June 15, 1999

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 February, 1999 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 February 1999 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 91of 97 (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 who are 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 February 15, 1999 Performance Evaluation Results for *M. tuberculosis* Nucleic Acid Amplification Testing Reported to the Centers for Disease Control and Prevention by Participating Laboratories

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 February, 1999. Testing results were received from 91 of 97 (94%) laboratories participating in this shipment. This program was developed to provide laboratories with assessment and evaluation of test methods and results. To maintain participant confidentiality the CDC only analyzes 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 avoided to allow this program to specifically assess *M.tb* NAA testing procedures (1,5).

Experiments were performed to document sample viability and test reactivity after holding samples at refrigeration and room temperature for varying periods of time. 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 six reference laboratories before shipping.

Figure 1 shows the laboratory classification represented by 90 of the 91 participants. Participants consisted of 44 hospitals, 27 health departments, 13 independents, and 6 other types of laboratories.

Figure 2 provides the distribution of the volume of specimens tested with *M.tb* NAA by participating laboratories between January 1 and December 31, 1998. The volume of specimens tested is represented in ranges that are multiples of 50 to approximate the average weekly test volume for participant laboratorie during 1999.

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. In the section for laboratory results the “in-house” and “other” *M.tb* NAA test procedures were combined and labeled as in-house test results. All “in-house” and “other” *M.tb* NAA test procedures reported were based on polymerase chain reaction (PCR). Although the CDC does not recommend the use of non-FDA cleared *M.tb* NAA test procedures (2,4) laboratories using in-house methods are encouraged to participate in this evaluation program to assess performance.

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 (3rd 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 23% (21/91) of participant laboratories indicated that they process *M.tb* specimens (such as performing digestion/decontamination procedures) in the same BSC that is used for *M.tb* NAA testing. Among the 29% (26/91) of participants that indicated "other" uses for the *M.tb* NAA testing BSC, 11 performed *M.tb* culture work (biochemicals, drug susceptibility testing, Accuprobe® identification, etc.), 13 performed mycology, and 2 performed other microbiology or clinical specimen work. Laboratories should be aware of recommendations (3) 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 (3) that emphasize considerations of laboratory design for NAA testing, both of the manufacturers (Roche Amplicor® and Gen-Probe® MTD) recommend the use of unidirectional workflow.

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 (5 reported using ethidium bromide, and 2 used radioactive detection methods). Both the Gen-probe® MTD and Roche Amplicor® tests have 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 selected words, "inconclusive" for Gen-Probe® MTD and "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 (negative, equivocal/inconclusive, and positive) are indicated for the 2 *M.tb*-positive and 3 *M.tb*-negative samples. The overall analytic sensitivity of results reported for the 2 *M.tb*-positive samples was 97.3% (179/184): 97.8% (133/136) sensitivity for Gen-probe® MTD; 100% (26/26) sensitivity for Roche Amplicor®; 90.9% (20/22) sensitivity for In-house methods. The overall analytic specificity of results reported for the 3 *M.tb*-negative samples samples was 93.1% (257/276): 92.2% (188/204) specificity for Gen-probe®; 100% (39/39) specificity for Roche Amplicor®; 90.9% (30/33) specificity for In-house methods).

One of the three inconclusive interpretations reported on samples positive for *M.tb* was inconsistent with the quantitative Gen-Probe® MTD result, which indicated a value in the positive range. Although 10% of the Gen-Probe® MTD interpretations reported for sample TB99-1-1,

containing only *M. abscessus,* were either false positive or inconclusive, the reference laboratory results did not indicate any cross-reaction of the Gen-Probe® MTD test with *M. abscessus.*

Figures 8a and 8b are graphical representations 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. The shaded band on each scale represents the "inconclusive" range as defined by the manufacturer. For the positive samples, TB99-1-2 and TB99-1-4, the median values of all data were 2,409,302 relative light units (RLU) and 2,384,266 RLU, respectively. The median values for the negative samples consisiting of *M. avium*, TB999-1-3 and TB99-1-5, were 3,320 and 3,740 RLU, respectively. For the sample consisting of *M. abscessus*, TB99-1-1, the median value was 2,646 RLU.

Figure 9 is a graphical representation of all quantitative results reported for each sample by participant laboratories using the Roche Amplicor® test. The total volume of data was insufficient to provide a box-plot of results for each sample. The solid line through each set of data represents the median value for each sample. The shaded band represents the equivocal range. For the positive samples, TB99-1-2 and 99-1-4, the median values were 3.020 (A_{450}) and 3.210 (A_{450}), respectively. The median value for all the negative samples, TB99-1-1, TB99-1-3 and TB99-1-5, were 0.0600 (A_{450}), 0.0575 (A_{450}), and 0.0565 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. This suggests overall improvement in correlation between quantitative results and qualitative interpretations reported over previous *M.tb* NAA Performance Evaluation challenge shipments. The sensitivity in detecting positive samples was good for participants using the Gen-Probe® MTD, Roche Amplicor®, and in-house *M.tb* NAA tests.

References

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

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

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

4. 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.

5. 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.

6. Butler, WR, O'Conner SP, Yakrus MA, and Gross WM. Cross-reactivity of genetic probe for detection of *Mycobacterium tuberculosis* with newly described species *Mycobacterium celatum*. J. Clin. Microbiol. 1994; 32: 536-538.

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

Figure 1. Primary Classification of Participating Laboratories

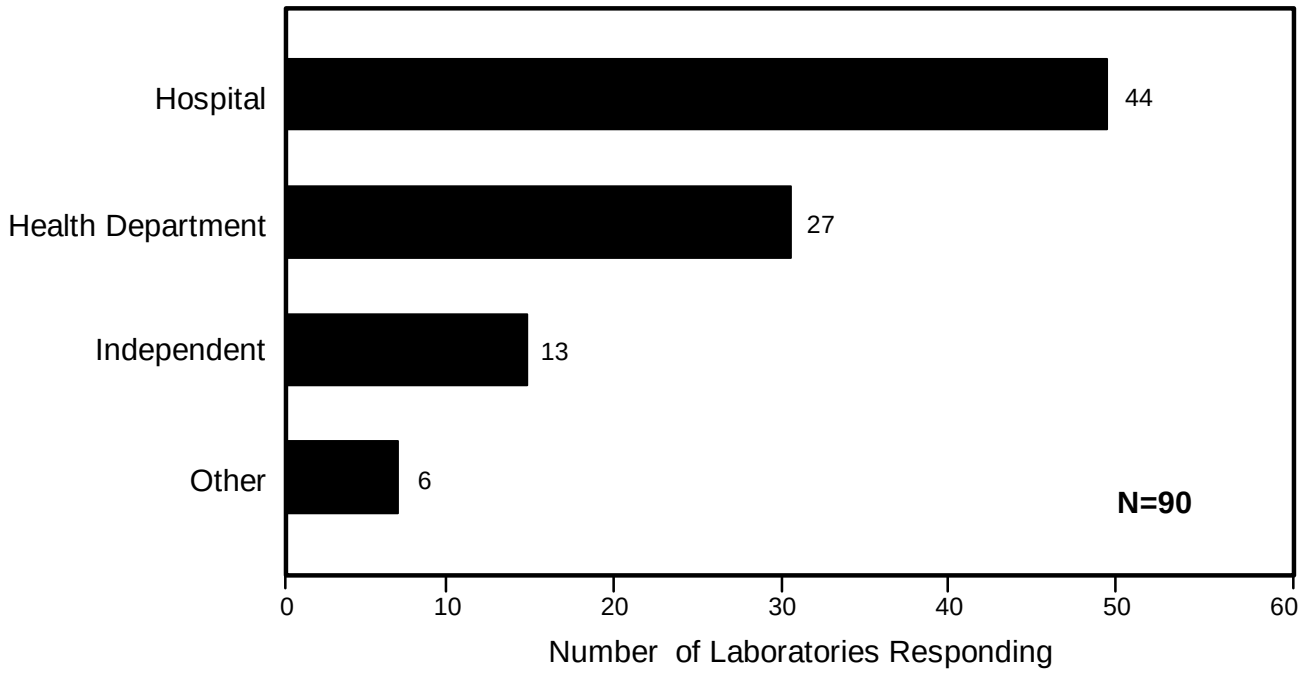

Figure 2. Number of Patient Specimens Tested for *M.tb* Using TB NAA between January 1, 1998, and December 31, 1998

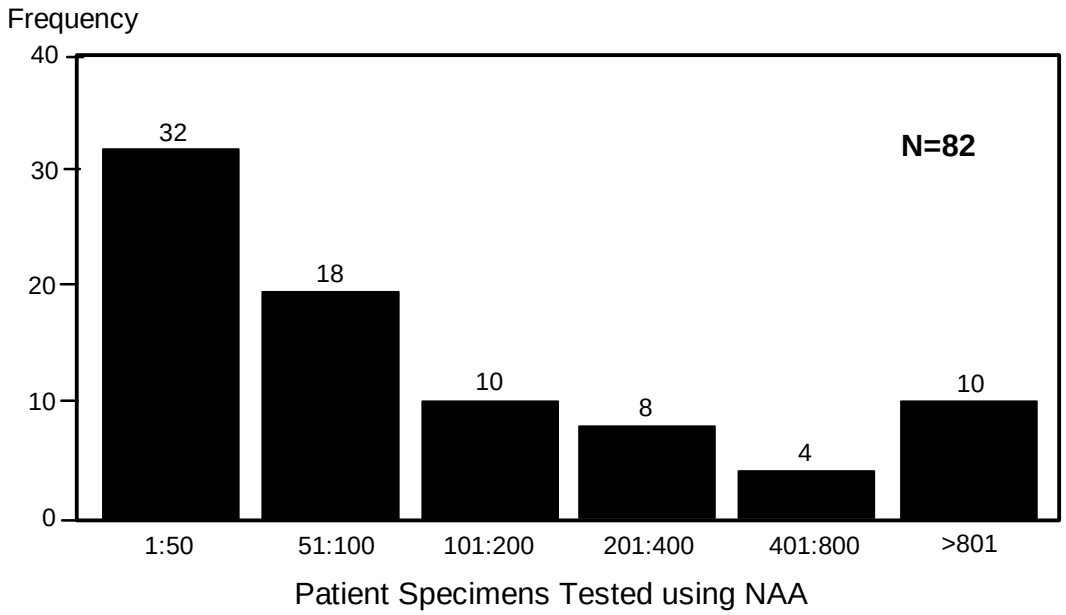

Figure 3. Amplification Procedure Used for Direct Detection of *M.tb*

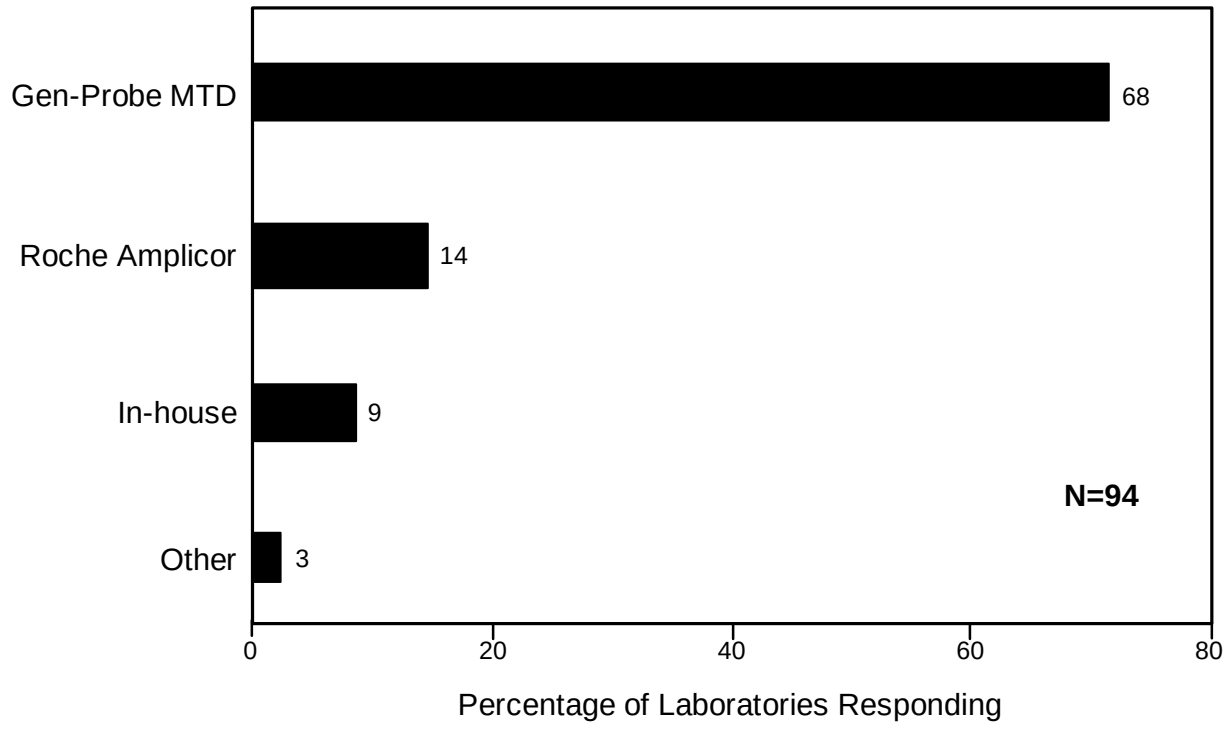

Figure 4. Biosafety Levels of Participant Laboratories

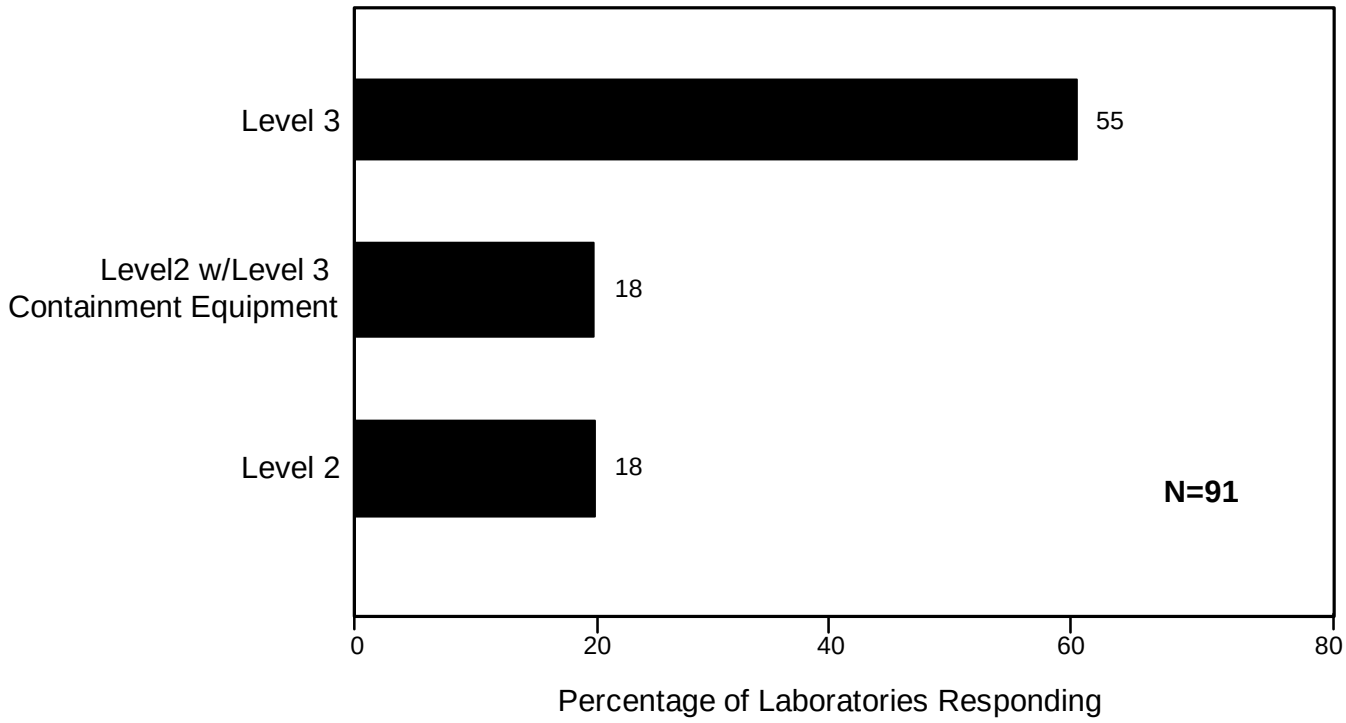

Figure 5. Is the Biological Safety Cabinet that is Used for TB NAA Testing Used for Other Purposes?

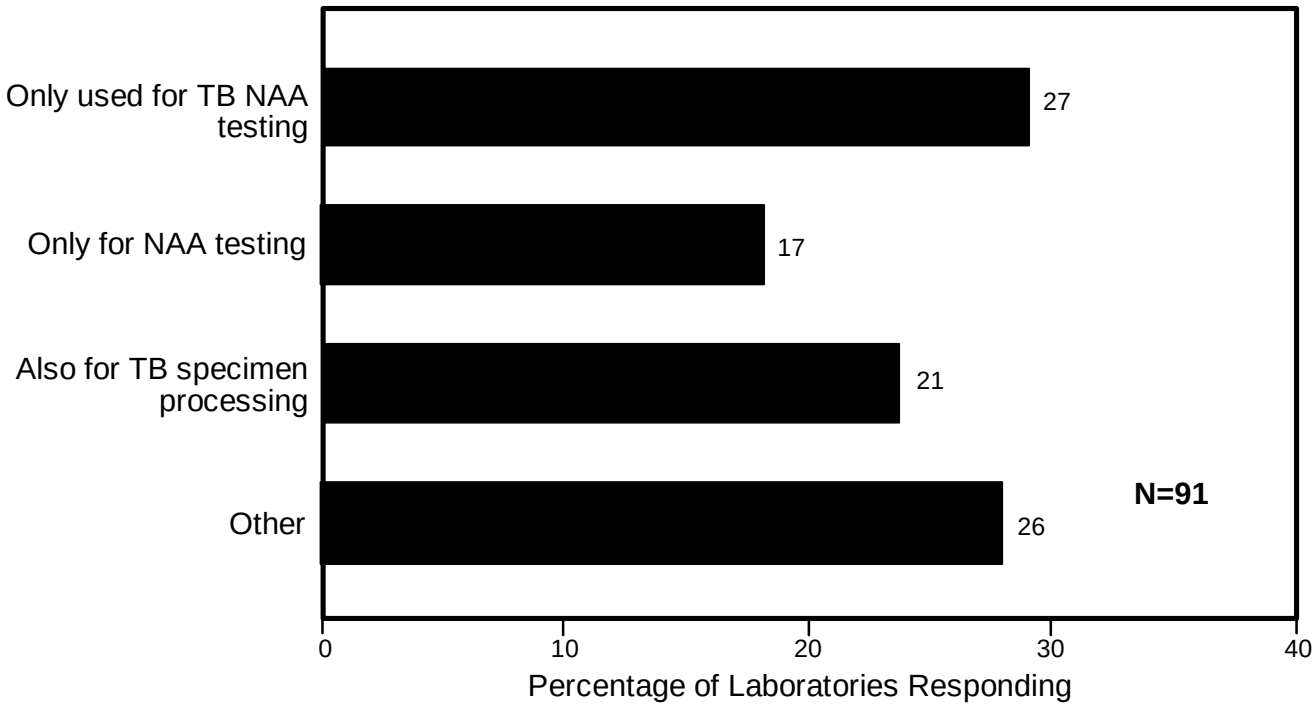

Figure 6. Use of Uni-directional Workflow by Participating Laboratories

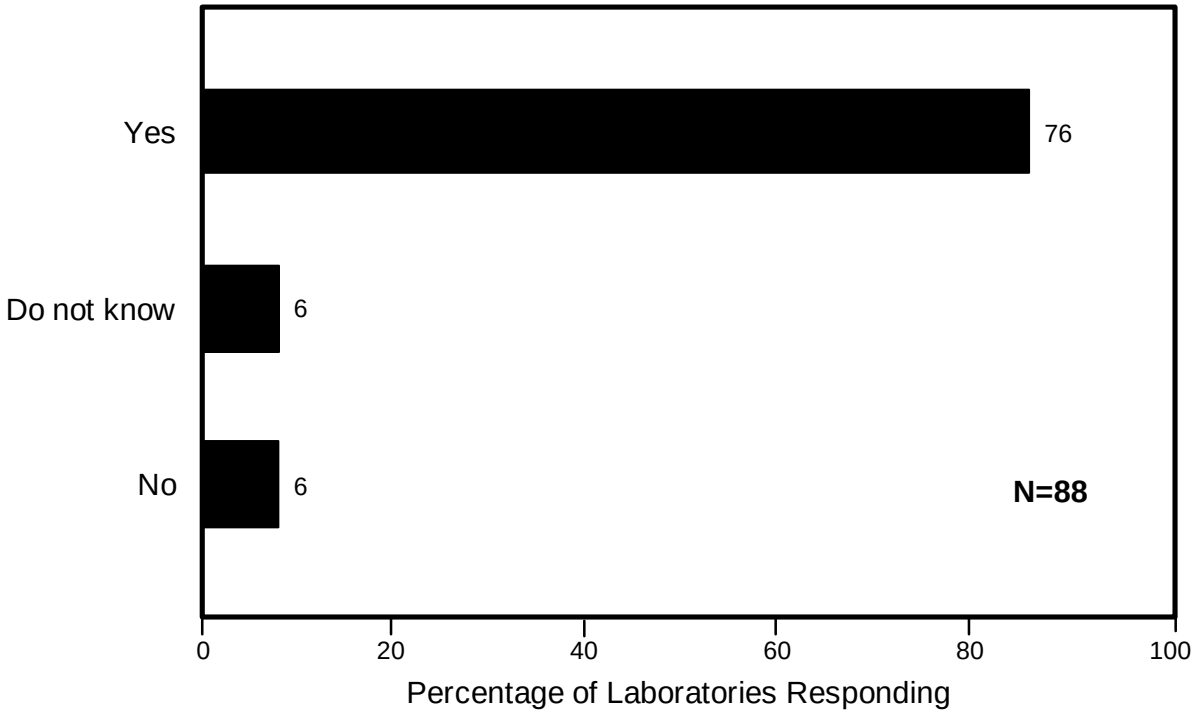

Figure 7. Frequency of TB NAA Qualitative Test Results by Sample Type for the Gen-Probe MTD, Roche Amplicor, and In-House Methods

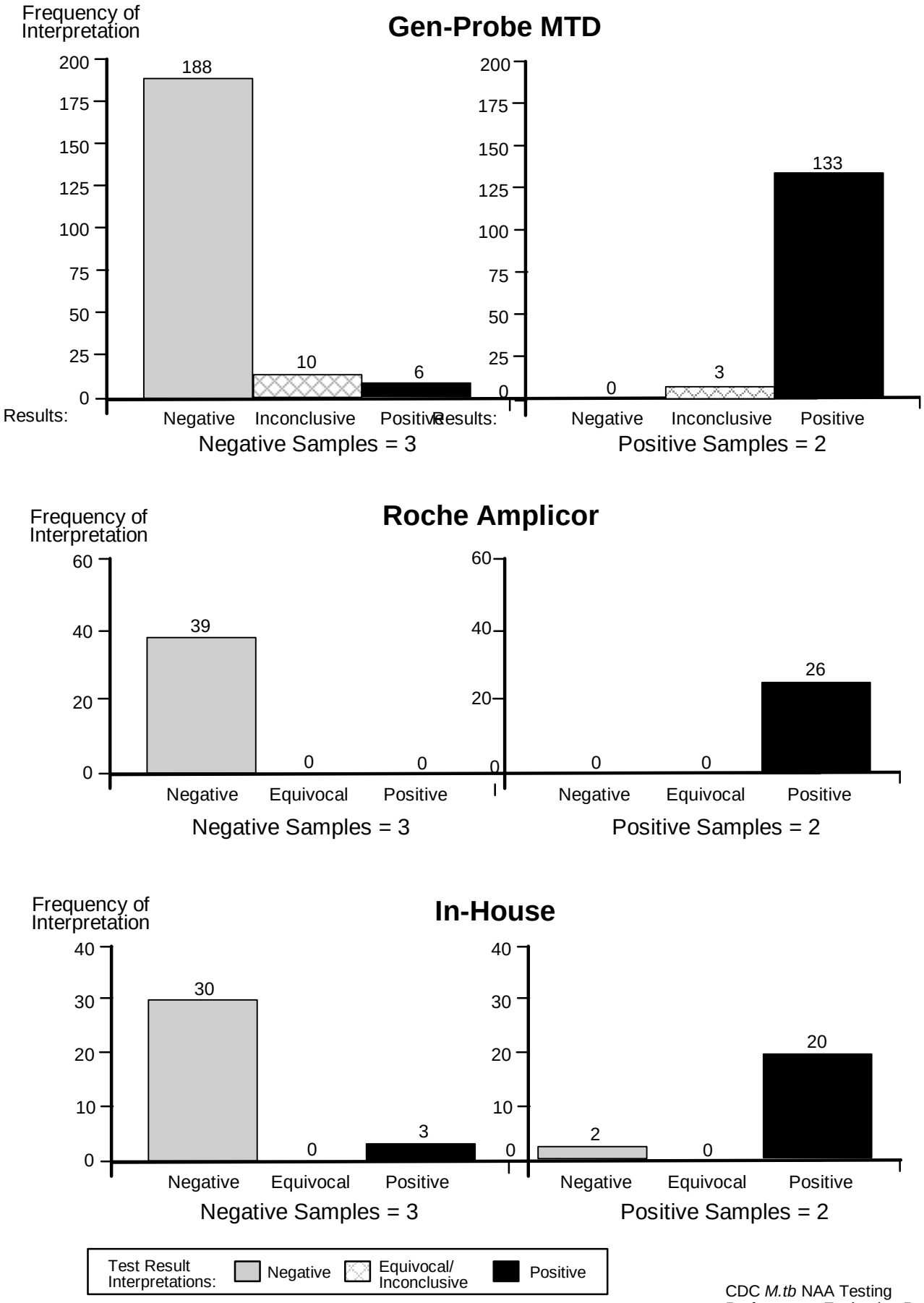

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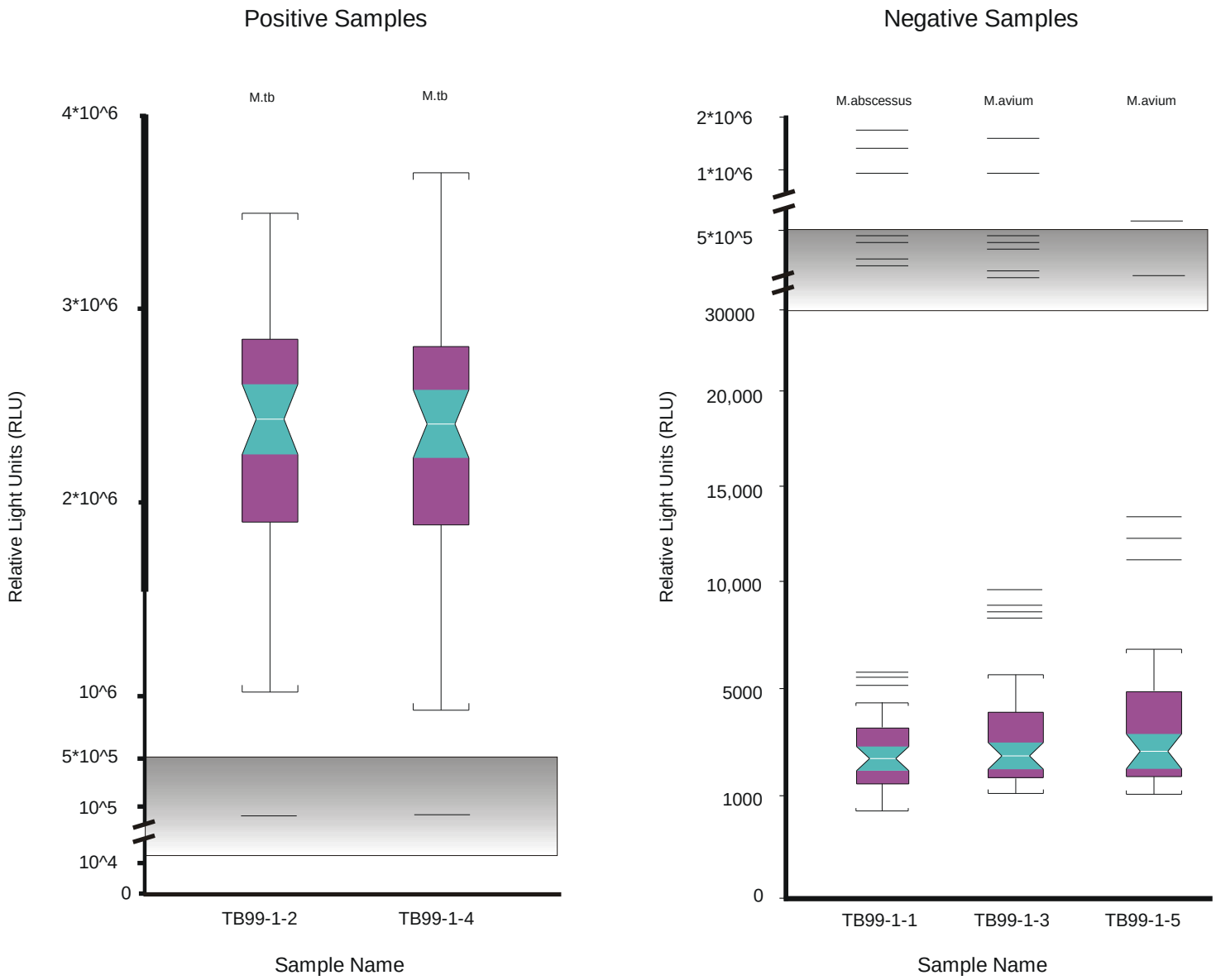
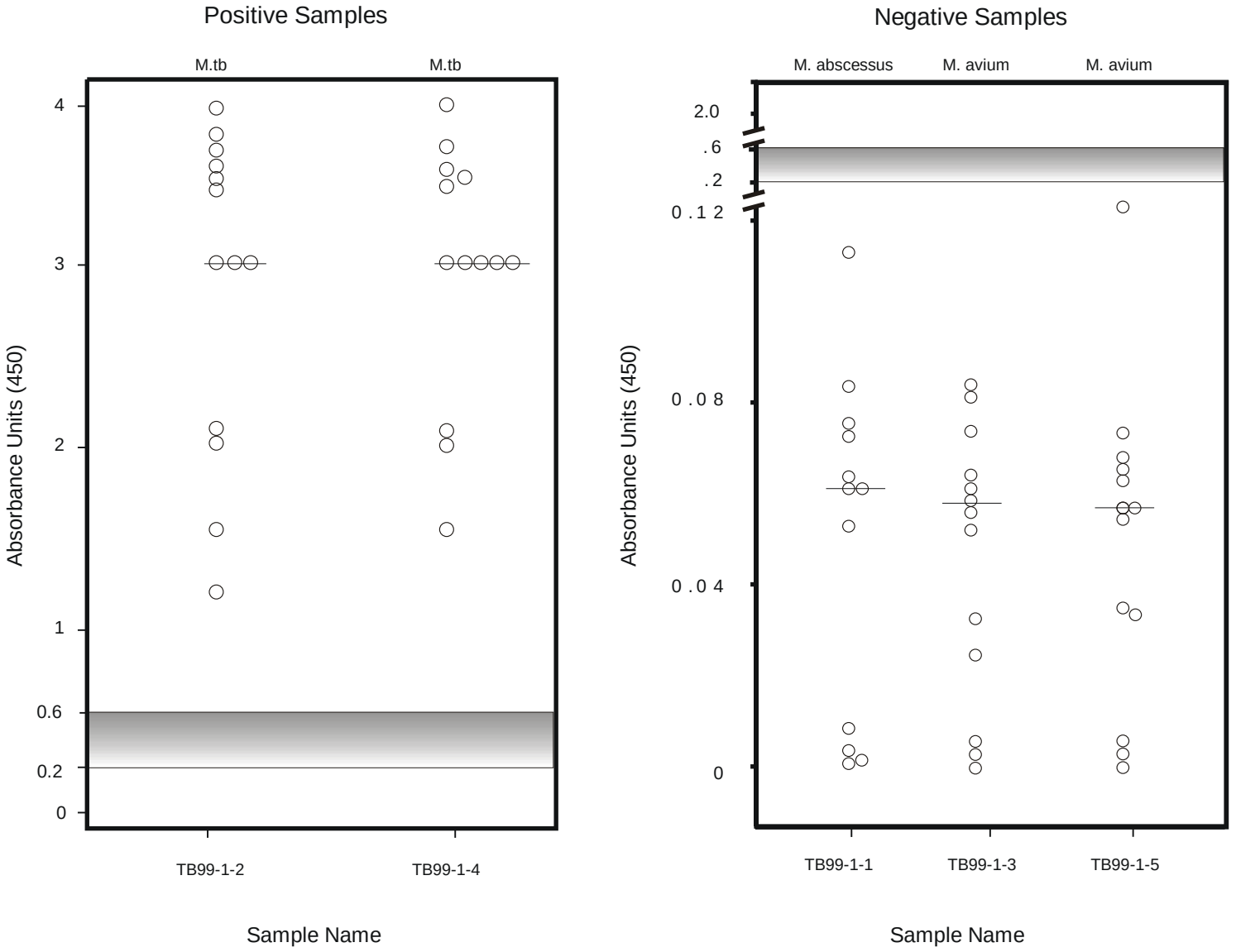
Figure 8a. Quantitative Results for GenProbe® MTD



Note: Shaded areas represent inconclusive range.

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 February 1999 shipment of samples for the *M. tb.* NAA testing performance evaluation program.

Table 1. Sample TB99 1-1 contained only *Mycobacterium abscessus*

Test Methods	No. Tests Performed	Positive No.	Positive %	Inconclusive No.	Inconclusive %	Negative No.	Negative %
Gen-Probe	68	3	4.4	4	5.9	61	89.7
In-house	11	0	0	0	0	11	100
Roche	13	0	0	0	0	13	100
All methods	92	3	3.3	4	4.3	85	92.4

Table 2. Sample TB99 1-2 contained only *Mycobacterium tuberculosis*

Test Methods	No. Tests Performed	Positive No.	Positive %	Inconclusive No.	Inconclusive %	Negative No.	Negative %
Gen-Probe	68	67	98.5	1	1.5	0	0
In-house	11	11	100	0	0	0	0
Roche	13	13	100	0	0	0	0
All methods	92	91	98.9	1	1.1	0	0

Table 3. Sample TB99 1-3 contained only *Mycobacterium avium* complex

Test Methods	No. Tests Performed	Positive No.	Positive %	Inconclusive No.	Inconclusive %	Negative No.	Negative %
Gen-Probe	68	2	2.9	5	7.4	61	89.7
In-house	11	1	9.1	0	0	10	90.9
Roche	13	0	0	0	0	13	100
All methods	92	3	3.3	5	5.4	84	91.3

Table 4. Sample TB99 1-4 contained only *Mycobacterium tuberculosis*

Test Methods	No. Tests Performed	Positive No.	Positive %	Inconclusive No.	Inconclusive %	Negative No.	Negative %
Gen-Probe	68	66	97.1	2	2.9	0	0
In-house	11	9	81.1	0	0	2	18.2
Roche	13	13	100	0	0	0	0
All methods	92	88	95.7	2	2.2	2	2.2

Table 5. Sample TB99 1-5 contained only *Mycobacterium avium* complex

Test Methods	No. Tests Performed	Positive No.	Positive %	Inconclusive No.	Inconclusive %	Negative No.	Negative %
Gen-Probe	68	1	1.5	1	1.5	66	97.1
In-house	11	2	18.2	0	0	9	81.8
Roche	13	0	0	0	0	13	100
All methods	92	3	3.3	1	1.1	88	95.7