December 10, 2001

Participant
Centers for Disease Control and Prevention (CDC)
Mycobacterium tuberculosis Nucleic Acid Amplification Testing
Performance Evaluation Program

Subject: Analyses of Participant Laboratory Results for the June 2001 Shipment

Dear Participant:

Enclosed are analyses of laboratory test results reported to the Centers for Disease Control and Prevention (CDC) by participant laboratories for the June 2001 shipment of samples for the CDC *M. tuberculosis* Nucleic Acid Amplification (*M.tb* NAA) Testing Performance Evaluation Program. Participant laboratories received five individual samples. Testing results were received from 84 of 89 (94%) enrolled laboratories that received this shipment.

The enclosed aggregate report is prepared in a format that will allow laboratories to compare their results with those obtained by other participants for the same sample using the same *M.tb* NAA test method.

We encourage you to circulate this report to all personnel involved with *M.tb* NAA testing, interpreting, or reporting. If you have any comments or suggestions on the format selected for the results, or questions regarding this report, you may call Laurina Williams at (770) 488-8130.

Sincerely yours,

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Enclosures

Analyses of the June 18, 2001 Performance Evaluation Results for *M. tuberculosis* Nucleic Acid Amplification Testing Reported to the Centers for Disease Control and Prevention

This report is an analysis of laboratory test results reported to the Centers for Disease Control and Prevention (CDC) by participant laboratories for the samples containing *M. tuberculosis* or other mycobacteria shipped in June 2001. Testing results were received from 84 of 89 (94%) laboratories participating in this shipment. The *M.tb* NAA Performance Evaluation Program provides laboratories with assessment and evaluation of test methods and results. To maintain participant confidentiality, the CDC analyzes only participant data from which all laboratory identifiers have been removed by the contractor, Wisconsin State Laboratory of Hygiene.

Participant laboratories received five individual samples. Participants were requested to test the samples without the decontamination and concentration routinely performed on respiratory specimens prior to *M.tb* NAA testing. The specimen decontamination/concentration preparation steps for *M.tb* NAA testing were eliminated to allow this program to specifically assess *M.tb* NAA testing procedures (2,6).

Experiments were performed to document sample viability and test reactivity. Due to specific concerns of cross-contamination between *M.tb* NAA-positive and *M.tb* NAA-negative test samples, the negative samples were produced in a separate area. Additionally, 10% of both positive and negative samples were randomly selected and tested by the contractor to validate *M.tb* NAA test results. The test samples were also tested by five reference laboratories before shipping.

Figure 1 shows the laboratory classification represented by 82 participants. Participants consisted of 37 hospitals, 29 health departments, 12 independents, and 4 other types of laboratories.

Figure 2 provides the distribution of the volume of specimens tested with *M.tb* NAA by participating laboratories during the 3 months prior to reporting results. *The volume of specimens tested is represented in ranges that are multiples of 13 to estimate the average weekly test volume for participant laboratories during that period.

Figure 3 provides a breakdown of the *M.tb* NAA test procedures reported by the participating laboratories. Participants were asked to check all of the test methods used. Most of the participants (7/9) reporting the use of In-house and "Other" *M.tb* NAA test procedures used methods based on polymerase chain reaction (PCR). Although the CDC does not recommend the use of non-FDA cleared *M.tb* NAA test procedures (3,5), laboratories using In-house methods are encouraged to participate in this evaluation program to assess performance (2).

Figure 4 lists the biosafety levels reported by participant laboratories. All laboratories should routinely consult the CDC/NIH manual, Biosafety in Microbiological and Biomedical Laboratories (4th edition), for recommendations and for determining their correct biosafety level.

Participants were also asked to provide information on specific quality control practices related to the prevention of cross-contamination and subsequent false positives with NAA testing. Figure 5 provides the participant laboratory responses to a question about whether the biological safety cabinet (BSC) used for *M.tb* NAA testing is used for other purposes. One concern is that 18% (15/82) of participant laboratories indicated that they process *M.tb* specimens in the same BSC that is used for *M.tb* NAA testing. Among the 30% (25/82) of participants that indicated "Other" uses for the *M.tb* NAA testing BSC, 12 performed *M.tb* culture work (biochemicals, drug susceptibility testing, Accuprobe® identification, etc.), 9 performed mycology, and 4 performed other microbiology or clinical specimen work. Three laboratories reported using the same BSC for bioterrorism-related work and other procedures. Laboratories should be aware of recommendations (4) to perform specimen processing and NAA testing in separate work areas with separate equipment.

Figure 6 provides participant responses to a question on the use of uni-directional workflow for *M.tb* NAA testing. In addition to recommendations (4) that emphasize considerations of laboratory design for NAA testing, both manufacturers (Roche Amplicor® and Gen-Probe® MTD) recommend the use of unidirectional workflow. It is concerning that 11% (9/82) responding laboratories reported that unidirectional workflow is not being used.

Separate figures and tables are provided to show either the qualitative or quantitative results reported for each sample by the participant laboratories. Quantitative results for the In-house methods could not be presented in a consistent format since participants used a variety of detection systems and test interpretation criteria. The Roche Amplicor® test has interpretive criteria for quantitative results that reflect some probability that the sample is positive but are below the recommended threshold for positivity. The result form and this report use the term "equivocal" for Roche Amplicor®, to reflect the manufacturer's recommendation for reporting indeterminate quantitative test results.

Figure 7 provides a summary of the participant qualitative results reported for all five samples by test method. The aggregate participant qualitative results are indicated for the 2 *M.tb*-positive and 3 *M.tb*-negative samples. The combined analytical sensitivity of all methods was 88.8% (151/170) for the 2 *M.tb*-positive samples: 99.2% (119/120) sensitivity for Gen-Probe® MTD; 63.9% (23/36) sensitivity for Roche Amplicor®; 64.3% (9/14) sensitivity for In-house methods. The combined analytical specificity of all methods was 98.4% (251/255) for the 3 *M.tb*-negative samples: 97.8% (176/180) specificity for Gen-Probe®; 100% (54/54) specificity for Roche Amplicor®; 100% (21/21) specificity for In-house methods.

The low sensitivities observed for the Roche Amplicor® and In-house methods were primarily due to the 15 false-negative and 2 equivocal results reported for sample TB01-06-4. This was a mock sample diluted to contain 300 *M. tuberculosis* cells per ml, a very low concentration that may resemble some AFB smear-negative specimens. Thus, the sensitivity threshold of these methods was

approached using this very low concentration sample. CDC recommendations do not endorse the use of methods which have not been specifically evaluated and cleared by the FDA for testing smear-negative specimens. (1)

The four positive interpretations reported for negative samples using Gen-Probe® MTD were apparently random. Neither the participant results nor the reference laboratory results indicate cross-reaction of the Gen-Probe® MTD test with *M. terrae* or *M. gordonae.*

Figure 8 is graphical representation of the quantitative results reported for each sample by participant laboratories using the Gen-Probe® MTD test. The indention in each box-plot indicates the median value. The shaded area within the box represents the results between the 25th percentile and 75th percentile of the data. The bracketed areas designate either 1.5 times the interquartile range of the data or the most extreme data point on either side of the median, whichever is the least distance from the median. Each value reported which was outside these ranges is signified by one of the solid lines drawn outside the brackets. For the positive samples, TB01-06-2 and TB01-06-4 the median values of all data were 2,692,228 and 2,918,114 relative light units (RLU), respectively. The median value for the negative sample containing *M. gordonae*, TB01-06-1, was 3,040 RLU. For the samples containing *M. terrae*, TB01-06-3 and TB01-06-5 the median values were 2,949 and 2,914 RLUs, respectively.

Figure 9 is a graphical representation of all quantitative results reported for each sample by participant laboratories using the Roche Amplicor® test. The solid line through each set of data represents the median value for each sample. The shaded band represents the equivocal range. For all the positive samples, TB01-06-2 and TB01-06-4 the median values were 3.000 (A_{450}) and 0.086 (A_{450}), respectively. The very low median value for sample TB01-06-4, compared with other positive samples used in this program, was due to the 11 false-negative and 2 equivocal interpretations reported for this sample. The range of values reported for this sample was 0.004 (A_{450}) - 3.075 (A_{450}). All interpretations were correct, based upon the Roche Amplicor® criteria for interpreting quantitative results. The median values for the negative sample containing *M. gordonae*, TB01-06-1, was 0.056 (A_{450}). The median values for the samples containing *M. terrae,* TB01-06-3 and TB01-06-5, were 0.060 (A_{450}), and 0.064 (A_{450}), respectively.

Tables 1-5 provide the qualitative results reported for individual samples by participants. In most instances the laboratories used the manufacturer's recommended interpretations of quantitative test results. The low overall sensitivity in detecting positive samples was affected by 13/18 false-negative or equivocal interpretations reported for sample TB-06-4 using the Roche Amplicor® method, and 4/7 false-negative interpretations using In-house methods. This sample was designed to contain a very low concentration of *M.tb*, 300 org/ml, such as might be encountered with some smear-negative specimens. The sample contained viable organisms as determined through plate counts. The false-negative results could have been related to the low sample volume used in some procedures. Nevertheless, the sensitivity threshold for Roche Amplicor® and In-house methods was apparently approached with this sample. Based upon these results, the use of methods not specifically cleared by the FDA with smear-

negative specimens is contraindicated, and may lead to false-negative results. The overall specificity was similar to previous results in challenge shipments.

References

1. CDC. Update: Nucleic Acid Amplification Tests for Tuberculosis. MMWR 2000; 49:593-594.

2. CDC. Nucleic acid amplification tests for tuberculosis. MMWR 1996; 45:950-951.

3. CDC. Notice to Readers. Diagnosis of tuberculosis by Nucleic Acid Amplification methods applied to clinical specimens. MMWR 1993; 42:686.

4. NCCLS - Molecular Diagnostic Methods for Infectious Diseases; Approved Guidelines (NCCLS Document MM3-A, 1995).

5. Noordhoek GT, van Embden JDA, Kolk AHJ. Reliability of nucleic acid amplification for detection of *Mycobacterium tuberculosis*: an international collaborative quality control study among 30 laboratories. J. Clin. Microbiol. 1996; 34:2522-2525.

6. Noordhoek GT, Kolk AHJ, Bjune G, Catty D, Dale JW, Fine PEM, Godfrey-Faussett P, Cho S-N, Shinnick T, Svenson SB, Wilson S, van Embden JDA. Sensitivity and specificity of PCR for detection of *Mycobacterium tuberculosis*: A blind comparison study among seven laboratories. J. Clin. Microbiol. 1994;32:277-285.

7. CDC. Multiple misdiagnoses of tuberculosis resulting in laboratory error-Wisconsin, 1996. MMWR 1997; 46:797-801.

Figure 1. Primary Classification of Participating Laboratories

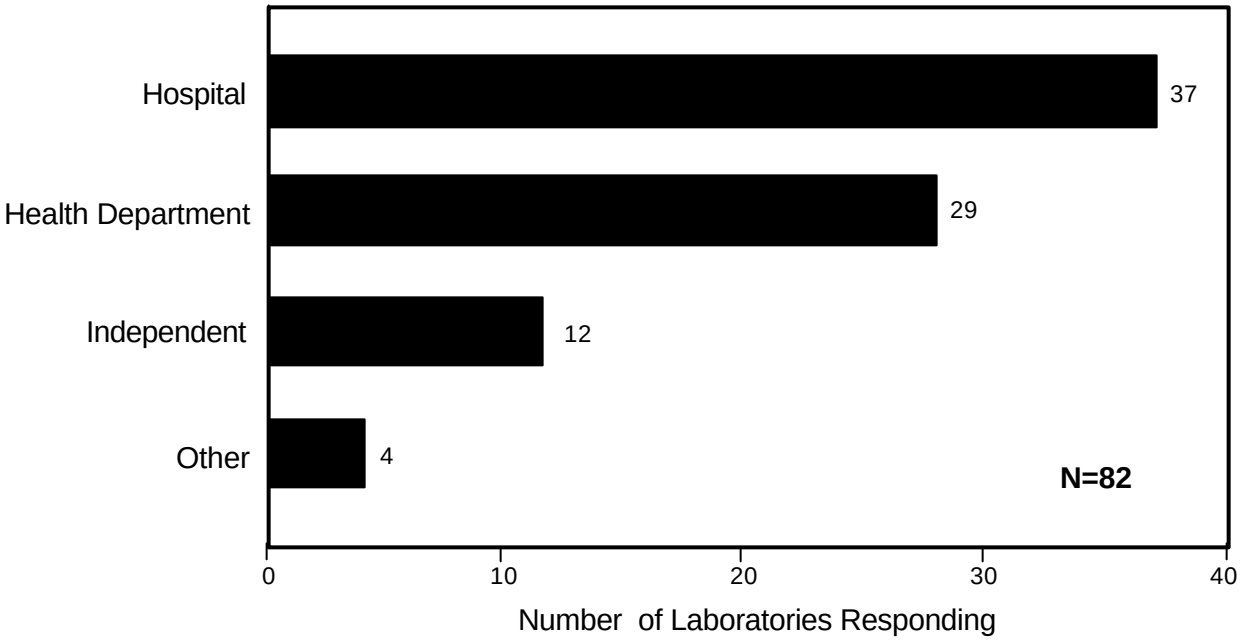
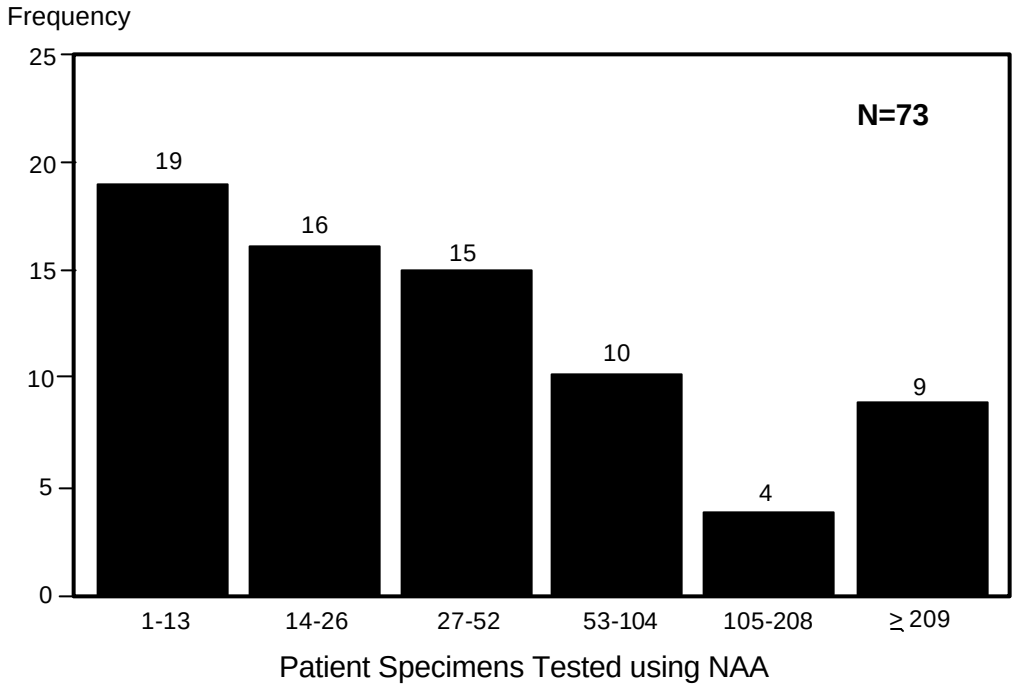


Figure 2. Number of Patient Specimens Tested for *M.tb* Using TB NAA during the Previous Quarter.*



*See explanation in the analysis.

Figure 3. Amplification Procedure Used for Direct Detection of *M.tb*

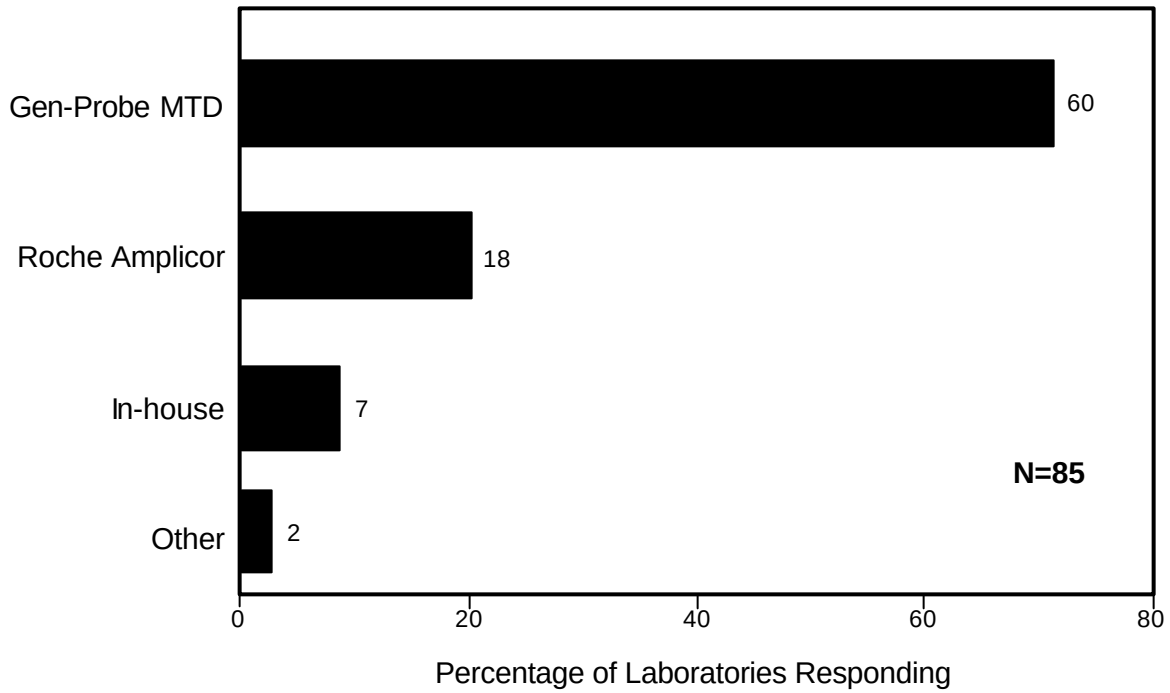


Figure 4. Biosafety Levels of Participant Laboratories

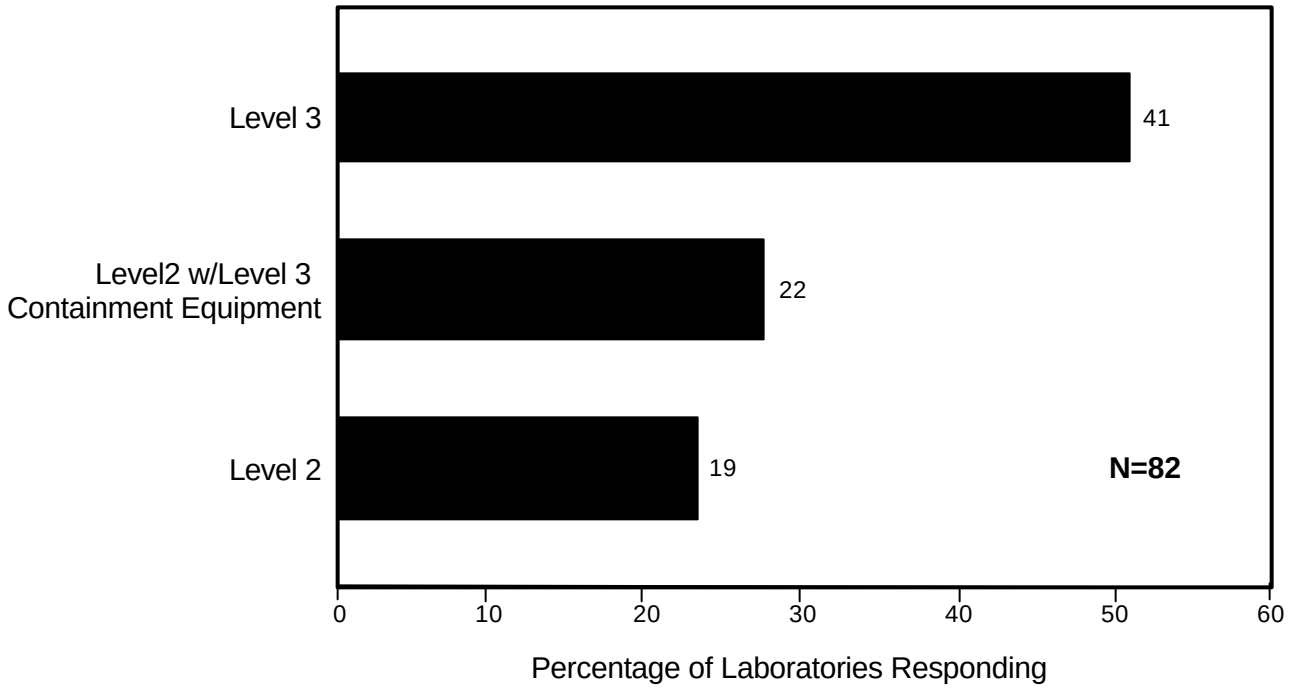


Figure 5. Is the Biological Safety Cabinet that is Used for TB NAA Testing Used for Other Purposes?

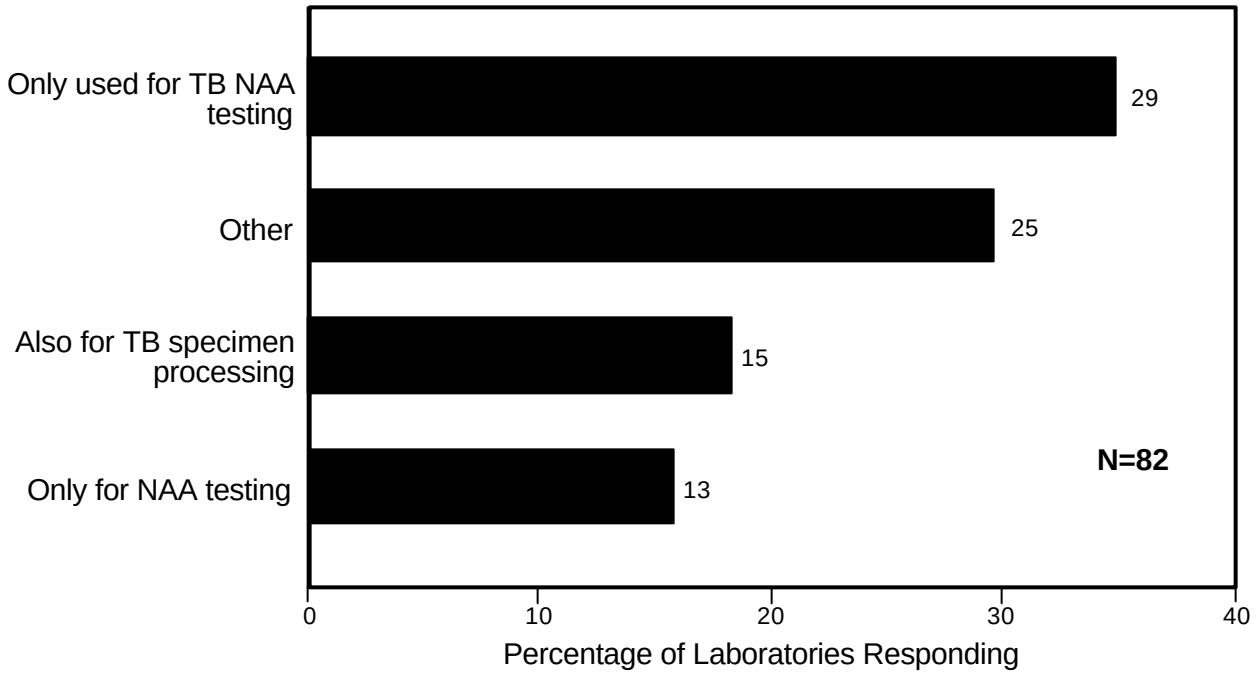


Figure 6. Use of Uni-directional Workflow by Participating Laboratories

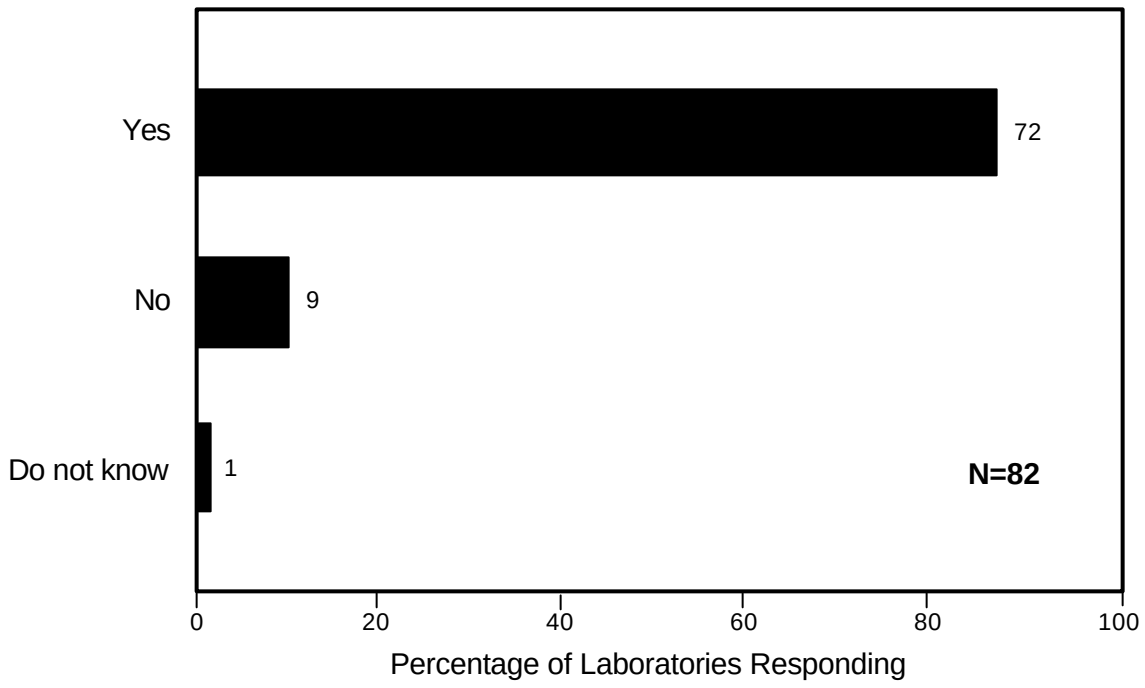


Figure 7. Frequency of TB NAA Qualitative Test Results by Sample Type for the Gen-Probe MTD, Roche Amplicor, and In-House Methods

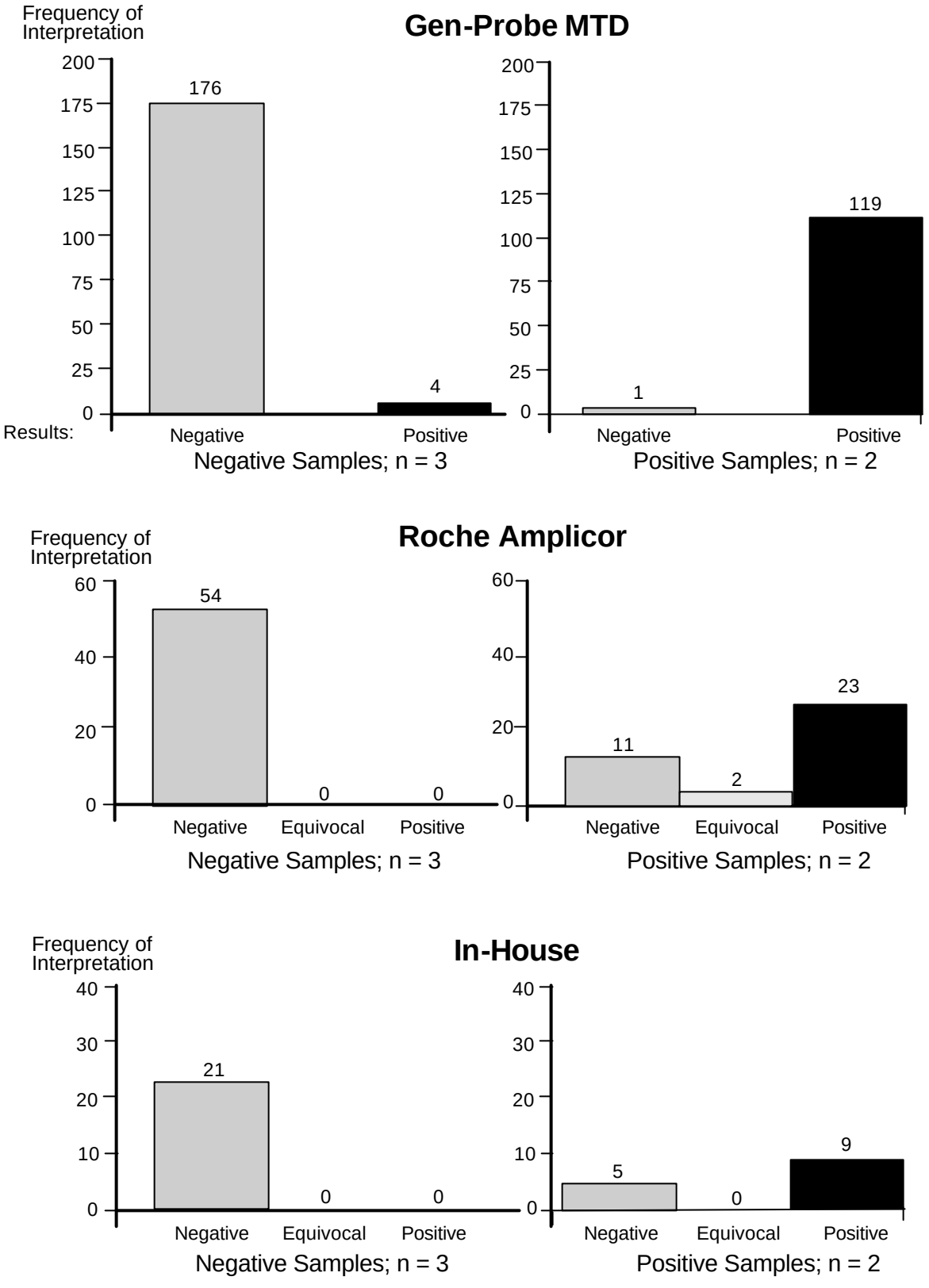
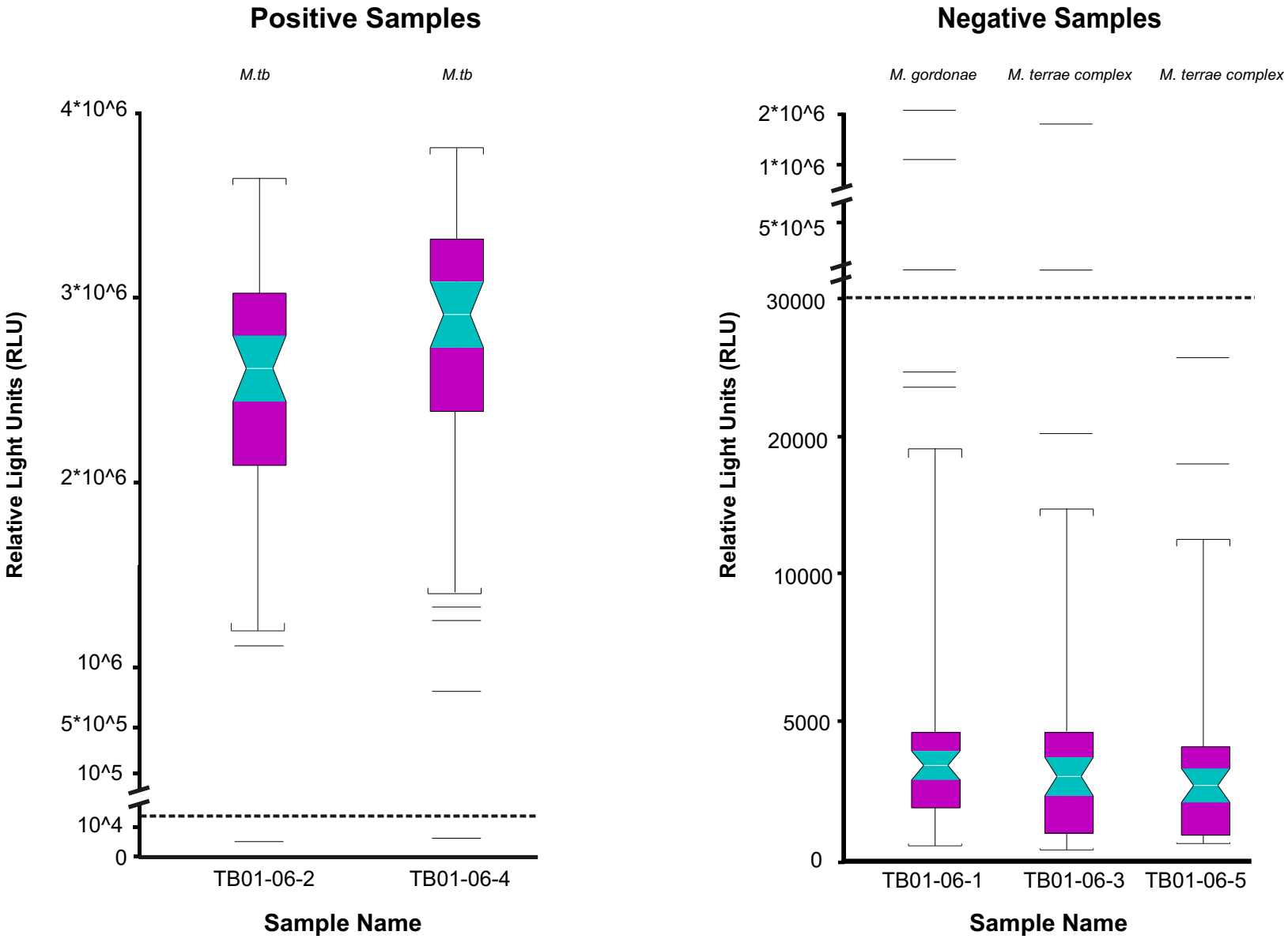
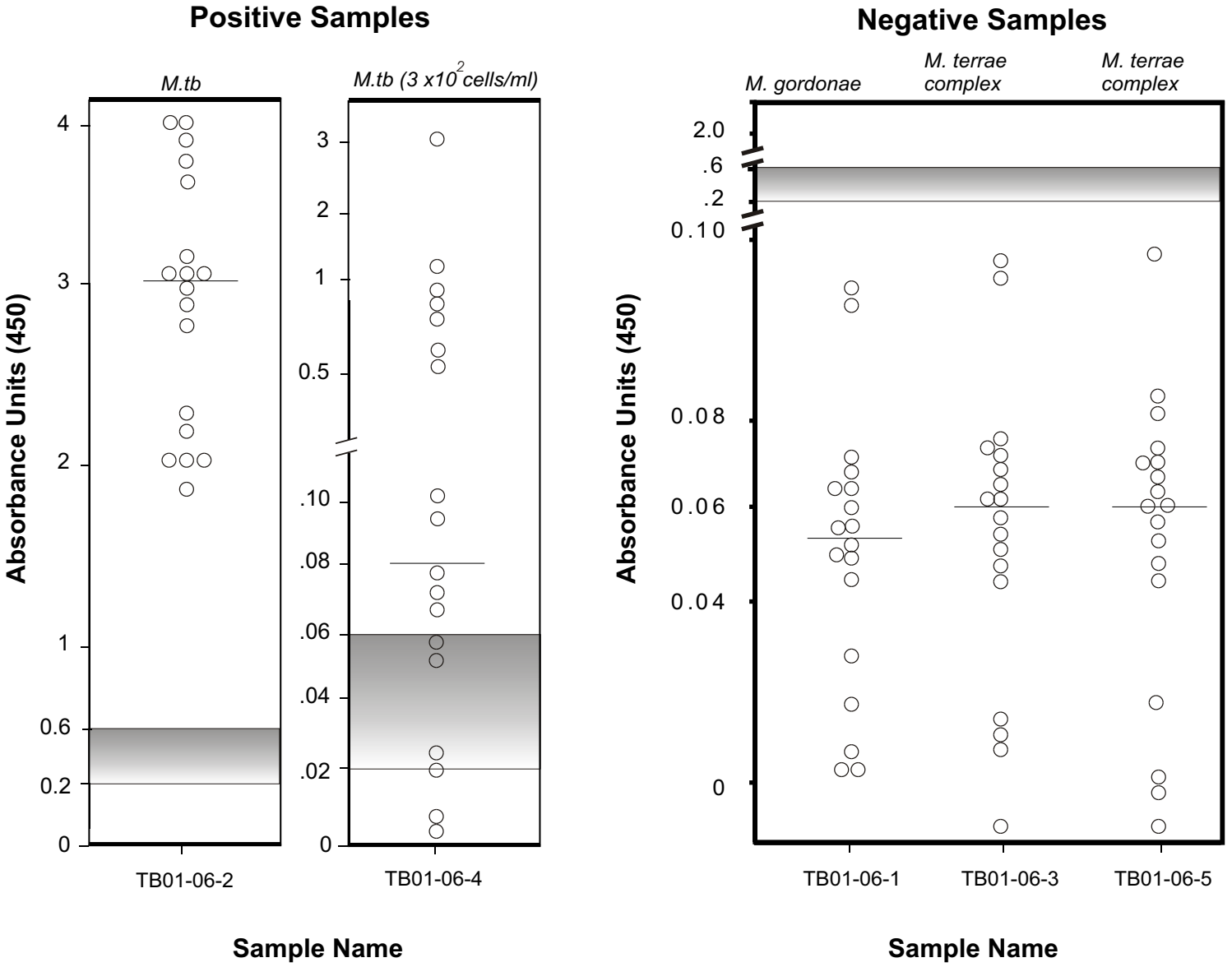


Figure 8. Quantitative Results for GenProbe® MTD



Note: Dashed line (**---**) represents cut-off between positive and negative values (30,000 RLUs).

Figure 9. Quantitative Results for Roche Amplicor®



Note: Shaded areas represent equivocal range.

The following tables summarize qualitative results reported by participant laboratories for the June 2001 shipment of samples for the *M. tb.* NAA testing performance evaluation program.

Table 1. Sample TB01-06-1 contained *Mycobacterium gordonae*

Test Methods	No. Tests Performed	Positive		Equivocal		Negative	
		No.	%	No.	%	No.	%
Gen-Probe	60	3	5.0	0	0	57	95.0
In-house	7	0	0	0	0	7	100
Roche	18	0	0	0	0	18	100
All methods	85	3	3.5	0	0	82	96.5

Table 2. Sample TB01-06-2 contained *Mycobacterium tuberculosis*

Test Methods	No. Tests Performed	Positive		Equivocal		Negative	
		No.	%	No.	%	No.	%
Gen-Probe	60	59	98.3	0	0	1	1.7
In-house	7	6	85.7	0	0	1	14.3
Roche	18	18	100	0	0	0	0
All methods	85	83	97.6	0	0	2	2.4

Table 3. Sample TB01-06-3 contained *Mycobacterium terrae*

Test Methods	No. Tests Performed	Positive		Equivocal		Negative	
		No.	%	No.	%	No.	%
Gen-Probe	60	1	1.7	0	0	59	98.3
In-house	7	0	0	0	0	7	100
Roche	18	0	0	0	0	18	100
All methods	85	1	1.2	0	0	84	98.8

Table 4. Sample TB01-06-4 contained *Mycobacterium tuberculosis*

Test Methods	No. Tests Performed	Positive		Equivocal		Negative	
		No.	%	No.	%	No.	%
Gen-Probe	60	60	100	0	0	0	0
In-house	7	3	42.9	0	0	4	57.1
Roche	18	5	27.8	2	11.1	11	61.1
All methods	85	68	80.0	2	2.4	15	17.6

Table 5. Sample TB01-06-5 contained *Mycobacterium terrae*

Test Methods	No. Tests Performed	Positive		Equivocal		Negative	
		No.	%	No.	%	No.	%
Gen-Probe	60	0	0	0	0	60	100
In-house	7	0	0	0	0	7	100
Roche	18	0	0	0	0	18	100
All methods	85	0	0	0	0	85	100