

The information listed is required by CLIA. This information must be present or must be solicited when not included. Obtain information that is essential to the provision of accurate results before reporting. 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 may be more extensive, and with good reason. However, if you are requesting information that is *not* necessary or is not usually supplied, it may be time to review your submission form. As a way to monitor the relevance and necessity of requested information, compare the information sought and the information received on a sample number of specimen forms. Documentation provides a record of the monitoring activity.

Scheduled quality assurance documentation should include an ongoing means to assess the quality of the information received by the laboratory. An example of an indicator plan would be to "review 10% of the specimen forms every other week for completeness." You could develop a simple form to record the observations and record ongoing results in the QA manual. With an indication of poor compliance from any one or several providers, you could initiate a plan of action.

If your monitoring system indicates a non-compliance problem, examine your specimen form to determine the value of the information requested:

- Is this information necessary?
- How is the information used?
- What would be the consequences of deletion?
- Can the information requested reasonably be supplied?
- What information is needed by the TB Control Program?

After evaluating each field of the specimen information form, it may be possible to reduce or eliminate the request for information of little consequence to the laboratory or the medical staff. This action will be recognized by the clinic or hospital as an effort by the laboratory to reduce unnecessary record keeping. It also helps the laboratory by reducing the number of fields that must be recorded.

CLIA does not detail specific QA monitors nor the remedial action. Any plan adopted by your organization, however, should be appropriate to define and resolve patient test management problems.

**4. Does your Mycobacteriology Laboratory provide transport containers and specimen submission forms to health care providers upon request?**

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

**5. Does your Mycobacteriology Laboratory supply new, sterile, 50ml plastic conical centrifuge tubes with screw-cap closures for collecting respiratory specimens?**

The vial or tube for collecting a mycobacteriology specimen must be sterile and leakproof. Preferably all respiratory specimens are collected in 50ml centrifuge tubes for ease of handling, consistency, and safety. These tubes are designed with screw caps for watertight closure. Urine cups are not suitable for collecting TB specimens.

50ml plastic, disposable centrifuge tubes are recommended; they are optimal for collection and processing specimens for these reasons:

- Eliminate an opportunity for mislabeling.
- Allow space for adequate mixing of the specimen and the digesting agent.
- Allow the specimen to be diluted in the specimen container.
- Can be centrifuged without transfer or aerosol formation.

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

**6. Does your Mycobacteriology Laboratory monitor the number of specimens collected per patient (3-6) as part of the quality assurance program?**

Monitoring the number and succession of specimens 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. The number of bacilli in a specimen varies from patient to patient and from day to day. Since guidelines recommend the collection of three to six specimens per patient, 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.

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.

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

The challenge of this assessment tool is the reduction of laboratory turnaround time for reporting mycobacteriology specimens. To assure that specimens are processed quickly and accurately, they must be received in a timely manner. The CLIA Quality Assurance (QA) guidelines require "accurate, reliable, and prompt reporting of test results." Patient treatment can be significantly enhanced by a timely acid-fast smear report.

*TB specimens should be delivered to the laboratory as soon as possible, but no longer than 24 hours after collection.*

Specimens should be delivered to the laboratory as soon as possible after collection so the smear and culture process can begin while the specimen is fresh. Holding specimens while waiting for more specimens to accumulate 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 of these times by sample for a measured number or percent of specimens will provide documentation. When necessary, this documentation can be used to work with provider/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 (41)." (See Appendix 5, *The Resurgence of Tuberculosis: Is Your Laboratory Ready?*)** A change in the transport system can only be effected through close cooperation with the provider and a commitment from the laboratory management team.

**8. Does your Mycobacteriology Laboratory communicate regularly with provider to promote understanding and cooperation?**

Personal communication with providers underscores the importance of an acceptable specimen and helps to educate the health care professional about the laboratory specimen requirements. Communication enhances the health care team approach and reinforces the value of each individual's contribution to total patient care.

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

Your laboratory must have available and follow a written policy for proper labeling of specimens. Specimens that are being processed, transferred and/or subcultured must be labeled appropriately to assure the integrity of the specimens.

*Specimens that are being processed, transferred, or subcultured must be labeled appropriately to assure the integrity of the specimens.*

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



**10. Does your Mycobacteriology Laboratory furnish the healthcare provider with a copy of the laboratory's:**

- a. Criteria for rejecting specimens?
- b. Reporting policy?

**10a.** The provider must be given a copy of the laboratory's criteria for rejection of specimens. The laboratory must report any information regarding the condition and disposition of specimens that do not meet the laboratory's criteria for acceptability. Report unsatisfactory specimens to the provider as quickly as practical, but no later than 24 hours after receipt.

*The laboratory must report any information regarding the condition and disposition of specimens that do not meet the laboratory's criteria for acceptability.*

Better cooperation will be obtained if the provider understands the laboratory's rejection policy. Below are listed some usual reasons for specimen rejection:

- a. Specimen not labeled.
- b. Name on specimen and requisition form do not match.
- c. Specimen leaking.
- d. Insufficient quantity (urine, sputum, bronchial washing).
- e. Specimen in nonsterile container.
- f. Gastric specimen more than two hours old or not neutralized. [1.5 ml of 40% disodium phosphate ( $\text{Na}_2\text{HPO}_4$ ) dehydrated in collection vial will neutralize 45ml of specimen with pH equal to 1/10N HCl].
- g. Tissue specimen more than one hour old or not refrigerated

**10b. Does your Mycobacteriology Laboratory furnish the healthcare provider with a copy of the laboratory's reporting policy?**

CLIA regulations require that an adequate system is in place to report test results in a timely, accurate, and confidential manner. Unsatisfactory specimen results must be recorded on the report, along with the reason for rejection. The laboratory must develop and follow a written procedure for reporting life-threatening or "panic" laboratory results.

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

*Report acid-fast microscopy results within 24 hours of specimen receipt in the laboratory.*

The following format for reporting positive acid-fast microscopy and TB culture results is recommended:

- a. Report positive microscopy results within 24 hours of specimen receipt.
- b. Report results confirming the identification of *M tuberculosis* complex as soon as available, but within 14-21 days of specimen receipt.
- c. Report drug susceptibility results as soon as available, but within 30 days of specimen receipt.
- d. Report all final positive or negative test results in writing, including a repeat of all preliminary reports.

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 clinician should communicate to establish a protocol that secures confidentiality under all circumstances.

**11. Does your Mycobacteriology Laboratory include the following information on the specimen report form:**

- a. **Name and address of your laboratory?**
- b. **Date and time the specimen was received in your laboratory?**
- c. **Name and address of testing laboratory, if different from "a"?**
- d. **Test results?**
- e. **Drug susceptibility results, when performed?**

These data elements are the minimal information required by CLIA regulations. Your laboratory, through consultation with the medical staff and TB Control Division of the state, should decide additional relevant information to be included on your report form.

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

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

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

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

Because some laboratories, or even some individuals within a laboratory, adopt a policy of leniency toward specimen acceptability, the number rejected may represent only the worst specimens, or one or two marginal specimens may be accepted for every one rejected. Monitor unsatisfactory specimens by recording individual specimens rejected over time, e.g., for 1 month. Record the number, the source, and the reason for rejection. Repeat the surveillance quarterly, notifying providers with a compliance problem of ways they can improve specimen quality.

*Report form should include:*

- name and address of your laboratory.
- date and time the specimen was received in your laboratory.
- name and address of testing laboratory if different than "a."
- test results.
- drug susceptibility results.

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

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 transmission of TB if the patient has active disease. 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. If it is impossible to call, it is very important to mail the report of an unsatisfactory specimen the day that the specimen is received.

## SAFETY

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:

- Pouring liquid culture or supernatant fluids.
- Using fixed volume automatic pipettors.
- 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.
- Centrifuging without safety carriers.
- Sonicating.

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

**14. Does your Mycobacteriology Laboratory follow a written biosafety plan that:**

- a. Defines safe laboratory practices?**
- b. Includes procedures for handling spills and other emergencies?**

**14a.** Federal "Bloodborne Pathogens" Regulations (17) require that a biosafety manual be prepared or adopted by every laboratory working with infectious material. Personnel must be advised of special hazards and are required to read and to follow instructions on practices and procedures. This plan, along with an explanation of its contents, must be available to employees.

**14b. 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. Since the immediate response will have an impact on the final outcome of the incident, there needs to be rehearsal by supervisor(s) so employees will react in an effective manner.

Any spill and/or accident which results in exposure of an employee to infectious material must immediately be reported to the laboratory director. Appropriate medical evaluation, surveillance, and treatment must be made available, and a written record maintained in the employee's personnel file, the laboratory safety records, and/or the director's safety records.

*The written laboratory safety plan must include instructions on how to handle a spill or a laboratory emergency.*

If your biosafety plan does not have detailed information on how to handle a laboratory emergency, information may be found in the *CDC LAB MANUAL, Isolation and Identification of Mycobacterium tuberculosis: A Guide for the Level II Laboratory*, pages 140-142 (39).

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

*Laboratory personnel must receive annual training on handling infectious material.*

Federal regulations regarding occupational exposure to infectious material require a written biosafety plan which includes the safe handling of infectious agents. Federal regulations as they apply to medical laboratories defer to the *CDC/NIH Biosafety in Microbiological and Biomedical Laboratories* manual (12). Laboratory personnel must receive appropriate training in:

- a. The potential hazards associated with the work.
- b. The necessary precautions to prevent exposures.
- c. The exposure evaluation procedures.

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

**16. 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 (18). Employees must be informed of the chemical hazards in the workplace and be prepared for emergency action if exposure occurs.

**17. Does your Mycobacteriology Laboratory evaluate the risk associated with the procedures performed in your laboratory?**

*Evaluation of the risk associated with each procedure is the basis of the laboratory infection control plan.*

TB control measures for each laboratory should be based on an assessment of job risk within the area of work. Since mycobacteriology laboratory workers may be at increased risk of becoming infected with *M. tuberculosis*, they should be evaluated periodically. A risk assessment program follows a protocol of evaluation based on documented tuberculin skin test (TST) conversions in the laboratory.

The risk assessment should be conducted by a group that may include laboratorians, microbiologists, hospital epidemiologists, infectious disease specialists, or pulmonary disease specialists. Further information on developing a risk assessment plan can be found in the Federal Register, Vol.59, No.208, October 28, 1994, *Guidelines for Preventing the Transmission of Tuberculosis in Health Care Facilities; Notices* (15).

Using special engineering controls and personal protection equipment (PPE), the laboratory staff may become comfortable in their own environment and develop an artificial sense of security. 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.

Additional measures may be necessary in laboratories that process large numbers of specimens that contain *M. tuberculosis* and thus, have a greater potential for exposing workers to TB. The frequency of tuberculin skin testing (TST) of laboratory workers using purified protein derivative (PPD) should be based on the level of risk within the immediate work group.

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

**18. Does your Mycobacteriology Laboratory monitor the Mantoux tuberculin skin test (TST) conversion rate of personnel as a part of a risk assessment plan?**

If insufficient data for determining risk have been collected on TST conversions using purified protein derivative (PPD) among laboratory staff, these data should be compiled, analyzed, and reviewed expeditiously. Until such data are analyzed and found to warrant a lesser risk rating, laboratory workers should be considered high risk (15).

*If insufficient data on tuberculin skin test (TST) conversions among laboratory workers is available, such data should be compiled, analyzed and reviewed expeditiously.*

**19. Does your Mycobacteriology Laboratory provide for new employees:**

- a. A two-step Mantoux tuberculin skin test (TST)?
- b. A medical evaluation, including a chest radiograph, if TST is positive?

**19a.** All new employees should be evaluated at the time of employment to establish a baseline for future tuberculin skin testing. Use a two-step method to detect the boosting phenomenon that might be misinterpreted as skin test conversions. Those with a history of vaccination with Bacillus of Calmette and Guerin (BCG) should also be skin tested (15).

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 should be exempt from TST screening. Employees with a positive initial skin test should be referred for chest radiograph and evaluation for possible isoniazid (INH) prophylaxis.

**19b.** Does your Mycobacteriology Laboratory provide a medical evaluation, including chest radiograph, if the tuberculin skin test is positive?

Laboratory workers with positive TST(s) 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.

**20. Does your Mycobacteriology Laboratory provide for all employees:**

- a. An annual Mantoux skin test on tuberculin negative employees?
- b. A chest radiograph and medical evaluation if the tuberculin skin test converts to positive or symptoms of tuberculosis are exhibited?

- c. Medical evaluation, follow-up, and counseling for any known exposure event or PPD conversion?
- d. A permanent record of skin testing results?

**20a.** For laboratories with careful documentation of tuberculin skin tests for a 3-5 year period with no conversions, annual skin testing for employees is appropriate. In a laboratory where transmission of tuberculosis has recently occurred, tuberculin testing should be repeated every three months until no additional conversions have been detected for two consecutive three-month intervals (15).

**20b. Does your Mycobacteriology Laboratory provide a chest radiograph and medical evaluation if the skin test converts to positive or symptoms of tuberculosis are exhibited?**

If chest radiograph suggests tuberculosis, immediate investigation and follow-up should be initiated, including medical evaluation and treatment. Tuberculin skin tests on other employees should also be initiated. Employees with symptoms of tuberculosis should be examined immediately by the medical staff.

**20c. Does your Mycobacteriology Laboratory make medical evaluation, counseling, and follow-up available for any known exposure event or TST conversion?**

All laboratory workers with newly recognized positive tuberculin conversions should be promptly evaluated for clinically active TB, to include a chest radiograph and clinical evaluation. Those without clinical TB should be evaluated for possible INH prophylaxis according to published guidelines.

*All laboratory workers with newly recognized TST conversions should be promptly evaluated for clinically active TB.*

If an employee becomes tuberculin positive, a history of possible exposure should be obtained in an attempt to determine the potential source of exposure. When the source of exposure is known, the drug susceptibility pattern of the particular strain *M. tuberculosis* should be determined to implement appropriate preventive therapy for the worker.

Review laboratory activities and practices for possible errors in technique. Test all equipment for safe operation and review safety procedures with all employees.

**20d. Does your Mycobacteriology Laboratory maintain a permanent record of skin testing results?**

Results of tuberculin skin tests should be confidentially recorded both in the individual employee health records and in a retrievable aggregate database of all workers' TST 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.

*An aggregate record of employees' TST results should be confidentially maintained.*

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

To minimize the risk of infection of self and others, personnel should be selected with care. Employees should receive instructions on how to handle a laboratory accident, and be instructed to report any real or suspected break in procedure to the supervisor.

All employees with occupational exposure to infectious agents must participate in a training program. The training must be provided during working hours at no cost to the employee. The training must be provided at the time of initial assignment to tasks where occupational exposure may take place.

As the best protection against becoming infected, severely immunosuppressed employees should avoid exposure to *M. tuberculosis*. Employees with severely impaired cell-mediated immunity (due to HIV infection or other causes) who may be exposed to *M. tuberculosis* should consider a change in job setting. For further information, see *Federal Register, October 28, 1994, Section II-I, "Education and Training of Health Care Workers (15)."*

*Personnel working in the TB laboratory should be selected with care and properly trained.*

**\*22. Does your Mycobacteriology Laboratory use a Class I, II, or III biological safety cabinet (BSC) that has been certified 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 equipment item necessary for reducing the possibility of laboratory acquired infections. If a BSC is not available or not working properly, all mycobacteriology smear preparation and culture processing must be referred to a Level II or III laboratory for examination. The Occupational Safety and Health Act (OSHA) Standard (28), Section A, details the employer's responsibility for protecting the employee from harm. (See Appendix 6 for a list of the regional OSHA offices.).

*A well-maintained certified biological safety cabinet is a pre-requisite for handling specimens on patients suspected of having tuberculosis.*

If you have a BSC, but are not sure how effectively it is functioning, check to see if it has been certified within the past year. If not, have it certified.. See Resource List (Appendix 6) for the number to call for more information. Also, see Appendix 7(27) for assistance in selecting, maintaining and certifying a BSC.

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



COMPARISON OF BIOLOGICAL SAFETY CABINETS (12)

Cabinets

Application

Type	Face velocity (lfpm)	Airflow Pattern	Radionuclides/ Toxic Chemicals	Biosafety	Product Protection
Class I*, open front	75	In at front; out rear and top through HEPA filter	NO	2,3	NO
Class II:					
Type A	75	70% recirculated through HEPA; exhaust through HEPA	NO	2,3	YES
Type B1	100	30% recirculated through HEPA; exhaust via HEPA and hard-ducted	YES (Low levels/volatility)	2,3	YES
Type B2	100	No recirculation; total exhaust via HEPA and hard-ducted	YES	2,3	YES
Type B3	100	Same as IIA, but plena under negative pressure to room and exhaust air is ducted	YES	2,3	YES
Class III	NA	Supply air inlets and exhaust through 2 HEPA filters	YES	3,4	YES

\*glove panels may be added and will increase face velocity to 150 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, 3rd Edition, HAS Publication No. (CDC) 9308395, May, 1993. U.S. Department of Health and Human Services, Centers For Disease Control and Prevention.

### **Class I Biological Safety Cabinet:**

A ventilated cabinet for personnel and environmental protection with an unrecirculated inward airflow away from the operator. Suitable for working with *M. tuberculosis* (Biosafety Level 2/3, depending on whether pure cultures are transferred or manipulated.) This BSC can be used when no product protection is required.

### **Class II Biological Safety Cabinet:**

A ventilated cabinet for personnel, product, and environmental protection having an open front and inward airflow for personnel protection, downward HEPA-filtered laminar airflow for product protection and HEPA filtered exhausted airflow for environmental protection. This BSC is suitable for working with *M. tuberculosis* (Biosafety Level 3). Several types of Class II BSCs are available, including types A, B1, B2 and B3. The difference in types depends on the filtered airflow and the recirculation of air. All types are applicable for use with biohazards, including *M. tuberculosis*; B2 is designed for "total (100%) exhaust" and is not required for the mycobacteriology laboratory.

A BSC should not be installed unless it is vented into a nonrecirculating exhaust system or directly to the outside. There is documentation that laboratorians have been infected with *M. tuberculosis* circulating in faulty exhaust systems.

**All Microbiology Laboratories providing service at the ATS Level II or Level III must provide Biosafety Level 3 protection for the laboratory workers (12).**

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

Studies have shown that the risk of *M. tuberculosis* infection is three to five times greater among workers in the mycobacteriology laboratory than other laboratory workers. The production of aerosols containing tubercle bacilli can pose an unrecognized hazard to workers who are not trained in safety practices. The most dangerous aerosols are those that produce droplet nuclei, particles of less than 5 $\mu$ m in size. These droplet nuclei remain suspended almost indefinitely in air unless they are removed by controlled airflow or ventilation. If droplet nuclei are not contained or eliminated, they are capable of entering a pulmonary alveolus and establishing the primary site of infection (24).

*All procedures that create aerosols should be performed only in a Biological Safety Cabinet.*

All procedures that create aerosols should be performed only in a fully functioning BSC in an isolation room. Examples are: processing clinical specimens, preparing slides, inoculating plates or tubes, performing *in-vitro* tests, pouring or pipetting cultures or supernatant fluids, mixing or diluting fluid cultures or concentrates. All workers are responsible for the safety of themselves and others with whom they work.

The BSC must be located in an area with limited or controlled access and with little movement of air around the BSC while it is in operation. The BSC should be enclosed in a room where access is controlled while culture work is performed. Air currents generated by the opening and closing of doors or by workers moving around can disturb the airflow of the cabinet.

The BSC should have the capacity to draw 75 to 100 linear feet of air per minute (lfpm) across the entire front opening. The magnehelic gauge on the front of the cabinet will indicate if it is delivering the appropriate amount of air. A strip of tissue paper taped to the front of the BSC may be used to indicate if the direction of air movement is correct. The space inside the BSC should be kept clean and free of racks and stored material that may limit or distort the airflow within the cabinet.

If the airflow is less than 75 lfpm, as detected by the magnehelic gauge on the front of the BSC, or by an air meter, the HEPA filters may be clogged and need replacement. The BSC should be decontaminated with paraformaldehyde (Appendix 8) or this service can be contractually purchased before filters are replaced.

**Ultraviolet (UV) Lights:** UV lights afford a minimal protection to laboratory workers. While it has been proven that UV lights are moderately effective for decontaminating work surfaces and killing airborne microorganisms, they should only be turned on in the BSC after work is completed. The UV light has little penetrating power and is easily blocked by dust, grease, or organic material. To remain effective the intensity of UV lights should be checked every three months, and the bulbs dusted or cleaned regularly with alcohol-soaked gauze. Replace the bulbs when the initial output is decreased to 70% of the initial reading. Do not work in the BSC with UV lights on or look directly at the burning bulbs. Direct or reflected UV lights can cause severe burns to the eyes and other exposed parts of the body.

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

Protective laboratory coats, gowns, smocks, or uniforms designed for laboratory use must be worn while in the laboratory. The gowns or laboratory coats preferably have back closures and fitted sleeve cuffs that fit snugly over the wrist. Personal protection equipment (PPE) is removed before leaving the laboratory for non-laboratory areas (e.g., cafeteria, library, administrative offices.) Gowns or coats, face protectors, gloves and other PPE worn in the dedicated culture room must be removed and disposed of appropriately before assuming duties in the laboratory at large. 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.

Face protection (goggles, masks, faceshield or other splatter guards) is used when splashing or sprays of infectious or other hazardous materials to the face are possible. The "dust/mist" molded face mask with adjustable metal clip across the bridge of the nose has been widely used for respiratory protection short of wearing

a HEPA filtered respirator. This face mask is designed to filter 90% of particles in the  $>0.5\mu\text{m}$  range. However, not all molded face masks are equivalent, so check the specifications to see that the ones you are using are effective.

*Face protection gives an additional measure of safety when splash or sprays of infectious materials are possible.*

If there are any questions about the quality of the air provided by the present engineering controls in your mycobacterial culture area, it is better to be safe and use the protection provided by a HEPA filtered respirator(s). Take immediate steps to define the status of the engineering controls for worker safety in your laboratory, if not known. Engineering controls include the following:

- Separate room or suite specifically designed for mycobacterial culture
- One-pass (nonrecirculating) ventilation system
- Annually certified Class I, II, or III biological safety cabinet
- Exhaust air from BSC discharged through HEPA filters or recirculated only after passing through filters certified to remove 99.97% of particulates  $0.3\mu\text{m}$  or larger
- Negative pressure in culture area and a means to monitor (Smoke tubes or differential pressure-sensing devices can be used to monitor negative pressure.)
- Proper airflow pattern (Directional airflow should be from clean to least clean area.)
- Appropriate number of air exchanges per hour (Six to twelve room air exchanges per hour provide removal of 99% of airborne particulates within 30 - 45 minutes.)

Selection of the maximum respiratory protection for the task is important, but equally important is *using* the selected device correctly. Both supervisors and health care workers should be trained in the selection, proper use, and maintenance of respiratory protection appropriate for personal use against airborne tubercle bacilli.

Currently, there is much discussion among safety experts over the type and kind of respiratory protection required for culturing mycobacteria in a properly controlled environment. Guidelines published in the Federal Register October 28, 1994, Section II M-c., *Guidelines for Preventing the Transmission of Tuberculosis in Healthcare Facilities* (15) defer considerations for laboratories processing specimens for mycobacterial studies (e.g., AFB smears and cultures) to criteria specified by CDC and NIH. **(A single copy of the 3rd Edition of the CDC/NIH Biosafety Guidelines (12) is available from CDC upon request. See resource list in Appendix 6.)**

## **SPECIAL CONSIDERATIONS:**

When performing high risk procedures such as cleaning liquid spills, NIOSH Type A ( $\geq 99.97\%$  efficiency) powered air purification respirators (PAPR) with HEPA filters are the most effective protection. Persons responsible for cleaning up spills or handling other emergencies with aerosol exposure risk must be trained and fit-tested for respirator use.

In certain clinic and hospital settings, the laboratorian may be present or involved in the collection of expectorated or induced sputum specimens. Employees who have been adequately trained and fit tested with National Institute of Safety and Health (NIOSH) Type C ( $\geq 95\%$  efficiency) respirators will be protected from TB infection. Strict management procedures and reliable engineering controls are needed to reduce the risk of exposure (15).

**25. Does your Mycobacteriology Laboratory decontaminate all personal protection equipment before it leaves the 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 washed for reuse. CDC approved methods for decontaminating disposables include autoclaving, chemical disinfection, and incineration.

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.

## LABORATORY PRACTICE

With the exception of aseptically collected specimens, most clinical mycobacteriology specimens are contaminated to varying degrees by more rapidly growing organisms. These specimens must be subjected to harsh decontamination and digestion procedures that liquefy organic debris and eliminate unwanted normal flora organisms. The success of these procedures depends on:

- The resistance of the mycobacteria to strongly digesting solutions
- The length of time the mycobacteria contact the digesting-decontaminating agent
- The temperature buildup in the specimen during centrifugation
- The efficiency of the centrifuge to sediment the mycobacteria

All operating procedures should be designed for optimum isolation of acid-fast organisms. You must develop your own standard operating procedures based on the standards recommended by CDC and other expert mycobacteriologists. Also, update as necessary.

The proficiency and the dedication of staff working in the laboratory makes all of the difference in the success of the mycobacteriology program. One laboratory (Appendix 2) reduced the average turnaround time of its TB specimens processing from 40 days to 10-22 days in sixteen months (2). They credit the following factors as making the difference:

- Having a determined and dedicated laboratory supervisor and staff
- Working with the mail delivery service to arrange for timely receipt of specimens in the laboratory
- Reporting testing results via telephone and fax, followed by a written report
- Starting drug susceptibility testing immediately (usually daily) after isolation/identification procedures
- BACTEC read 7 days a week; NO batching

### **Study your program. You, too, can report a SUCCESS STORY!**

#### **26. 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. 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 which is appropriate for the level of testing provided for patient specimens.

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

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

*Written standard operating procedures must be readily available and followed by all technical personnel.*

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

The dates of initial use and discontinuance of each procedure must be documented. A copy of a discontinued procedure must be retained for two years after the date of discontinuance. The laboratory must follow a written quality control protocol to monitor and evaluate the quality of the analytical testing process. All quality control readings must be documented and the records maintained for a minimum of two years.

**28. Does your Mycobacteriology Laboratory label all reagents to indicate identity, strength or concentration, storage requirements, preparation and expiration dates?**

*Label all reagents and chemicals before storage.*

Reagents, solutions, culture media, control materials, calibration materials and other supplies must be labeled to indicate:

- a. Identity
- b. Titer, strength, or concentration, when significant
- c. Recommended storage conditions
- d. Preparation and expiration dates
- e. Date received and date opened for purchased reagents

**\*29. Does your Mycobacteriology Laboratory prepare and examine >10 acid-fast smears per week?**

*Laboratories processing fewer than 10 smears should consider sending specimens to a reference laboratory.*

It is recognized by the ATS and CDC that to maintain proficiency in smear examination (acid-fast microscopy), laboratories should examine a minimum of 10-15 smears a week. Specimens must be processed (decontaminated and concentrated) to provide meaningful stain results. Positive and negative control smears should be stained at least once each day of use to provide an indication of stain performance.

Laboratories that examine fewer than 10 acid-fast smears per week should consider using a reference laboratory for smear examination. Because of the reduced sensitivity of smear examination, **all** sputa collected for examination for acid-fast

bacilli should be tested in a laboratory with the capacity for culturing mycobacteria. With a secure and reliable transportation system and 24 hour service, the practical aspect of examining acid-fast smears must be questioned.

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

The fluorochrome method is preferred to screen for acid-fast bacilli because of increased sensitivity and the ease of reading the smears. Low power magnification allows more rapid examination of smears, because a greater area of the slide can be examined at one time. Laboratories need not confirm results of positive fluorochrome stains with the Ziehl-Neelsen or Kinyoun's acid-fast stain unless there is doubt about results of the initial smear. The routine use of the fluorochrome stained smear for the examination of TB specimens is recommended by both the CDC and the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD). Use of the Ziehl-Neelsen or Kinyoun's acid-fast stain is recommended only to detect AFB or contamination in cultures.

*Routine use of the fluorochrome stain to screen for acid-fast organisms is recommended by CDC and ASTPHLD.*

**31. Does your Mycobacteriology Laboratory check reactivity of fluorochrome stain each day of use by staining and examining known acid-fast and non-acid-fast organisms?**

Laboratories performing acid-fast staining procedures in mycobacteriology laboratories should check the fluorochrome acid-fast stain for positive and negative reactivity each day staining is done. *The Clinical Microbiology Procedures Handbook (25)* published by the American Society for Microbiology(ASM), describes a method for preparing and examining acid-fast control slides. Control slides for acid-fast stains are also commercially available.

*Acid-fast and non-acid fast controls must be stained and examined each day patient smears are stained and examined.*

ASM recommends that the stained control slides be reviewed before the patient smears are read to confirm that the acid-fast mycobacteria are staining properly. Record the results of the control slides. If they are acceptable, evaluate the patient smears. If the control slides are unacceptable, review procedures and reagent preparations. Unacceptable control slides include the following:

- a. Negative control fluoresces
- b. Positive control does not fluoresce, or is dull
- c. Background is not properly decolorized or fluoresces

When the problem is resolved, restain the original control slides, as well as all patient slides from the problem run (25) .

An increase of >2% in the number of specimens positive by smear, but negative on culture suggests the possibility of false positive acid-fast smears. All components of the digestion and staining procedure, including the water, should be examined as sources of contaminating mycobacteria. Digestant solutions, buffer or water diluents, bovine albumin solution or powder, and staining reagents are examples of possible sources of contamination by all mycobacteria (including *M. tuberculosis*). (See Appendix 9.)



If you question smear examination results, have a second, experienced reader examine the slides (36).

**32. Does your Mycobacteriology Laboratory examine acid-fast smears the day they are stained?**

In addition to reducing turnaround time by reading fluorochrome stains the day they are prepared, fluorochrome-stained smears should be observed within 24 hours of staining because the fluorescence may fade. Stained smears that cannot be read immediately should be stored at 2-8°C until read, but no longer than 24 hours.

**33. Does your Mycobacteriology Laboratory report an approximation of the number of acid-fast organisms viewed on the slide?**

Staining of specimen smears is used to assess patient infectivity, because the smear demonstrates a semi-quantitative estimate of the number of bacilli being excreted. A quantification of the numbers of acid-fast organisms per field should be rated 1+ - 4+(1,24,36). The number of tubercle bacilli in pulmonary secretions is directly related to the risk of transmission.

*The number of tubercle bacilli in pulmonary secretions is directly related to the risk of transmission of disease.*

**METHOD FOR REPORTING NUMBERS OF ACID-FAST BACILLI OBSERVED IN STAINED SMEARS\*(Appendix 1)**

<u>Number of AFB Observed</u>	<u>CDC Method Report</u>
None	Negative for AFB (-)
1-2/300 fields	Number seen (±)**
1-9/100 fields	Average No./100/F (1+)
1-9/10 fields	Average No./10F (2+)
1-9/field	Average No./F (3+)
>9/field	>9/F (4+)

\*Examination at 800-1000X is assumed. Magnification less than 800X should clearly be stated. If microscopist uses consistent procedure for smear examination, relative comparisons of multiple specimens should be easy for the clinician, regardless of magnification used. To equate numbers of bacilli observed at less than 800X with those seen under oil immersion, adjust counts as follows: for magnifications about 650X, divide by 2; near 450X, divide by 4; near 250X, divide by 10, e.g., if 8 AFB per F were seen at 450X, the count at 1000X would be about 2/F (8 ÷ 2) (36).

\*\*Counts less than 3/300F at 800-1000X are not considered positive; another specimen (or repeat smear of same specimen) should be processed if available.

Although *M. tuberculosis* may present characteristic morphology as viewed on the microscope slide (e.g., cording), other species may do the same; therefore, do not use microscopy alone to identify individual species of mycobacteria.

*Do not try to speciate mycobacteria based on microscopy alone.*

**\*34. Does your Mycobacteriology Laboratory telephone, fax, or electronically report all positive acid-fast smear results to the health care provider as soon as results are known, but within 24 hours from specimen receipt?**

To halt the continuing spread of tuberculosis across the United States and to help control transmission within the community, laboratories must recognize the urgency and optimize their procedures to report results of acid-fast smears, culture identification, and drug susceptibility tests to clinicians. Maintain a written record of direct calls and a record of the fax reports of smear results as a part of the QA record. A hard copy of the smear examination results should follow within 24 hours.

*Report positive acid-fast smear results within 24 hours of specimen receipt.*

***BOTH CDC AND ASTPHLD RECOMMEND REPORTS OF POSITIVE SMEAR RESULTS BY TELEPHONE, FAX OR ELECTRONIC MAIL WITHIN 24 HOURS OF RECEIPT OF THE SPECIMEN IN THE LABORATORY. EVERY LABORATORY SHOULD BE COMMITTED TO COMPLYING WITH THIS IMPORTANT REPORTING MECHANISM. THE MEDICAL COMMUNITY EXPECTS THIS RESPONSE.***

On smears in which no acid-fast bacilli are seen, report "Negative for acid-fast bacilli" by hard copy only. Reserve direct communication for positive results.

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

Logging telephone calls of positive results to physicians or maintaining a copy of fax results is an integral part of the quality assurance plan. Documentation is necessary to establish this link in the system. Since telephoned results are critical to patient care, it is important to establish the authority and record the identity of the person receiving the report. Confidentiality of the test result must be maintained. The following information must be documented when telephone reports are given:

- a. Patient name
- b. Type of culture or stain
- c. Specimen type and date obtained
- d. Findings
- e. Person called
- f. Person making the call
- g. Date and time of call

To meet CLIA requirements for reporting results, written procedures must be developed for reporting imminently life-threatening results or panic values. The presence of AFB in a smear should be considered a critical value and handled as such. The individual who orders or uses the test result must be alerted immediately.

- 36. Does your Mycobacteriology Laboratory telephone, fax or electronically report all positive acid-fast smear results to the public health department as soon as the results are available, but no longer than 24 hours from specimen receipt?**

Report patients who are suspected on clinical grounds of having tuberculosis or those with acid-fast bacilli (AFB) present on smears to the local health department promptly so that appropriate public health management (including contact investigation) can be initiated. If TB is to be controlled, it is imperative that immediate and appropriate responses to new cases be initiated by infection control experts and public health practitioners.

- 37. Does your Mycobacteriology Laboratory deliver or mail a written microscopy report to the health care provider within 48 hours of specimen receipt in the laboratory?**

The report must be sent promptly to the authorized person ordering or using the result. Interim reports of smear positive and smear negative are exceedingly important in the management of patients, whether they are new patients, recovering patients, or not infected. The laboratory manager can assist in this effort by having a written procedure that sets a time limit for reporting acid-fast smear results and assuring the staff are meeting the requirement.

Frequently, an interim or preliminary report will contain significant, but not definitive, information. This report should be identified as interim or preliminary, with a note that the final report will follow. The final report should repeat preliminary findings. All reports must contain proper patient identification.

- 38. Does your Mycobacteriology Laboratory maintain all patient reports and test records for two years?**

The original report or an exact duplicate must be retained for two years. The "exact duplicate" need not be paper, but may be retrieved from a computer system, microfilm or microfiche record, so 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 original test requisition and test records must be retrievable for two years.*

- 39. Does your Mycobacteriology Laboratory assure confidentiality of all patient information?**

If the laboratory uses a laboratory information system, i.e., an electronic reporting system, what security measures 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? Selective access by codes to the laboratory information system will prohibit unauthorized users from gaining entry.

Your laboratory must communicate with users for correct addresses and routing codes 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. A written policy for reporting by telephone, fax, or electronic mail is necessary to meet the CLIA intent for confidentiality of reporting.

## LEVEL I LABORATORIES ANSWER 40 & 41

### 40. Does your Mycobacteriology Laboratory limit access into the laboratory when specimens are being processed?

The TB laboratory must meet specifications of biosafety level 3 and doors must be closed while work is in progress in the BSC. If the BSC is shared for work with other microorganisms, limit access to the area during smear preparation to those who need to be present. After the specimens have been disinfected or decontaminated/concentrated and the smears are drying, continue work in the area. Heat fix the smears as soon as possible.

### \*41. Does your Mycobacteriology Laboratory send *all* specimens to a full service laboratory for culture within 24 hours of receipt?

The Level I laboratory performing only smear examination should immediately send 5-10ml of specimen to a reference laboratory for culture. Preliminary results of smear examination can be reported to the physician/provider, including information that the specimen has been sent to a reference laboratory for culture.

*All specimens collected for examination for TB should be cultured.*

**All** specimens collected to examine for *M. tuberculosis* should be cultured. The acid-fast smear alone is not a reliable predictor of disease due to *M. tuberculosis*.

Reports estimate that 30-50% of patients with pulmonary tuberculosis have negative sputum smears. For a definitive diagnosis of tuberculosis the bacillus must be isolated and identified. **Laboratories with no capacity for identification of mycobacteria can seriously delay reporting of a case of tuberculosis by delaying the culture of the specimen(s).**

- False negative smear reports on single sputum specimens can be misleading. Sputum for smear and culture collected on 3-6 consecutive days provides better information.
- Preparing and examining AFB smears, culturing specimens, and sending original positive slants, plates, or bottles to a reference laboratory for further work unnecessarily delays identifying *M. tuberculosis*.
- Preparing and examining AFB smears, inoculating cultures on media and sending mycobacterial subcultures (pure isolates) to a reference laboratory will unnecessarily delay identification of *M. tuberculosis*. For best results, send specimens to reference laboratories without delay.

## LEVEL II LABORATORIES ANSWER QUESTIONS 1-39; 42-80

### \*42. Does your Mycobacteriology Laboratory process and culture >20 specimens per week?

*It is recommended that laboratories culturing fewer than 20 specimens per week send specimens to a reference laboratory.*

Proficiency in culture and identification of *M. tuberculosis* may be maintained by digestion and culture of 20 specimens per week, provided adequate controls are used. Application of state-of-the-art laboratory technology for the laboratory identification of *M. tuberculosis* is costly and requires sophisticated instrumentation and highly trained laboratorians. Available rapid methods require high specimen numbers to be performed reliably and cost-effectively. Compliance with CLIA regulations, particularly those relating to safety, quality assurance, and proficiency testing, are also fiscal considerations for mycobacteriology laboratories.

The American Thoracic Society recommends that the full spectrum of bacteriologic support be concentrated in only a few laboratories in a given community or region where professional expertise and complete and safe facilities are available. Thus, laboratories with a low volume of work should refer specimens/cultures to laboratories that have chosen to maintain capabilities in mycobacteriology. This will save the time, effort, and expense of setting up and maintaining quality control standards for tests that are performed only rarely (1). If your laboratory cannot meet today's standards for quickly and accurately identifying and reporting *M. tuberculosis* complex, consider one of the alternatives:

- a. Update your laboratory equipment and procedures to shorten the time from collection until reporting.
- b. Refer your TB specimens to a laboratory that meets strict standards for quality testing and rapid reporting.

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

*Every effort must be taken to assure that no contamination or carry-over occurs between specimens.*

There is no substitute for meticulous technique when working with live microorganisms. When culturing *M. tuberculosis*, many opportunities exist to introduce contaminating organisms from the environment or, an even greater hazard, from another specimen. The laboratory technique used in the TB laboratory must be attuned to perfection. Every effort must be taken to assure that no contamination or carry-over occurs. In a retrospective study, 16% of 140 cases of MDR tuberculosis were the result of laboratory cross-contamination (35)! Transfers or inoculation of cultures must be accomplished by using individual transfer pipettes, single delivery diluent tubes or disposable labware to ensure that the specimen integrity is maintained. Examine your procedure and make certain there is no opportunity for cross-contamination between specimens. Be suspicious of several successively positive specimens or of a culture with very few colonies that follows a specimen culture that is 4+ positive. Meanwhile, use the following suggestions to protect the integrity of your specimens:

- a. Perform all culture work, especially liquid culture, over towels soaked with disinfectant spread over the work surface. Working with a tray lined with disinfectant-soaked towels permits easy disposal of all autoclavable material at the same time. Towels will absorb any droplets or splatters that may inadvertently occur during culture manipulations.
- b. Discard contaminated fluids into a splash-proof container.
- c. Use aseptic technique when removing caps from tubes, never laying them on a tabletop. Work with only one specimen or culture open at a time.
- d. 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 cultures.
- e. To avoid droplet aerosol cross-contamination, open tubes one line at a time. Opening several tubes at once creates an opportunity for crossover of droplet nuclei.
- f. Pipette bovine serum albumin or sterile buffer with a sterile pipette. Do not pour from the tube or bottle into centrifuged sediment because splash-back can and does occur.
- g. Make transfer and dilutions with sterile pipettes.
- h. Make smears after all inoculations are complete.
- i. Daily, after completing culture procedures, wipe down the work surfaces inside the BSC, and the tabletops, and centrifuges with towels moistened in a disinfectant with tuberculocidal action (note claims on the label).
- j. Autoclave all discard materials daily, after culturing is completed.
- k. Perform daily QC on the BACTEC instrument.
- l. Change BACTEC needles daily.
- m. Keep BACTEC media trap, filters, and tubing clean.
- n. Review all cultures which are **only** BACTEC positive to ensure that there has been no cross-contamination.

**44. Does your Mycobacteriology Laboratory routinely process and culture specimens seven days a week?**

Laboratories with no weekend work seriously delay reports of identification and susceptibility testing because they are not operational for 28.6% of the time. Added to this built-in delay are the practices of batching until a certain number of specimens for mycobacterial testing have been received or only processing 3 or 4 days per week.

With the use of the BACTEC and other rapid methods, more efficient and timely reporting of positive identification and drug susceptibility results can occur if a 7 day work week is adopted. Many institutions, hospitals in particular, have expanded their laboratory coverage out of necessity.

Would your patients benefit from weekend coverage in mycobacteriology? Have you considered an alternative work schedule in your laboratory?

*More efficient and timely reporting of positive results and drug susceptibility results can occur if a 7 day work week is adopted.*

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

Since heat buildup is extremely destructive to viable mycobacteria, it is highly recommended to use only refrigerated centrifuges as a means to prevent excess heat buildup. Also, a RCF of at least 3000 must be held for 15 minutes to achieve 95% sedimentation rate and effectively concentrate *M. tuberculosis* from sputum specimens. The centrifuge must be monitored for centrifugal efficiency and the RCF posted and known by the operating staff. [The staff must understand the difference between "revolutions per minute" (RPM) and "relative centrifugal force" (RCF or gravity force)]. Many old centrifuges still used in mycobacteriology laboratories commonly spin at 2300-3000 RPM (1500-2000 RCF); most users of such equipment centrifuge their digested specimens for only 15 minutes and thus attain theoretical sedimenting efficiencies ranging from 75-84% that may be further reduced by lethal heat buildup (24).

Angle head rotors are preferred for use in mycobacteriology to reduce frictional air resistance during centrifugation.

**46. Does your Mycobacteriology Laboratory use *only* safety carriers equipped with O-ring closures when centrifuging specimens?**

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.

Microcentrifuges should not be placed under the BSC for operation because air convection during operation compromises the integrity of the BSC. Safety cups for microcentrifuges are now available.

A good discussion of centrifugal safety and efficiency can be found in *PUBLIC HEALTH MYCOBACTERIOLOGY: A Guide to the Level III Laboratory*, pp 31-35 (24).

**47. Does your Mycobacteriology Laboratory prepare, stain, and examine acid-fast smears from all specimens sent for mycobacterial culture?**

Routine procedure for the examining any specimen submitted to the laboratory for TB culture should include AFB smear preparation and examination. AFB smear findings will provide information of value to the physician whether they are positive or negative. Microscopic observation of acid-fast bacilli in stained smears may be the first evidence that mycobacteria are present in a clinical specimen.

Although smear examination is less sensitive than the culture method for detecting mycobacteria and does not allow the observed organisms to be identified to the species level, it is the easiest and most rapid procedure that can be performed in the laboratory. AFB smears can be helpful in several ways:

*Routine procedure for the examination of any specimen submitted for TB culture should include AFB smear examination.*

- Provide a presumptive diagnosis of mycobacterial disease, making it possible to rapidly identify most infectious patients (i.e., those that are smear positive).
- Use to follow the progress of tuberculosis patients on chemotherapy.

**48. Does your Mycobacteriology Laboratory have an isolation room for mycobacteriology that is separate from the rest of the laboratory?**

Laboratories performing mycobacteriology at ATS/CDC Level II or III must meet CDC/NIH Biosafety Level 3 requirements. An appropriate secondary barrier is provided by separating the mycobacteriology laboratory from areas open to unrestricted traffic flow within the building. Within this room, in which the BSC is located, specimens that may contain *M. tuberculosis* are decontaminated, centrifuged, and cultured. Smears are also prepared and cultures are inoculated in the BSC.

An "isolation room" prevents the escape of droplet nuclei from the room, thus preventing entry of *M. tuberculosis* into the corridor and other areas of the facility. It provides an environment that will allow reduction of the concentration of droplet nuclei through various engineering controls, primarily through negative air pressure and an efficiently operating BSC. Negative pressure ventilation dilutes and removes aerosolized pathogens and will prevent the contaminated room air from flowing into other rooms. The air flow into the operating BSC will create a proper egress for contaminated air into the HEPA filter system, which will then be emitted from the building or recirculated after proper filtration.

*Smears are prepared in the safety of the BSC, preferably in a room that is separate from the rest of the laboratory.*

**49. Does your Mycobacteriology Laboratory keep the laboratory doors closed when mycobacterial specimens are being processed?**

Passage through two sets of self-closing doors is the basic requirement for entry into the mycobacteriology laboratory from access corridors or other adjoining areas. A clothes change room (optional shower) may be included in the passageway. The door should always be closed, and access to the area should be limited to the laboratory staff performing the work.

**\*50. Does your Mycobacteriology Laboratory have a one-pass (non-recirculating) ventilation system that establishes an airflow pattern, moving from clean (e.g., corridor) to least clean area (e.g., isolation laboratory)?**

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 a lower (negative) pressure in the area into which flow is desired. Negative pressure is attained by exhausting air from the area at a higher rate than it is being supplied. The level of negative pressure necessary to achieve the desired air flow will depend on the physical configuration of the ventilation system and area, including the air flow path and flow openings, and should be determined by an experienced ventilation engineer on a case-by-case basis. Six to twelve air changes per hour are acceptable and provide removal of 99% of airborne particulate within 45 minutes (24).

*Air in the TB culture room should move from an area of low infectivity to an area of higher infectivity.*



The isolation room should have negative pressure relative to the adjacent area with air moving from an area of low infectivity to an area of higher infectivity. The work area should contain no air sources such as open windows or through-the-wall ventilation ducts. Doors between the isolation 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 (15).

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

Review the air handling systems 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, environmental engineers or experts in ventilation engineering should reinspect the mycobacteriology ventilation system to determine the air quality in the area. Record the event as you would any equipment maintenance event.

Use smoke tubes or an air velocity measuring device with smoke tubes to monitor negative pressure on a quarterly basis in the mycobacteriology laboratory. Record your findings as part of the preventive maintenance monitoring plan. Check and record the reading of the magnehelic gauge on the BSC as a daily operational QC check.

A simple way to daily ensure that a room has negative air pressure 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.

**52. Does your Mycobacteriology Laboratory control access to the laboratory when working with mycobacterial specimens?**

A secondary containment strategy to reduce the hazard of working with viable mycobacteria in a Biosafety Level 3 containment area is to control the access to the culture area. This is done by restricting access except to persons whose presence is required for program or support purposes.

**\*53. Does your Mycobacteriology Laboratory inoculate all digested/decontaminated, concentrated sediments into a selective broth, e.g., the BACTEC System, for the primary culture?**

Laboratories are being challenged to use liquid culture as the primary method for isolating *M. tuberculosis*. Primary culture in a selective broth medium enhances the growth of *M. tuberculosis* and is preferred to culture on solid media alone. The BACTEC 460 TB radiometric system (Becton-Dickinson, Sparks, MD) is being used with great success in many hospitals and reference laboratories. This system uses liquid growth medium containing radiolabeled palmitic acid as the substrate, and improves recovery and decreases the time required for detection of mycobacteria.

Growth of mycobacteria is detected within 7-14 days by measuring the  $^{14}\text{CO}_2$  released from the substrate by metabolizing mycobacteria. Once growth is detected, the organism can be identified by using specific procedures developed to rapidly identify *M. tuberculosis*. The sensitivity and specificity of the BACTEC method of isolation have proven to be the highest of any method to date to detect the strains of the *M. tuberculosis* complex quickly and accurately.

**Advantages:**

- Average time from primary inoculation to detection of positive growth is 8-12 days
- Isolation and identification of *M. tuberculosis* complex requires 4-5 days after growth detection
- Primary drug susceptibility testing from broth culture requires 4-6 days
- Growth occurs more often in liquid medium than on Lowenstein-Jensen or other solid media

*Primary culture in a selective broth medium enhances the growth of TB and is preferred to exclusive culture on solid media.*

**Disadvantages:**

- Capital investment for instrumentation in laboratories culturing fewer than 20 cultures per week is impractical
- The liquid culture method is hazardous because of potential for needle sticks
- The disposal of radioactive waste material is expensive and troublesome
- The method is labor intensive

The N-acetyl L-cysteine-sodium hydroxide (NALC-NaOH) digestion-decontamination method is the procedure of choice for preparing specimen for inoculation to BACTEC. The sodium hydroxide, oxalic acid, and sodium lauryl sulfate methods can be used, but the Zephiran-trisodium phosphate, benzalkonium chloride, or cetylpyridium chloride methods cannot be used with the BACTEC procedure, as the residual quantities of these substances in the inoculum inhibit mycobacterial growth in the BACTEC system (29).

Contamination may be indicated by any sudden increase in the growth index (GI) reading or by the presence of turbidity. Growth of AFB should be confirmed by a Ziehl-Neelsen or Kinyoun's stain. *M. tuberculosis* should not be reported based on smear examination only. Rapid identification of *M. tuberculosis* complex can be made from a concentrated sample of a primary BACTEC 12B broth culture that is AFB positive. Cross-contamination of BACTEC culture vials because of inadequate heat sterilization of the sampling needle has been reported and may pose a problem (25).

The Septi-Chek AFB System (Becton-Dickinson, Cockeysville, MD) is a biphasic culture system, consisting of modified Middlebrook 7H9 broth and a three-section paddle containing chocolate, an egg-based medium, and modified Middlebrook 7H11 solid agar. Comparison of Septi-chek growth to growth of mycobacteria in BACTEC revealed that while the sensitivity was similar, the average time for the detection of mycobacteria from BACTEC was 11.8 days, Septi-Chek 18.8 days, and Lowenstein-Jensen (L-J) 23.5 days (32). The Septi-Chek system does not require specialized instrumentation or the use of radioisotopes.

Both the Septi-Chek and the BACTEC systems are superior to conventional methods (L-J) for recovery and time to detection of mycobacterial growth (32).

Other systems [such as BACTEC 9000 and Midget System (Becton-Dickinson), ESP-AFB (Difco) and the BacT-Alert (Organon Teknika Corporation, Durham, NC)] are in development and should be available soon.

**54. Does your Mycobacteriology Laboratory inoculate all specimens not requiring decontamination into a broth, e.g., BACTEC System, for the primary culture?**

Specimens collected aseptically or from normally sterile sites can be inoculated directly to BACTEC 12B vials without being decontaminated. Aseptically collected specimens with volume greater than 10ml should be centrifuged at 3000 RCF before inoculation. Specimens that are thick or mucoid should be liquefied before centrifugation.

**55. Does your Mycobacteriology Laboratory inoculate digested/decontaminated, concentrated sediments of mycobacterial specimens to at least one solid medium?**

In addition to the inoculation of liquid culture medium good laboratory practice requires that at least one selective solid medium be inoculated at the same time. Growth on solid media can be used to detect mixed colony morphology and to provide a source for isolation of a pure culture. If there is no growth on the original solid medium, a second solid medium should be inoculated from the primary BACTEC vial at the time the AFB smear(s) is prepared (growth index >100 units). Compare growth on BACTEC with results on solid medium and the original AFB smear for quality control purposes.

**56. Does your Mycobacteriology Laboratory inoculate a negative control each day specimens are inoculated?**

*Using negative controls on the media under conditions identical to those of the cultures assures that no contamination has been introduced during the culture process.*

Inoculating a negative control under conditions identical to those used for the specimens assures that conditions are appropriate for recovery of *M. tuberculosis*, and may indicate if contamination has been introduced during the culture process. Record results as a part of the quality control record. Any growth of mycobacteria on the negative control is an alert for **immediate** investigation of positive cultures on that process run, including notification of physician(s) if questionable results have been reported.

**57. Does your Mycobacteriology Laboratory perform direct drug susceptibility testing with the primary drugs using the concentrated sediment of smear positive specimens?**

The BACTEC procedure for susceptibility testing against primary drugs [streptomycin, isoniazid, rifampin, and ethambutol (SIRE)] provides the most rapid results of any method in use today. Both direct and indirect susceptibility tests may be performed using the BACTEC method. The BACTEC direct susceptibility tests were reportable in 10.7 days from more than 67% of AFB smear positive cultures, and from 86% of *M. tuberculosis* culture positives (33). Agreement of the BACTEC direct susceptibility test results with those obtained by the conventional techniques has been reported to be very high (22).

When using "conventional" agar testing methods, direct susceptibility testing against primary drugs, SIRE, should be performed on all initial smear positive specimens. Both the agar dilution method and the disk-elution method have been used with success in laboratories performing direct susceptibilities to primary drugs. The disk-elution method provides equivalent results without errors associated with weighing, diluting, or labeling and provides a method for preparing only the number of plates needed for short-term use. The direct method for testing susceptibility from the initial concentrated sputum specimen is preferred since the indirect method may not represent the true patient cell population. If the indirect method from growth on solid medium is used to test susceptibility, the inoculum should be prepared from cells collected by a swipe across the medium to maintain as much as possible a representation of patients original microbial population.

*When using solid agar testing methods exclusively, direct drug susceptibility against primary drugs (SIRE) should be performed on all initial smear positive specimens.*

Read and report results of drug tests on Middlebrook 7H11 medium at three weeks. If the colonies are fully matured and only if they show resistance, results may be reported in less than three weeks. Although colonies may be fully matured on control media in less than three weeks, "drug susceptible" reports should not be sent until the third week, because resistant colonies often grow more slowly than susceptible ones and may not be visible until the third week.

Pyrazinamide (PZA) is a primary drug, and current practice requires that isolates should be tested for susceptibility. PZA susceptibility is difficult to test in vitro (22). The effect of the drug can be demonstrated only in an acidic medium that does not adequately support the growth of many *M. tuberculosis* isolates. Using an inoculum from a fresh, actively growing culture, the BACTEC provides a method for testing PZA susceptibility. If the BACTEC system is not available, it is recommended that PZA be tested only in reference laboratories. NOTE: Only *M. tuberculosis* is susceptible to PZA. *M. bovis*, *M. bovis* (BCG) are resistant to PZA, as are all other mycobacteria species except *M. tuberculosis*.

**58. Does your Mycobacteriology Laboratory use a control strain of *M. tuberculosis* susceptible to all antimycobacterial agents being tested, each time a drug susceptibility test is performed?**

The laboratory must check susceptibility of each daily run with a strain of *M. tuberculosis* susceptible to all antimicrobial agents tested. *M. tuberculosis*, strain H37Rv, is completely susceptible to the SIRE drugs and pyrazinamide (25).

QC strains should be tested under conditions identical to those of the test strains to ensure the quality of the testing procedure and reagents. QC procedures for susceptibility check organism dilution, plating technique, and the ability of the test medium to obtain acceptable results. If results of QC are not as expected, tests of control strain(s) and isolates must be repeated. The incubation temperature should be monitored and kept at 35-36°C.

**59. Does your Mycobacteriology Laboratory examine broth cultures for evidence of growth every 2-3 days for weeks 1-3, and weekly thereafter for a total of 6-8 weeks?**

Rapid detection of *M. tuberculosis* is critical to diagnosing pulmonary tuberculosis and to the detecting of drug-resistance. BACTEC is more useful for the earlier

detection of *M. tuberculosis* than other systems currently available. Average detection time for mycobacteria is approximately 8-12 days, followed by an additional 4-5 days for identification of *M. tuberculosis* complex. The culture positivity rate in this broth medium is higher than it is on conventional solid medium.

Read the inoculated BACTEC culture vials on the BACTEC instrument every 3-4 days for the first 2-3 weeks and once a week thereafter for a total of 6-8 weeks. (Laboratories with small numbers of specimens may be able to read cultures three times a week for the first 3 weeks of incubation and weekly thereafter for a total of 8 weeks.)

BACTEC 12B vials that develop a GI of 25-50 and seem to stop growing may be transferred to a fresh 12B vial, as this sometimes stimulates growth. Smear positive specimens that fail to grow in 6 weeks should be held for 8 weeks.

**60. Does your Mycobacteriology Laboratory examine cultures on solid media for evidence of growth twice weekly for 1-4 weeks, and weekly thereafter for a total of 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 8 weeks, but if the original smear was positive and the cultures are negative at 8 weeks, further incubation is indicated. Careful scrutiny using an inverted or dissecting microscope or hand lens is helpful for early observation of typical colonies of mycobacteria and facilitates early identification of *M. tuberculosis* complex using rapid methods.

**61. Does your Mycobacteriology Laboratory use a microscope or hand lens to examine agar plates and/or tubes for earlier visualization of mycobacterial growth?**

The liquid broth method is not available to all laboratories at this time, because of limitations imposed by the lack of availability of instrumentation and the need for justification for capital investment. Techniques to facilitate a more rapid diagnosis of tuberculosis should be considered. Good results in isolating mycobacteria from clinical specimens have been achieved using Middlebrook 7H10 and/or 7H11 agar with microscopic examination.

Colonial morphology directs the selection of the specific nucleic acid probe for rapid culture confirmation of *M. tuberculosis*.

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 *Mycobacterium* on plated media. A 3-10x hand lens is useful to examine tubed media.

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 10-60x magnification and transmitted light, with the source below the stage so that it shines through the medium.

Welch et al.(42) used a brightfield or inverted microscope at 100-160x to view the microcolonies on solid media, examining the plates twice weekly for four weeks.

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

- Rapid growers are fully matured within 7 days on subculture.
- Slow growers require more than 7 days.

Suspend growth in sterile buffer or bovine albumin for further testing, or submit on L-J slant to reference laboratory for further study. A preliminary report to the physician will communicate the progress of the culture.

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

- BACTEC vials exhibiting growth?**
- Selected colonies at an early stage of growth on solid medium?**

**62a.** Once the GI is 100 or more, prepare a smear and stain with an acid-fast stain and examine the smear. If contamination is detected, decontaminate the BACTEC vial using one of the procedures for initial specimens and streak on a selective solid medium or inoculate into a fresh BACTEC vial made selective by the addition of antibiotics.

*Prepare and stain acid-fast smears from growth positive BACTEC vials and from selected colonies on solid media.*

Acid-fastness, cording and a slow growth rate in 12B medium are suggestive of *M. tuberculosis*. A DNA probe may be used to confirm the identification of *M. tuberculosis* complex or another species.

**62b. Does your Mycobacteriology Laboratory perform an acid-fast smear from selected colonies on solid medium at an early stage of growth?**

When visual examination of the growth on transparent agar or L-J medium reveals suspicious colonies, prepare and stain smear(s), using an acid-fast stain. Examine the staining characteristics and note the cellular morphology. Acid-fast staining results and colonial morphology guides the laboratorian in subsequent steps for confirmation and/or identification. (All plates should be resealed before removal from BSC.) As with the selective broth, DNA probe may be used to confirm the identification of *M. tuberculosis* complex on the surface of solid medium.

**63. Does your Mycobacteriology Laboratory subculture all BACTEC vials exhibiting acid-fast growth to solid medium?**

Inoculating Middlebrook 7H10 agar allows development of colonial morphology from growth in the BACTEC vial. Colonial types may be purified and further tested to identify other mycobacteria that may be present. A pure culture may be transferred for stock cultures, reference, or quality control. Transferring to an L-J slant also allows better discrimination of pigment for species identification.

**\*64. Does your Mycobacteriology Laboratory use a rapid method to presumptively or specifically confirm the presence of *M. tuberculosis* complex:**

- a. From BACTEC vials?
- b. from growth on solid media?

**\*64a.** Once growth is detected in BACTEC broth, the organism can be specifically identified by the High Pressure Liquid Chromatography (HPLC), AccuProbe nucleic acid probe, (GenProbe, San Diego, CA) or presumptively identified as *M. tuberculosis* complex by growth inhibition in the BACTEC NAP test. Any acid-fast isolate that is not identified at a Level II Laboratory must be sent to a Level III Laboratory for identification.

**\*64b. Does your Mycobacteriology Laboratory use a rapid method to presumptively or specifically confirm *M. tuberculosis* complex from growth on solid media?**

Colonial morphology and acid-fast smear result directs the selection of the specific Accuprobe nucleic acid probe or other method for rapid culture confirmation of organism from the *M tuberculosis* complex, allowing the species confirmation within hours of detecting the colony. The probe has excellent sensitivity and the identification can be made within two hours from liquid or solid culture media. Level II and III laboratories should be prepared to use DNA probes for the identification of *M. tuberculosis* complex. Unusual or problem isolates should be sent to state public health laboratories for resolution, including possible consultation with CDC.

#### **The AccuProbe:**

This is the only probe method currently available for identifying isolates of several species of mycobacteria. The system is based on the use of nucleic acid probes that are complementary to species-specific rRNA. Mycobacterial cells are lysed by sonication, and exposed to DNA that has been labeled with a chemiluminescent tag. The labeled DNA probe combines with the organism's rRNA to form a stable DNA:RNA hybrid. A selection reagent hydrolyzes the signal on all unbound DNA. Chemiluminescence produced by a DNA:RNA hybrid is measured in a luminometer. Nucleic acid probes provide the most rapid identification of mycobacteria (25).

Any actively growing culture less than one month old recovered on any solid or broth medium can be used with the AccuProbe. The accuracy of identification of *M. tuberculosis* from an actively growing BACTEC vial with the AccuProbe is nearly 100%. Reportable results can be achieved within 2-4 hours, but up to  $10^5$  -  $10^6$  -bacilli are required for reliable, reproducible results. A known positive and negative control should be included with each batch of organisms tested to control the performance of the equipment, the procedure, and the reagents.

"Batching" of probe tests prolongs turnaround time of laboratory results. To respond to the increased incidence of tuberculosis, laboratories should commit to doing probe tests more frequently to obtain results as quickly as possible.

The *M. tuberculosis* complex probe does not differentiate between *M. tuberculosis*, *M. bovis*, *M. bovis* (BCG), *M. africanum*, and *M. microti*. The AccuProbe has probes for the identifying *M. tuberculosis* complex, *M. avium*, *M. intracellulare*, *M. gordonae*, *M. avium* complex, and *M. kansasii* (25).

#### **The BACTEC NAP test:**

The BACTEC NAP test utilizes  $\rho$ -nitro- $\alpha$ -acetylamino- $\beta$ -hydroxypropionophenone (NAP) to inhibit the growth of mycobacteria belonging to the *M. tuberculosis* complex, while not inhibiting or only partially inhibiting the growth of mycobacteria other than the *M. tuberculosis* complex. Confirmation of the NAP test and further identification require additional testing by conventional or other methods.

Use an actively growing culture in an early growth phase of BACTEC 12B vial for the NAP test. Growth from solid media can also be used for inoculum with the NAP test (25).

- Never report NAP test result if the growth index values in the control vial increases slowly or not at all (25).
- Mixed cultures of two mycobacteria or contaminated cultures give erroneous results. In such cases, an acid-fast stain helps to confirm a mixed culture or culture contamination. Examine the solid media cultures that were inoculated simultaneously from the initial concentrated specimen for evidence of differing colonial morphology and subculture to liquid media from the solid media.
- Tests should be performed at the temperature that provides the best growth in the control. If poor growth in the control is observed, the possibility of another optimum temperature should be investigated (33).
- Among mycobacteria other than *M. tuberculosis* complex, certain strains of *M. kansasii*, *M. gastri*, *M. szulgai*, *M. terrae*, and *M. triviale* are partially inhibited by the NAP test. In such instances there is a longer lag phase in the presence of NAP, and results interpreted in the first 2-4 days may be misleading. Incubate further and test for an additional 2-3 days before reporting (25).

#### **High Pressure Liquid Chromatography (HPLC):**

HPLC has been used as a rapid method to detect mycobacteria and identify them to the species level using standard chromatographic patterns. A method using HPLC analysis of the  $\rho$ -bromophenacyl esters of mycolic acids extracted from saponified whole cells has demonstrated the distinctly different mycolic acid patterns produced by different species (4). The method is proving helpful, especially in reference laboratories with many positive specimens of differing species. The initial cost of the equipment makes the method impractical for most smaller laboratories.

#### **65. Does your Mycobacteriology Laboratory telephone or fax a confirmed report of *M. tuberculosis* to provider as soon as the results are available?**

Verbally communicate to physician or provider any information that is crucial to diagnosis and treatment: morphology and pigmentation, results of the BACTEC NAP test or AccuProbe test, presence of mixed cultures, isolation of *M. tuberculosis* from multiple sites. Report resistance to antituberculosis drugs as



soon as the results are known. A verbal report should be documented and followed by a written report on the same or the next day. The final report should include a record of the verbal and/or preliminary report.

- \*66. Does your Mycobacteriology Laboratory average 14-21 days between receiving specimen and reporting *M. tuberculosis* complex on positive specimens?**

***Reporting of *M. tuberculosis* complex should average 14-21 days from receipt of specimen.***

*Reporting of *M. tuberculosis* complex should average 14-21 days from receipt of specimen.*

Primary culture in a selective broth (e.g., BACTEC 12B) medium (7-14 days), confirmation of positive growth by AFB smear (2-4 hours), and confirmation by the TB AccuProbe (2-4 hours) or the BACTEC NAP test (4-6 days) will permit a report of *M. tuberculosis* complex within 14-21 days.

- 67. Does your Mycobacteriology Laboratory monitor the turnaround time of test results to ensure that the majority of *M. tuberculosis* complex are identified within 21 days of specimen receipt?**

Management's decision to monitor, on a monthly or quarterly basis, the reporting of *M. tuberculosis* test results represents a commitment to better patient care. If the laboratory program fails to meet a 21 day average turnaround standard for identifying *M. tuberculosis* complex, management should focus on improving in this area.

- 68. Does your Mycobacteriology Laboratory retain positive mycobacterial cultures for one year?**

Epidemiologic investigations often use strains of *M. tuberculosis* that were isolated six to nine months previously. Therefore, it is good practice to keep isolates for at least one year. Outbreak investigations entail defining and identifying common patient exposures and the relatedness of *M. tuberculosis* isolates. Fingerprinting by the restriction fragment length polymorphism (RFLP) procedure is the most accurate method to determine strain relatedness.

Many laboratories maintain a subculture of the first isolate in the refrigerator or freezer that can be retested if the patient fails to respond to therapy. The laboratory can reexamine the initial isolate and a later one to determine if the susceptibility pattern has changed during therapy.

- 69. Does your Mycobacteriology Laboratory 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 specimens occur rarely, generally less than 2%. The occurrence of excessive numbers of smear positive, culture negative specimens suggests acid-fast contaminants in the system. Check tap and distilled water first, then diluent, buffer, stain solutions, and other reagents

(Attachment 9). The smear negative/culture positive specimen is usually associated with the number of organisms present. Cultures with  $>10^4$  mycobacteria per milliliter of original specimen are usually smear positive.

**70. Does your Mycobacteriology Laboratory document the percent of specimens producing contaminating growth on culture media inoculated with digested/decontaminated sediment as a way of monitoring the specimen preparation process?**

A contamination rate of 3-5% is considered acceptable in most laboratories receiving fresh specimens. If your laboratory is experiencing delays in delivery of specimens, the contamination rate may be greater than 5%. Although increasing the concentration of NaOH will help reduce the contamination rate, it will also increase the possibility of die-off of *M. tuberculosis* during the digestion/concentration process.

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 NALC concentration to digest thick, mucoid specimens.

If a contamination rate  $>5\%$  persists, increase the amount of NaOH slightly. With the BACTEC system, consider increasing the amount of the PANTA supplement only as a last resort, as increasing PANTA may inhibit slowly growing or fastidious mycobacteria.

If your laboratory is experiencing little or no contamination, your decontamination procedure may be too harsh. The laboratory processing may kill too many mycobacteria, diminishing the overall recovery rate. An excellent discussion of centrifugal efficiency and digestant toxicity may be found in the *CDC Guide for Level III Laboratories* (24).

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

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

If more than one colony type is observed, select colonies of each type, and prepare subcultures of each to establish purity of the cultures. Send acid-fast isolates to a Level III laboratory for identification if your laboratory cannot identify species with certainty.

**72. Does your Mycobacteriology Laboratory compare the prevalence of *M. tuberculosis* complex isolated in your laboratory to the prevalence in 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 housestaff of local trends and findings. Regional and state information is available from the local or state TB Control or from CDC.

**73. Does your Mycobacteriology Laboratory perform susceptibility testing on all initial isolates of *M. tuberculosis*?**

The CDC recommends that initial isolates from all patients be tested for drug susceptibility to confirm the anticipated effectiveness of chemotherapy.

*Initial isolates of TB from all patients must be tested for drug susceptibility.*

Susceptibility testing should be repeated if the patient continues to produce culture positive sputum after 3 months of treatment. Monitoring drug resistance patterns in specific locales can help to identify areas where infection control or public health interventions may be necessary to prevent MDR-TB outbreaks.

Local monitoring is also important for assessing the success of control programs in areas that have already established the presence of drug resistance in isolates (41).

**74. Does your Mycobacteriology Laboratory determine the susceptibility of *M. tuberculosis* to primary drugs using a liquid system such as BACTEC?**

*The BACTEC System provides the most rapid information on drug susceptibility against SIRE and pyrazinamide.*

Second only to the need to know whether a patient is infected with *M. tuberculosis*, is the need to know the most effective drug or chemotherapeutic agent(s) to use to treat the patient. All primary isolates should be tested for susceptibility against SIRE and pyrazinamide using the BACTEC method. This will provide the most rapid information for proper patient and contact treatment, if necessary.

Inoculum for the BACTEC indirect drug susceptibility test may be prepared from a positive 12B vial, from a solid agar medium, or from egg-based medium culture. Confirmation procedures and susceptibility tests on acid-fast growth should be carried out simultaneously to permit rapid identification of *M. tuberculosis* complex and rapid reporting of results. Drug susceptibility performed in BACTEC can be reported within 4-5 days, and is often available by the time confirmation is complete. Further susceptibility tests should be performed if resistance to one or more of the primary drugs is suspected. Secondary drug testing is usually performed in Level III laboratories having experience in *M. tuberculosis* susceptibility testing using secondary drugs.

Drug testing of *M. tuberculosis* isolates against additional antimicrobial agents can be performed in many Level III laboratories using the same techniques as for primary drugs. Second-line drugs and newer antituberculosis drugs being developed for clinical use are being screened in the BACTEC system in some larger Level III laboratories with results that correlate well with results in 7H10 agar. However, the experience of most laboratories with such testing is limited.

Drug susceptibility tests should be performed only in laboratories staffed by personnel who are proficient in identifying the species of *Mycobacterium* being tested and who perform a sufficient number of susceptibility tests to be aware of the problems associated with the procedures.

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

It is important to report drug susceptibility results to the attending physician or health care provider as soon as the information is available. An oral report of drug susceptibility should be documented and followed by a written report on the same or the next day. The final report should include a record of the oral and/or preliminary report.

*Susceptibility results on M. tuberculosis should be reported to provider as soon as available.*

If the patient has drug resistant tuberculosis, the information will be used by the physician to modify or change patient chemotherapy, especially if the patient is not improving on the assigned regimen. Many laboratories repeat "resistant" BACTEC susceptibility results using conventional media, especially until experience using the BACTEC system is gained. Correlation studies between the BACTEC system and the conventional method are still being documented, and it is possible that the recommended concentration of antimicrobials will be adjusted.

Send the comprehensive final report of preliminary information, final identification, and drug susceptibility results to the clinician, the infection control officer, local or state TB Control Division, and the medical records department. Computerized summary results may be sent to other laboratories, clinicians and public health practitioners, as appropriate.

Since the drug susceptibility patterns are not always known at the time *M. tuberculosis* is confirmed, information on drug resistance 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, isolation and managed care for those with treatment and/or compliance problems. As regionalization of tuberculosis testing occurs, this link between laboratory and local and state Public Health TB Control Programs must be emphasized and secured.

**\*76. Does your Mycobacteriology Laboratory average 15-30 days between receiving specimen and reporting primary drug susceptibility test results on *M. tuberculosis* complex?**

Laboratories must accept the responsibility of their role in the proper management of patients infected with tuberculosis. In the past, susceptibility testing has been minimized, and reports have been sent whenever the identification/susceptibility testing was complete. Drug susceptibility testing has been handled almost as an afterthought in too many institutions. The importance of and attention to this aspect of laboratory reporting have now become an essential link to patient management.

*Report drug susceptibility test results to the provider within 15-30 days of specimen receipt.*

All Level II and Level III laboratories must focus on reducing turnaround time for drug susceptibility testing to <30 days or consider referring all *M. tuberculosis* cultures to a reference laboratory.

With attention focused on the necessity for rapidly reporting of susceptibility testing results, the following approach should decrease turnaround time:

- BACTEC 12B vials with increasing growth index: Perform indirect drug testing using the five primary drugs at the same time the growth identification procedure(s) is performed.
- Conventional method: Direct susceptibility testing on acid-fast smear positive specimens produces results three to six weeks faster than indirect drug testing.
- Level I Laboratories: Rapid transport and monitoring reference laboratory results will help to reduce drug susceptibility turnaround time.
- Overnight mail to reference laboratory.

**77. Does your Mycobacteriology Laboratory telephone, fax, or electronically report result of confirmed positive *M. tuberculosis* complex and drug resistant strains of *M. tuberculosis* complex to the Tuberculosis Control Program in the state where the patient resides, as soon as the result are available?**

*Report confirmed positive and drug resistant strains of M. tuberculosis complex to Tuberculosis Control Program in the state where the patient resides, as soon as results are known.*

A real concern in the present environment of increasing numbers of tuberculosis cases and drug resistance is the interstate reporting of tuberculosis cultures. Commercial laboratories operating between states must be **committed to notifying the health officials in the state where the patient resides** to ensure that the link between diagnosis and investigation occurs.

Disseminating information that is essential for public health investigations must be faithfully and reliably initiated by all laboratories performing tuberculosis culture and identification. Appendix 10 lists the individual state requirements for reporting patient information to the state health department (10). State requirements for confinement, contact investigation, treatment, and follow-up must be met by the State TB Control Program.

Detection of resistant strains of *M. tuberculosis* must be reported directly to the physician or infection control staff, the local public health practitioners and the state TB Control Program. The epidemiologic follow-up and control of both drug-resistant and drug-susceptible tuberculosis depend on a cooperative approach that begins with the laboratory notifying the local and state health department. Duplicate notification is not a concern.

**78. Does your Mycobacteriology Laboratory monitor the turnaround time for reporting primary drug susceptibility test results on *M. tuberculosis* isolates to ensure that reports are sent within 30 days of specimen receipt?**

Management's decision to monitor the drug susceptibility testing on a monthly or quarterly basis will be viewed as a commitment. If the laboratory program fails to meet the prescribed standard of practice, management should focus on improving specific areas. An average 30 day turnaround time for reporting primary drug susceptibility results on *M. tuberculosis* is reasonable, and a longer average turnaround time shows need for improvement.

**79. Does your Mycobacteriology Laboratory monitor the resistance patterns of *M. tuberculosis* and make information available to house staff, local, and State Tuberculosis Control Program?**

The provider/physician has little opportunity to observe trends. By monitoring drug results in the laboratory, trends can be observed more quickly. This information will assist physicians in drug selection, permitting the medical community to deal more effectively with new patients or patient contacts. Implementing rapid technology that will reduce the turnaround time for reporting the drug susceptibilities of tuberculosis is critically needed to resolve the current MDR-TB problem.

**80. Does your Mycobacteriology Laboratory submit outbreak-associated strains of *M. tuberculosis* through the state public health laboratory to a restriction fragment length polymorphism (RFLP) reference laboratory for fingerprinting?**

Laboratories must be able to support outbreak investigations and special studies of MDR-TB. Isolates can be sent to regional laboratories performing a procedure for fingerprinting, restriction fragment length polymorphism (RFLP). Subtyping of *M. tuberculosis* by RFLP is a powerful tool for tracking the spread of individual strains and testing for laboratory cross-contamination when suspected.

RFLP is a means of "fingerprinting" the DNA. This DNA fingerprinting uses repetitive sequences located on the chromosome to characterize each isolate of *M. tuberculosis*. RFLP typing does not have the disadvantage of plasmid profile analysis which uses extra-chromosomal genetic material that can readily be lost (30). RFLP has been used to:

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

Eight strategically located regional centers for RFLP studies (Appendix 6) have been established in Level III laboratories across the United States with coordination through the state public health laboratory system. Level II and Level III laboratories may avail themselves of this service by contacting the state laboratory director in the patient's state of residence (Appendix 6).

## LEVEL III LABORATORIES ANSWER QUESTIONS 1-39; 42-84

**81. Does your Mycobacteriology Laboratory identify a broad range of mycobacterial species?**

Laboratories qualifying as Level III laboratories must work to develop and enhance their reference capability:

- Utilize currently available laboratories resources for providing services to meet patient needs according to risk/disease prevalence. Standardize electronic transfer of information through software to avoid duplication of effort.
- Recognize and standardize key elements to link testing laboratories and community users of results with CDC.
- Coordinate timely transfer of critical test results with those providers and agencies that need to know.

**82. Does your Mycobacteriology Laboratory perform drug susceptibility studies against other mycobacteria in addition to *M. tuberculosis*?**

Reference laboratories may or may not perform drug testing on mycobacteria other than *M. tuberculosis* (MOTT) bacilli. Although the modified proportion method has been used for testing most species of slowly growing mycobacteria, no method is yet accepted for susceptibility testing of mycobacteria other than tubercle (MOTT) bacilli. The difference in the susceptibility pattern of MOTT bacilli provided by BACTEC and conventional methods is being investigated. The clinical relevance of the in-vitro susceptibility test results obtained for MOTT bacilli has not been determined. Tests should be conducted in large or reference-type laboratories with sufficient competence to be aware of the limitations of current practices.

CDC reported in January , 1992 that most *M. avium* complex (*M. avium* or *M. intracellulare*) isolates are resistant to all of the antituberculosis drugs routinely tested, and there are no convincing published data that demonstrates therapeutic success with these drugs even when in vitro tests indicate susceptibility to a specific drug. It therefore serves no useful purpose to pursue susceptibilities against *M. avium* complex. CDC does not routinely accept reference strains of *M. avium* complex for susceptibility testing.

With the exception of *M. smegmatis*, which is susceptible to ethambutol, the pathogenic, rapidly growing mycobacteria are resistant to achievable serum and tissue levels of all first-line anti-tuberculosis drugs. Neither the broth microdilution, agar disk-elution, nor the disk-diffusion method has been approved by the National Committee for Clinical Laboratory Standards (NCCLS) for testing of the rapidly growing mycobacteria (22).

**83. Does your Mycobacteriology Laboratory use one of the following rapid methods to identify mycobacteria:**

- a. DNA probe?
- b. High performance liquid chromatography (HPLC)?

**83a.** AccuProbe, a DNA probe currently available for identifying cultures of *M. tuberculosis* complex, has excellent sensitivity. Identification can be accomplished within 2 hours on growth from liquid or solid culture (41). Level II and III laboratories isolating mycobacteria should be prepared to use DNA probes for identifying *M. tuberculosis* complex (30). An organism with colonial morphology on solid media consistent with *strains of the M. tuberculosis* complex, or an organism growing in liquid medium and suspected of being *M. tuberculosis* should be tested. Testing with nucleic acid probes should be combined with isolation of mycobacteria in liquid culture. Liquid cultures positive for *M. tuberculosis* nucleic acid can be subcultured to a solid medium to rule out mixed mycobacterial cultures. The growth of the subculture on Middlebrook 7H10 or 7H11S can also be used later for biochemical tests, susceptibility testing, and for stock culture(s). Manufacturers are being encouraged to develop a rapid, amplified testing system to provide direct identification of *M. tuberculosis* from the patients' specimens and a stand-alone screening test for other mycobacterial pathogens (30).

Nucleic acid probes are also available for the culture identification of *M. avium* complex, *M. avium*, *M. intracellulare*, *M. gordonae* and *M. kansasii*.

**83b. Does your Mycobacteriology Laboratory use high performance liquid chromatography (HPLC) to identify mycobacteria?**

HPLC applications for the identification of mycobacteria have been available to the clinical laboratory since the late 1980's. Extraction and derivatization of mycolic acids from the cell wall of mycobacteria and subsequent chromatographic analysis yield distinctive peaks which are identified by computer-assisted pattern recognition programs. This method identifies a wide range of mycobacterial species, the test takes less than 2 hours, and the procedure can be combined with BACTEC to offer a rapid detection/identification scheme. It is recommended that larger Level III laboratories adopt this rapid technology for routine use (30).

Although gas liquid chromatography (GLC) has been used in the past for analysis of the mycolic acid in mycobacteria, at least one study has found that GLC alone is insufficient to accurately identify mycobacteria (41). The GLC method is not as widely used for mycobacteriology applications as AccuProbe and HPLC.

**84. Does your Mycobacteriology Laboratory not report the identification of *M. tuberculosis* based solely on polymerase chain reaction (PCR) results?**

Although PCR holds great promise for the future, researchers have concerns with nucleic amplification methods that must be addressed before widespread use can be recommended. Concurrent microbiological procedures for the isolation of *M. tuberculosis* are now and probably always will be appropriate for quality control of amplification techniques. The laboratory community is awaiting Food and Drug Administration (FDA) approval to assure that sufficient data exists on the false positive and false negative rates, predictive values, and reproducibility of PCR test results. Mycobacteriology laboratories must continue to rely on established techniques until the FDA approves amplification technologies (30).

*Mycobacteriology reference laboratories must continue to rely on established techniques to identify M. tuberculosis until amplification techniques are approved by FDA.*



## **ASTPHLD**

The Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) represents the state public health laboratory directors throughout the United States and its territories.

ASTPHLD maintains a Washington, D.C. headquarters office and seven Area Resource Offices of the National Laboratory Training Network (NLTN). NLTN is a cooperative training system sponsored in conjunction with the Centers for Disease Control and Prevention (CDC) that forms alliances among federal, state and local health agencies and private sector organizations to develop and deliver localized laboratory training programs based on documented need. ASTPHLD organizes and presents scientific conferences and symposia relevant to testing activities of public health laboratories. This project was co-sponsored as a joint activity of the ASTPHLD and the CDC.

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## MYCOBACTERIA AUDIOVISUAL RESOURCES AVAILABLE IN THE NLTN AREA RESOURCE OFFICES

Note: Some offices may have additional resources; please contact the NLTN office serving your area for specific resource information.

### MANUALS

"Laboratory Manual for Acid-Fast Microscopy", 2nd Ed. (CDC '76)

"Isolation and Identification of Mycobacterium Tuberculosis: A Guide for the Level II Laboratory" (CDC)

"Public Health Mycobacteriology: A Guide for the Level III Laboratory" (CDC:1985)

### VIDEOTAPES OF LABORATORY PROCEDURES

"Safety in the Mycobacteriology Laboratory" (CDC: 1982: 18 min)

"In Case of Accident...Control of Airborne Mycobacteria" (CDC: 1981: 17 min)

"Primary Isolation of Mycobacteria Using the N-Acetyl-L-Cysteine-Sodium Hydroxide Method" (CDC: 1980: 12 min)

Biochemical Identification of Mycobacteria I (CDC: 1980)

- "3-day Arylsulfatase Test for Mycobacteria" (6 min)
- "3-day Tellurite Reduction Test for Mycobacteria" (6 min)
- "Sodium Chloride Tolerance Test for Mycobacteria" (6 min)

"Acid Fast Microscopy" (CDC: 24 min)

"Specific Identification of *Mycobacterium tuberculosis*" (CDC: 12 min)

"Rough Grouping of Mycobacteria" (CDC: 15 min)

"Drug Susceptibility Testing of *Mycobacterium tuberculosis*" (CDC: 27 min)

Biochemical Identification of Mycobacteria II (CDC: 1985/86)

- "Urease Test for Mycobacteria" (10 min)
- "Iron Uptake Test for Mycobacteria" (10 min)
- "MacConkey Agar Test for Mycobacteria" (10 min)
- "Tween Hydrolysis Test for Mycobacteria" (9 min)

"Meeting the Challenge of Multi-Drug-Resistant TB: The Laboratory DOES Make a Difference" (CDC: 1992 : 18 minutes)

"Isolation and Identification of Mycobacterium Tuberculosis Module VI: Acid-Fast Microscopy in Mycobacteriology"

"A New Era in Mycobacteriology: Identification by High Performance Liquid Chromatography"  
(Waters: 1994)

"TB The New Epidemic" (Fulton, Comie, Canfield, Inc.)

"Isolation and Identification of Mycobacterium Tuberculosis Module V: Isolation of Mycobacteria Using N-Acetyl-L-Cysteine-NaOH for Digestion and Decontamination" (CDC: 1980: 10 min)

"Isolation and Identification of Mycobacterium Tuberculosis Module VII: Identification of *M. tuberculosis*" (CDC: 1985: 28 min)

"Preventing Tuberculosis in Children: We All Can Help" (Duke & Associates: 1994: 12 min)

"Tuberculin Skin Testing" - Mantoux (CDC: 20 min)

### **TRAINING COURSE PACKAGES**

Isolation and Identification of Mycobacterium tuberculosis: A Workshop for the Level II Laboratory  
(CDC: 1991)

Manual (Vol. I)

Slide Set (Vol. II)

Editor: Billie Ruth Bird

Original Authors: Patricia T. Kent, B.S., George P. Kubica, Ph.D. (Now Retired)

This 2½ day wet workshop is designed to provide essential information and laboratory experience for training personnel in a Level II mycobacteriology laboratory in: microscopic identification of AFB, culture of clinical specimens, identification and drug susceptibility testing of *M. tuberculosis* isolates. Both classic and rapid diagnostic methods are presented.

Note: Includes 2 volumes (printed materials and kodachrome slides) and 4 videotapes, "Isolation of Mycobacteria Using N-Acetyl-L-Cysteine-NaOH for Digestion and Decontamination," "Acid Fast

Microscopy in Mycobacteriology," "Identification of *M. tuberculosis*," "Drug Susceptibility Testing of *M. tuberculosis*."

Multidrug-Resistant Tuberculosis: The Laboratory Does Make a Difference (CDC: 1992)

Authors: Billie Ruth Bird, Jack T. Crawford, Ph.D., Bess Miller, M.D.

This half-day seminar deals with the critical issue of multidrug-resistant tuberculosis (MDRTB) by focusing on the need for laboratories to reduce turnaround time for testing and reporting tuberculosis. Information is provided on currently available laboratory strategies that can reduce the time for TB testing and reporting. Problem solving exercises and interactive class discussions provide examples and suggestions on ways to use the strategies.

## REFERENCE LABORATORY SCORES ON SELF-EVALUATION

As part of the pilot testing of this document, nine Level III laboratories were identified by the Technical Advisory Committee as well-established reference laboratories with excellent reputations in diagnostic mycobacteriology. No laboratories of committee members were included in the nine. Personnel in these nine Level III laboratories were asked to evaluate their laboratories using this document. They were asked to share their scores so that future users of this assessment tool would have a reference for comparison.

Results from the nine reference laboratories are presented in the chart below. Expressed in percent are the **Mean** or average scores and the range of scores. The highest percent (**High**) scored by a laboratory and the lowest percent (**Low**) scored are given below for each category.

To compare your laboratory to the experts, determine your scores (express them in percent) for each category and for the overall total. Enter your scores into the chart rows below marked **Your Laboratory's Scores**. Compare your scores to those of the experts. If your scores fall within the experts' range, you can infer that your overall laboratory practices are equivalent to the practices of some of the best mycobacteriology laboratories in the United States. If your scores are below the experts, this indicates the need to review your practices and institute changes.

Self-assessment questions written in red are critical issues and the results to these questions should be evaluated separately. A "no" to any of these question indicates a need for improving practices in that area.

### PERCENT SCORES FROM REFERENCE LABORATORIES

	Coll/Han	Safety	Lab Prac	QA/QC	CDC Rec	Overall
<b>High</b>	96.7%	100%	96.1%	98.3%	95.6%	95.4%
<b>Low</b>	71.7%	88.2%	78.6%	81.2%	81.5%	82.9%
<b>Mean</b>	90.8%	94.4%	88.6%	89.9%	89.8%	90.0%

Your Laboratory's Score

Date	Coll/Han	Safety	Lab Prac	QA/QC	CDC Rec	Overall