Analyses of the January 27, 2003 Performance Evaluation Results for *Mycobacterium tuberculosis* Nucleic Acid Amplification Testing Reported to the Centers for Disease Control and Prevention

Report Highlights

Laboratories performed *Mycobacterium tuberculosis* nucleic acid amplification (*M.tb* NAA) testing very well on the January 2003 shipment samples.

Overall Summary of Results

			Positive Donors	Negative Donor	
Method	**Total # of laboratories**	**Total # of results**	**False-negative results**	**False-positive results**	**Overall Performance**
Gen-Probe MTD	60	302	2/122 (1.6%)	4/180 (2.2%)	98.0%
Roche Amplicor	17	85	1/34 (2.9%)	1/51 (2.0%)	97.6%
In-house/Other	8	40	None	None	100%

New Findings

• The range of Gen-Probe® MTD values for all negative samples in this shipment included a greater proportion of higher values (>20,000 RLUs) than usual.

• The three false-negative interpretations reported for positive samples appeared to be random, and there was no evidence of cross-contamination.

• The five false positive results for samples TB03-01-2 and TB03-01-4 containing *M. avium* were apparently random; however, these were more false positive results than in other recent shipments.

• To check for possible cross-reactivity, we included a sample containing *Corynebacterium striatum*, a bacterium with a similar genomic G/C ratio as *M. tuberculosis*, and a component of the normal upper respiratory tract flora. No cross-reactivity was observed.

Findings of note that also have been reported previously

• Of participant laboratories, 14% (12/85) indicated they process *M. tuberculosis* specimens in the same biosafety cabinet (BSC) that is used for *M.tb* NAA testing. Twenty-eight percent (24/85) of participants indicated "Other" uses for the *M.tb* NAA testing BSC.

• It is a concern that 13% (11/85) of responding laboratories reported that unidirectional workflow is not used, or that they do not know if it is used.

Introduction

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 samples shipped in January 2003. Testing results were received from 86 of 87 (99%) 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.

Challenge Samples

Participant laboratories received five individual samples. Participants were requested to test the samples without the decontamination and concentration procedures 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.

Results

Figure 1 shows the laboratory classification represented by 84 participants. Participants consisted of 36 hospitals, 34 health departments, 12 independents, and 2 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.

Figure 3 provides a breakdown of the *M.tb* NAA test procedures reported by the participating laboratories. Participants were asked to check all test methods used. All of the participants (8/8) 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 approved *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 (4^{th} 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 nucleic acid amplification (NAA) testing. Figure 5 provides the participant laboratory responses to a question about whether the biological safety cabinet used for *M.tb* NAA testing is used for other purposes. One concern is that 14% (12/85) of participant laboratories indicated that they process *M. tuberculosis* specimens in the same BSC that is used for *M.tb* NAA testing. Among the 28%

(24/85) of participants that indicated "Other" uses for the *M.tb* NAA testing BSC, 12 performed *M. tuberculosis* culture work (biochemicals, drug susceptibility testing, Accuprobe® identification, etc.), 11 performed mycology, and one performed other microbiology or clinical specimen work. Three laboratories reported using the same BSC for bioterrorism-related work. 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 a concern that 13% (11/85) of responding laboratories reported that unidirectional workflow is not being used, or that they do not know if unidirectional workflow is 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 is 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 positive and 3 negative samples. The combined analytical sensitivity of all methods was 98% (169/172) for the 2 positive samples: 98% (120/122) sensitivity for Gen-Probe® MTD; 97% (33/34) sensitivity for Roche Amplicor®; 100% (16/16) sensitivity for In-house methods. The combined analytical specificity of all methods was 98% (250/255) for the 3 negative samples: 98% (176/180) specificity for Gen-Probe®; 98% (50/51) specificity for Roche Amplicor®; 100% (24/24) specificity for In-house methods. Samples TB03-01-2 and TB-03-01-4 contained 3×10^3 theoretical cells/ml of *M. avium*. Sample TB03-01-5 contained 3×10^5 theoretical cells/ml of *Corynebacterium striatum*.

Figure 8 is graphical representation of the quantitative results reported for each sample by participant laboratories using the Gen-Probe® MTD test. The indentation in each box-plot indicates the median value. The shaded area within the box represents the results between the 25^{th} percentile and 75^{th} 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, TB03-01-1 and TB03-01-3, the median values of all data were 2,599,850 and 2,560,558 relative light units (RLU), respectively. The median value for the negative samples containing *M. avium*, TB03-01-2 and TB03-01-4, were 2,451 and 2,738 relative light units (RLU) respectively. For the sample containing *Corynebacterium striatum*, TB03-01-5, the median value was 2,598. A greater proportion of higher Gen-Probe® MTD RLU values (>20,000 RLUs) for negative samples was

observed, as is indicated by the solid lines above the bracketed ranges. The range of Gen-Probe® MTD values for all negative samples in this shipment included a greater proportion of higher values (>20,000 RLUs) than usual. The higher values for negative samples seemed to be randomly distributed between samples and laboratories. The reason for this is unclear. We will continue to track trends in future shipments.

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. The median value was 3.000 (A_{450}) for both positive samples, TB03-01-1 and TB03-01-3. The median values for the samples containing *M. avium*, TB03-01-2 and TB03-01-4, were 0.053 (A_{450}) and 0.055 (A_{450}) respectively. The median value for the sample containing *Corynebacterium striatum*, TB03-01-5, was 0.056 (A_{450}).

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; however, there were exceptions. The three false-negative interpretations reported for positive samples appeared to be random, and there was no evidence of cross-contamination. The 5 false positive results for samples TB03-01-2 and TB03-01-4 containing *M. avium* appeared random; however, there were more false positive results than in other recent shipments. In this shipment we included a sample containing *Corynebacterium striatum*, a bacterium with a similar genomic G/C ratio as *M. tuberculosis*, and a component of the normal upper respiratory tract flora, to check for possible cross-reactivity. All results for sample TB03-01-5, containing *Corynebacterium striatum*, were correctly interpreted as negative. Overall, composite results for all samples were relatively accurate and indicate that laboratories performed very well.

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

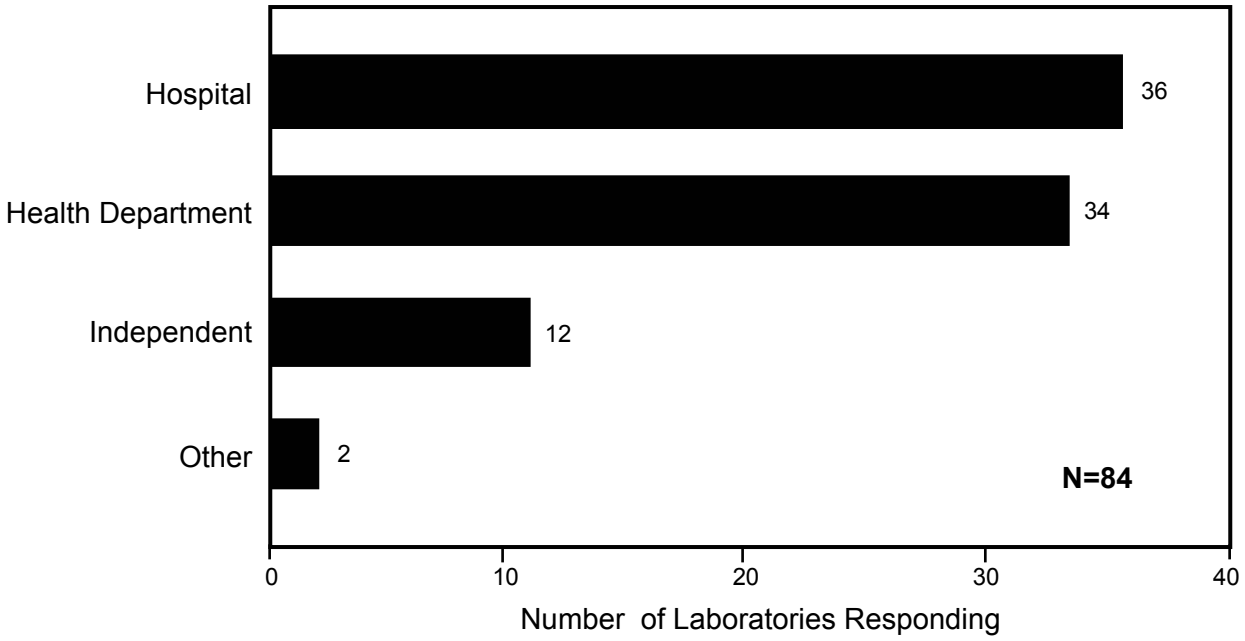
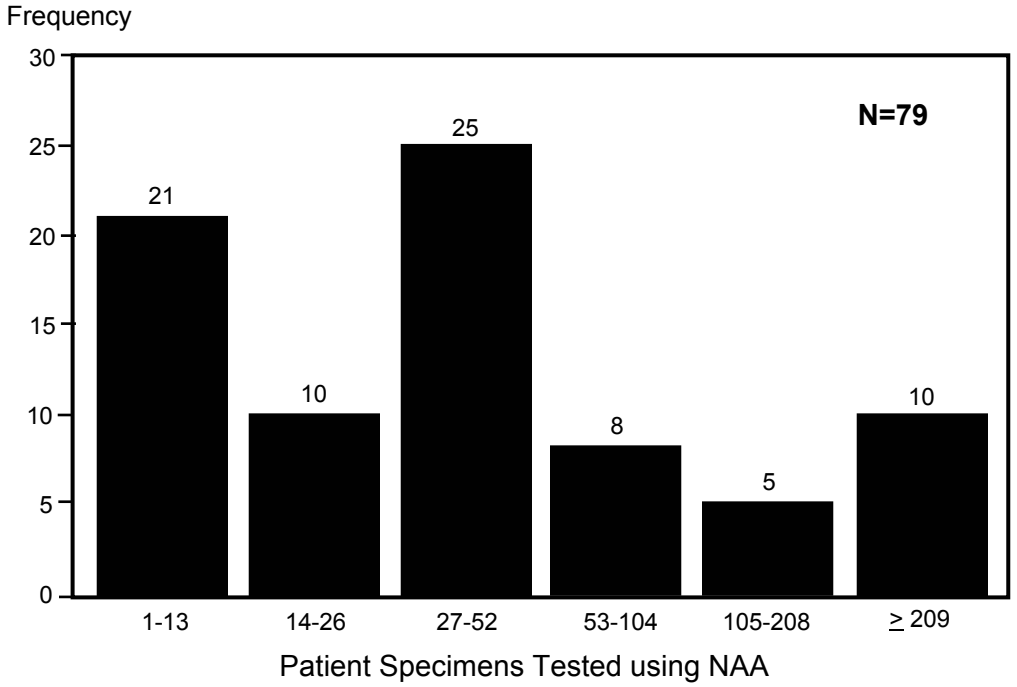


Figure 2. Number of Patient Specimens Tested for *M.tb* Using TB NAA during the Previous Quarter.*



*See explanation in the analysis.

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

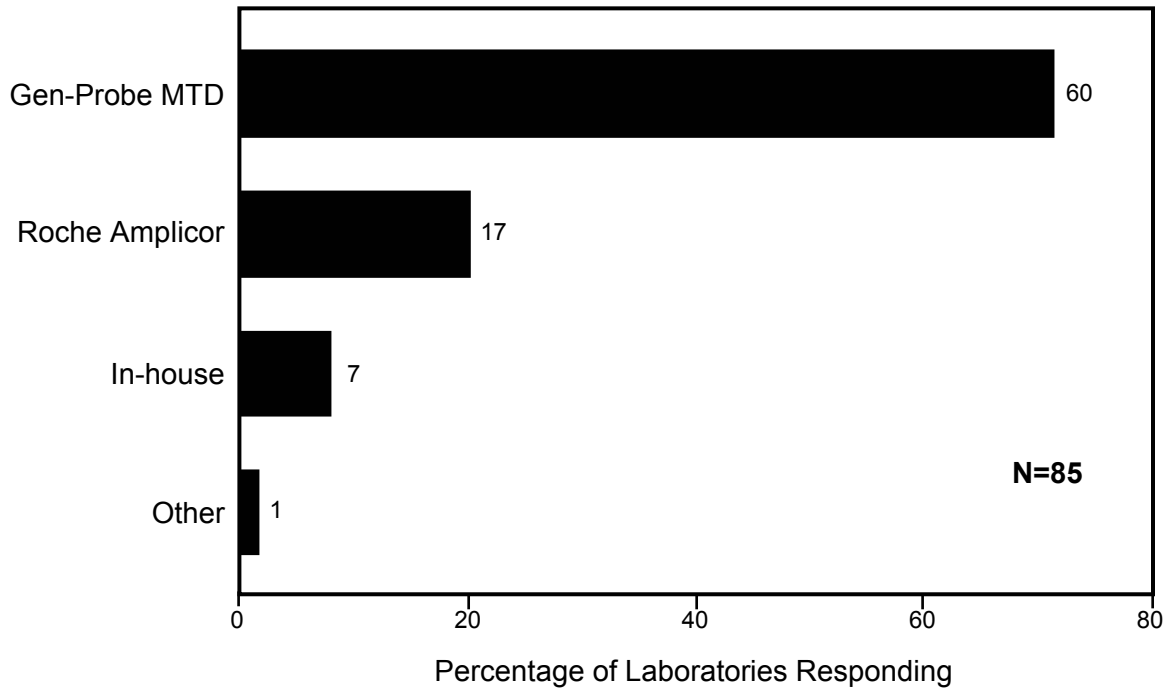


Figure 4. Biosafety Levels of Participant Laboratories

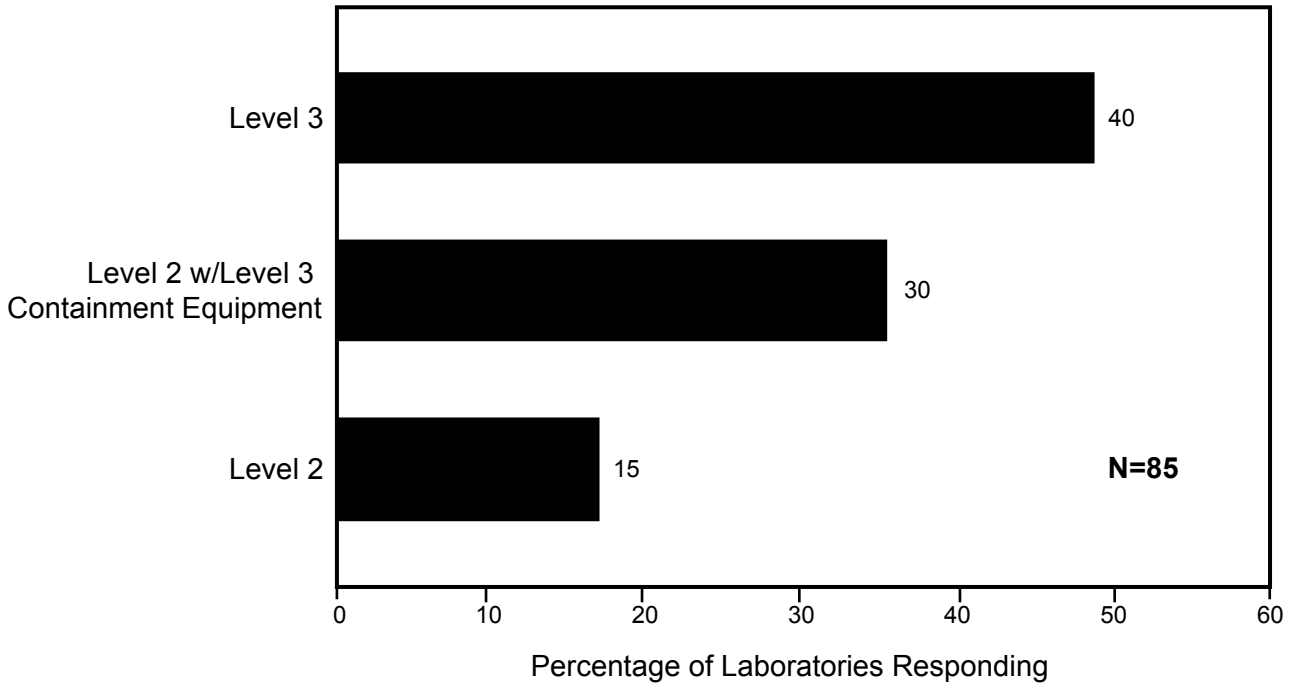


Figure 5. Is the Biological Safety Cabinet that is Used for TB NAA Testing Used for Other Purposes?

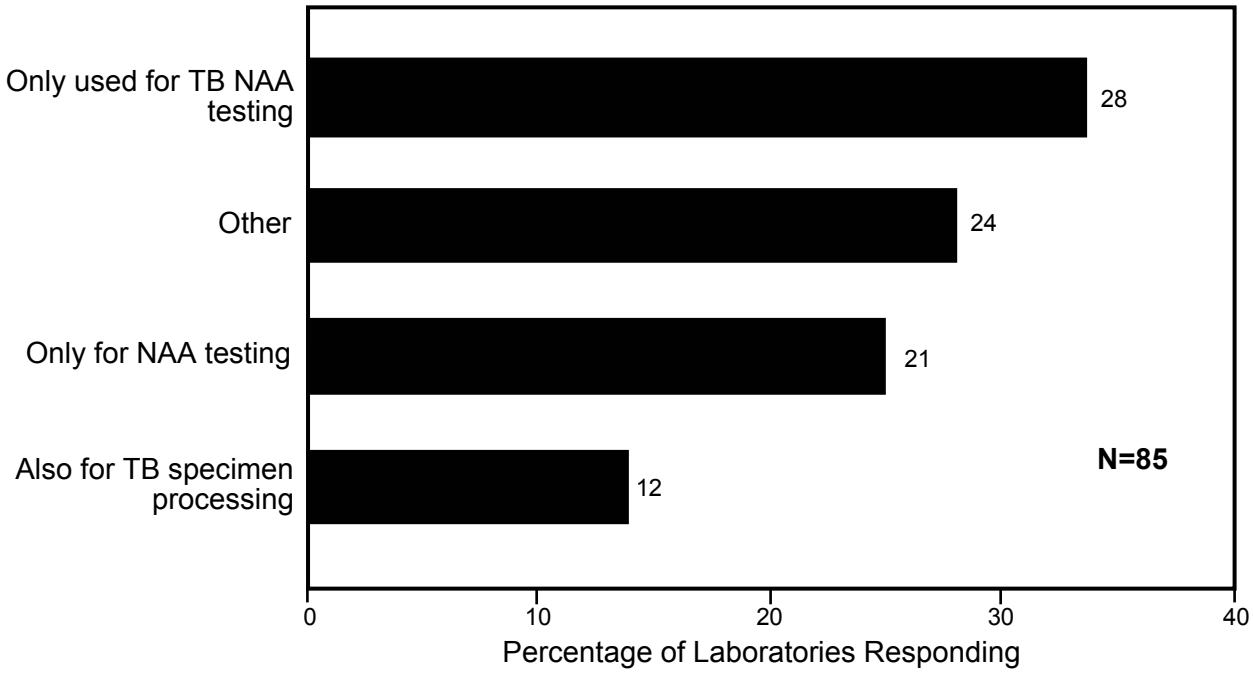


Figure 6. Use of Uni-directional Workflow by Participating Laboratories

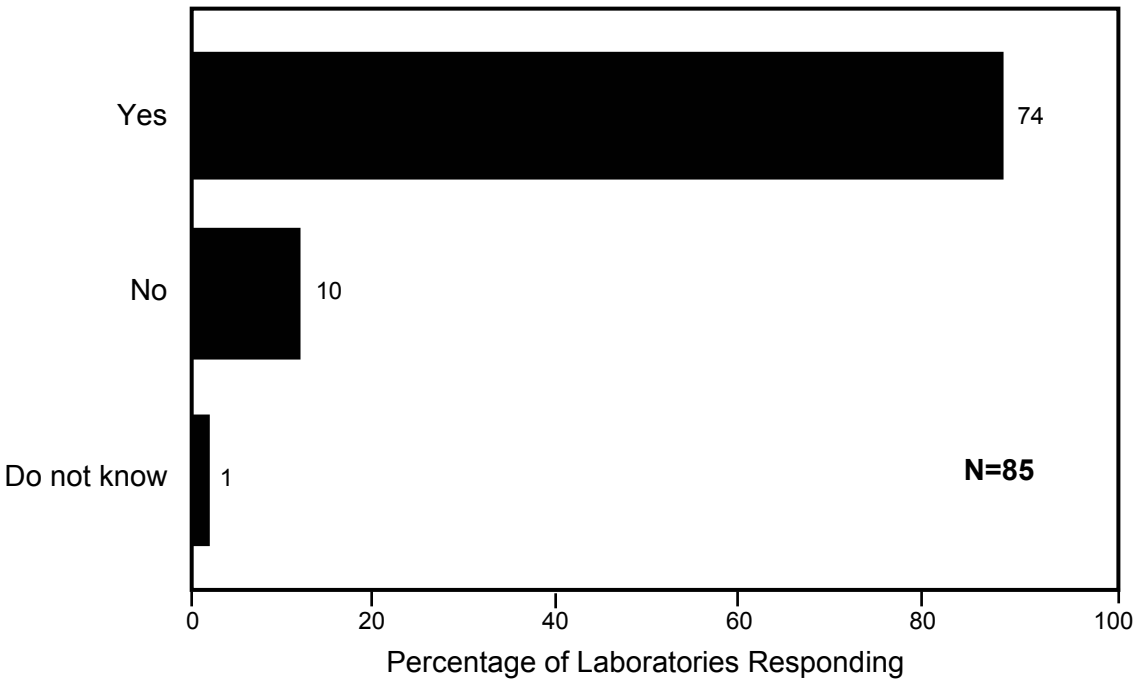


Figure 7. Frequency of TB NAA Qualitative Test Results by Sample Type for the Gen-Probe MTD, Roche Amplicor, and In-House Methods

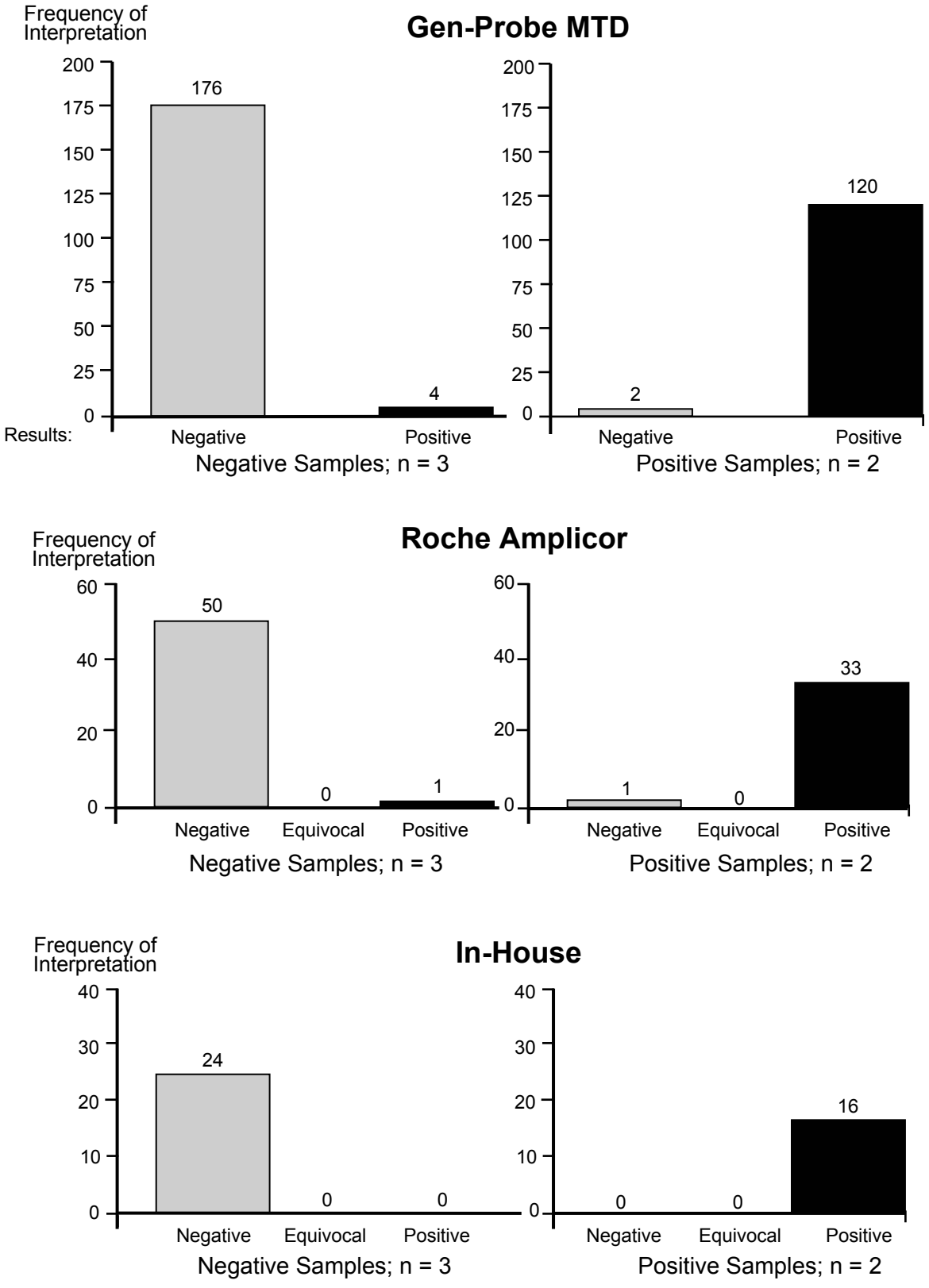
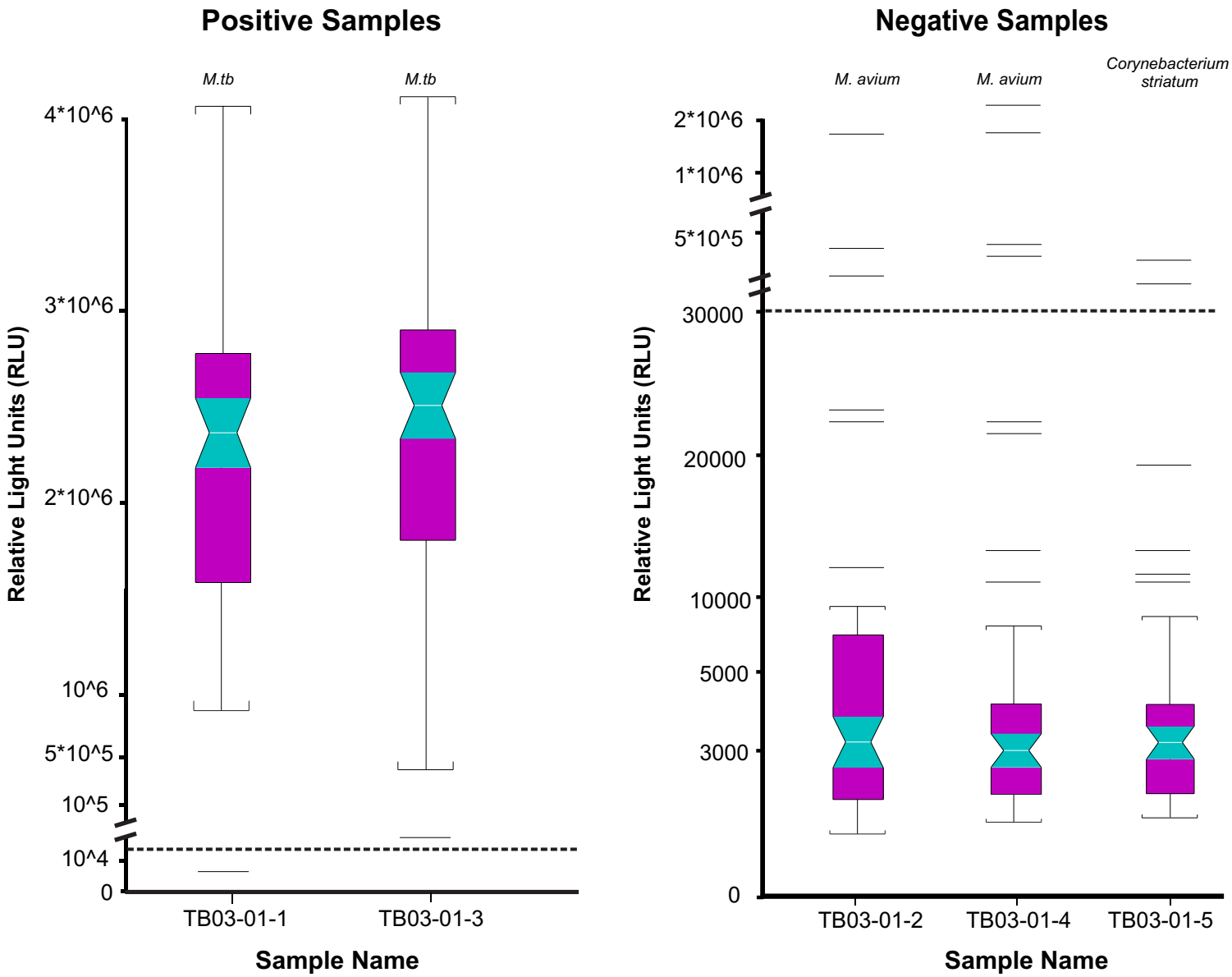
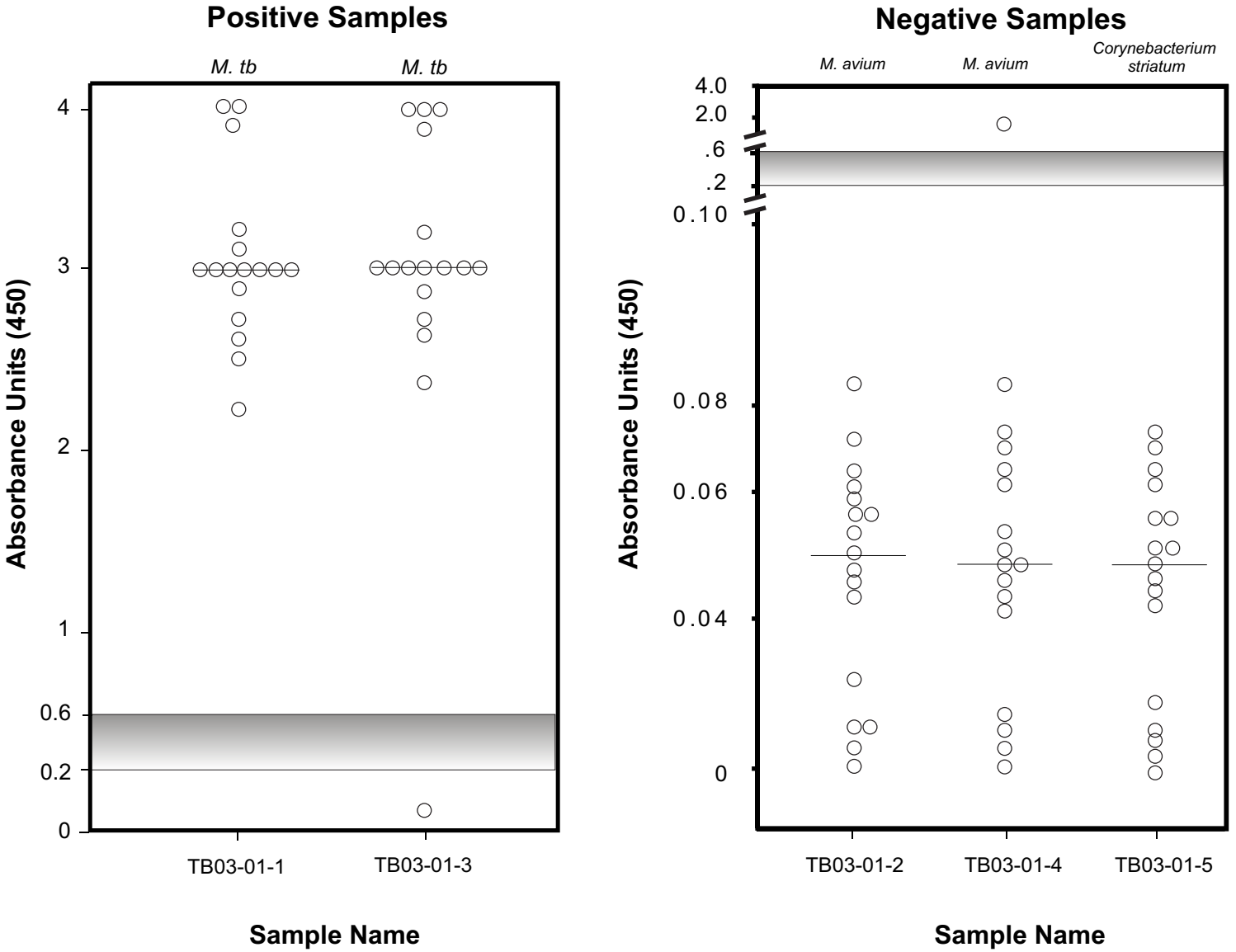


Figure 8. Quantitative Results for GenProbe® MTD



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 January 2003 shipment of samples for the *M. tb* NAA testing performance evaluation program.

Table 1. Sample TB03-01-1 contained *Mycobacterium tuberculosis*

Test Methods	No. Tests Performed	Positive No.	Positive %	Equivocal No.	Equivocal %	Negative No.	Negative %
Gen-Probe	61	60	98.4	0	0.0	1	1.6
In-house	8	8	100.0	0	0.0	0	0.0
Roche	17	17	100.0	0	0.0	0	0.0
All methods	86	85	98.8	0	0.0	1	1.2

Table 2. Sample TB03-01-2 contained *Mycobacterium avium* complex

Test Methods	No. Tests Performed	Positive No.	Positive %	Equivocal No.	Equivocal %	Negative No.	Negative %
Gen-Probe	60	1	1.7	0	0.0	59	98.3
In-house	8	0	0.0	0	0.0	8	100.0
Roche	17	0	0.0	0	0.0	17	100.0
All methods	85	1	1.2	0	0.0	84	98.8

Table 3. Sample TB03-01-3 contained *Mycobacterium tuberculosis*

Test Methods	No. Tests Performed	Positive No.	Positive %	Equivocal No.	Equivocal %	Negative No.	Negative %
Gen-Probe	61	60	98.4	0	0.0	1	1.6
In-house	8	8	100.0	0	0.0	0	0.0
Roche	17	16	94.1	0	0.0	1	5.9
All methods	86	84	97.7	0	0.0	2	2.3

Table 4. Sample TB03-01-4 contained *Mycobacterium avium* complex

Test Methods	No. Tests Performed	Positive No.	Positive %	Equivocal No.	Equivocal %	Negative No.	Negative %
Gen-Probe	60	3	5.0	0	0.0	57	95.0
In-house	8	0	0.0	0	0.0	8	100.0
Roche	17	1	5.9	0	0.0	16	94.1
All methods	85	4	4.7	0	0.0	81	95.3

Table 5. Sample TB03-01-5 contained *Corynebacterium striatum*

Test Methods	No. Tests Performed	Positive No.	Positive %	Equivocal No.	Equivocal %	Negative No.	Negative %
Gen-Probe	60	0	0.0	0	0.0	60	100.0
In-house	8	0	0.0	0	0.0	8	100.0
Roche	17	0	0.0	0	0.0	17	100.0
All methods	85	0	0.0	0	0.0	85	100.0