May 13, 2004

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 January 2004 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 January 2004 shipment of samples for the CDC *M. tuberculosis* Nucleic Acid Amplification (*M.tb* NAA) Testing Performance Evaluation Program. Participant laboratories received five individual samples. Responses were received from 89 of 93 (96%) 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 January 26, 2004 Performance Evaluation Results for *M. tuberculosis* Nucleic Acid Amplification Testing Reported to the Centers for Disease Control and Prevention

Report Highlights

Laboratories performed *Mycobacterium tuberculosis (M.tb)* nucleic acid amplification testing very well on the January 2004 shipment samples.

Overall Summary of Results

M.tb positive and negative samples:

			2 Positive Samples TB04-01-1 TB04-01-4	2 Negative Samples TB04-01-2 TB04-01-5	
Method	**Total # of laboratories**	**Total # of results**	**False-negative results**	**False-positive results**	**Overall Performance**
Gen-Probe MTD	67	268	2/134 (1.5%)	4/134 (3.0%)	97.8%
Roche Amplicor	16	64	None	None	100.0%
In-house/Other	6	24	None	None	100.0%

M.tb positive inhibited sample:

			Positive Sample with Inhibitors TB04-01-3	
Method	**Total # of laboratories**	**Total # of results**	**False-negative results**	**Overall Performance**
Gen-Probe MTD	67	67	46/67 (68.7%)	31.3%
Roche Amplicor	16	16	12/16 (75.0%)	25.0%
In-house/Other	6	6	2/6 (33.3%)	66.7%

New Findings

- In this shipment, sample TB04-01-3, contained 3.0 x 10^5 theoretical cells/ml of *M. tuberculosis* and inhibitors, i.e. high molarity phosphate buffer and other inhibitors. Of all participants, 67.4% (60/89) reported interpretations as either negative or equivocal, which are considered to be incorrect.

- Three laboratories, 3.4% (3/89) using Gen-Probe® reported sample TB04-01-5, *M. simiae,* as positive. These seem to be random errors.

- It is a concern that 11.4% (10/88) of responding laboratories reported that unidirectional workflow is not used.

Findings of note that also have been reported previously

- Forty-nine of eighty-four (58.3%) participants perform inhibition testing on *M.tb* NAA-negative specimens. The current *M.tb* NAA testing algorithm recommended by CDC includes recommendations for inhibition testing on negative specimens (1).

- Of the laboratories that received processed specimens for testing, 50.0% (32/64) indicated that they inquire about the sample submission buffer.

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 containing organisms other than *M. tuberculosis* shipped in January 2004. Responses were received from 89 of 93 (96%) laboratories participating in this shipment. The *M.tb* NAA Performance Evaluation Program (*M.tb* NAA MPEP) provides laboratories with a tool for external quality assessment. 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 86 participants. Participants consisted of 37 hospitals, 36 health departments, 11 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 (6/6) reporting the use of In-house *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 (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 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 16% (14/88) of participant laboratories indicated that they process *M.tb* specimens in the same BSC that is used for *M.tb* NAA testing. Among the 27% (24/88) of participants that indicated "Other" uses for the *M.tb* NAA testing BSC, 5 performed *M.tb* culture work (biochemicals, drug susceptibility testing, Accuprobe® identification, etc.), 9 performed mycology, and one performed other microbiology or clinical specimen work. Two 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 to avoid contamination problems.

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 11.4% (10/88) of 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 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, 2 negative, and one positive sample containing inhibitors. The combined analytical sensitivity of all methods was 98.9% (176/178) for the TB04-01-1 (3.0×10^5 theoretical cells/ml) and TB04-01-4 (3.0×10^4 theoretical cells/ml): 98.5% (132/134) sensitivity for Gen-Probe® MTD; 100% (32/32) sensitivity for Roche Amplicor®; 100% (12/12) sensitivity for In-house methods. The combined analytical specificity of all methods was 97.2% (173/178) for the 2 negative samples TB04-01-2 (3.0×10^3 theoretical cells/ml, *M. avium*) and TB04-01-5 (3.0×10^4 theoretical cells/ml, *M. simiae*): 96.3% (129/134) specificity for Gen-Probe®; 100% (32/32) specificity for Roche Amplicor®; 100% (12/12) specificity for In-house methods.

For sample TB04-01-3 (3.0×10^5 theoretical cells/ml, *M. tuberculosis*) 32.3% (29/89) of laboratories reported acceptable interpretations of either positive or inhibition: 31.3% (21/67) for Gen-Probe® MTD; 25.0% (4/16) for Roche Amplicor®; 66.7% (4/6) for In-house methods. Equivocal interpretations were included with negative interpretations for Roche Amplicor® and In-house methods, and were considered unacceptable.

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 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, TB04-01-1 and TB04-01-4, the median values of all data were 3,129,642 and 3,082,774 relative light units (RLU), respectively. The median value was 2,377 RLU's for sample TB04-01-3 which was positive for *Mycobacterium tuberculosis* and contained inhibitors. The distribution was similar to a negative sample. The median values for the negative samples containing *M. avium,* TB04-01-2, and *M. simiae,* TB04-01-5, were 2,343 and 2,499 RLU's 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. The median value was 3.000 (A_{450}) for both positive samples, TB04-01-1 and TB04-01-4. The median value for sample TB04-01-3, containing *Mycobacterium tuberculosis* and inhibitors was 0.054 (A_{450}), similar to the median for negative samples. The median values for the samples containing *M. avium,* TB04-01-2, and *M. simiae*, TB04-01-5, were 0.054 (A_{450}), and 0.057 (A_{450}) respectively.

In response to a question regarding inhibition testing, 58.3% (49/84) of participants performed inhibition testing on *M.tb* NAA negative specimens. The current *M.tb* NAA testing algorithm recommended by CDC includes recommendations for inhibition testing on negative specimens (1). Product inserts for both the Gen-Probe MTD test and the Roche Amplicor PCR test contain procedures for the testing of inhibitors in NAA-TB negative specimens.

Since specimen resuspension fluids are now commercially available which contain very high phosphate concentrations, we asked the participant laboratories, "If you receive processed specimens for *M.tb* NAA testing, do you ask what type of buffer was used for the concentration/decontamination procedure?" Of the laboratories that received processed specimens, 50.0% (32/64) indicated that they did inquire about the sample submission buffer. Since fluids containing very high molarity phosphate concentrations may inhibit amplification, laboratories receiving processed specimen sediments for NAA-TB testing should be aware of the buffer that was used to process the specimen.

In this shipment, we included an *M.tb*-positive sample, TB04-01-3, containing a high molarity phosphate buffer, (analogous to some currently available *M.tb* specimen resuspension buffers) and other inhibitors. There were 60 false negative interpretations reported for this sample (46/67 by laboratories using Gen-Probe MTD; 12/16 using Roche Amplicor PCR; 2/6 using In-house methods). Of the 60 false negative interpretations, 21 were from laboratories who

reported that they performed inhibition testing on negative specimens. It is unclear whether or not these laboratories actually performed inhibition testing on the MPEP samples. If laboratories are doing inhibition testing and find inhibition, the result should be reported as "inhibited" not "negative" for both clinical specimens and for quality assessment samples. Overall, the percentage of laboratories providing a correct interpretation of "positive" or "inhibition" for the inhibited sample was very low, i.e. 32.6% (29/89). Current CDC recommendations for *M.tb* NAA testing recommend testing negative specimens for inhibitors (1). If laboratories are not testing negative specimens for inhibition, there is an increased likelihood of reporting false negative results. In a clinical specimen, there would be no way of determining that the sample actually contained *M.tb*, but was inhibited, until culture results were completed. Laboratories not doing inhibition testing should consider reporting that inhibition testing was not done so that physicians and healthcare providers are aware that the negative results could be due to inhibition.

References

1. CDC. Update: Nucleic Acid Amplification Tests for Tuberculosis. MMWR 2000; 49:593-594. http://www.cdc.gov/mmwr/PDF/wk/mm4926.pdf

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

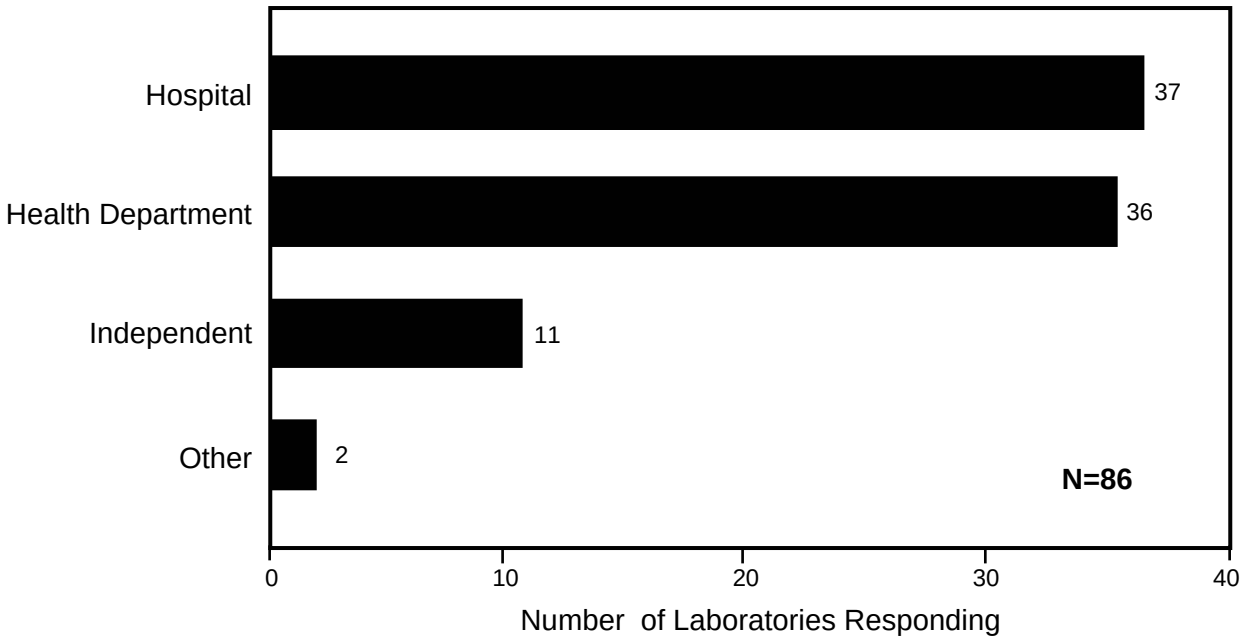
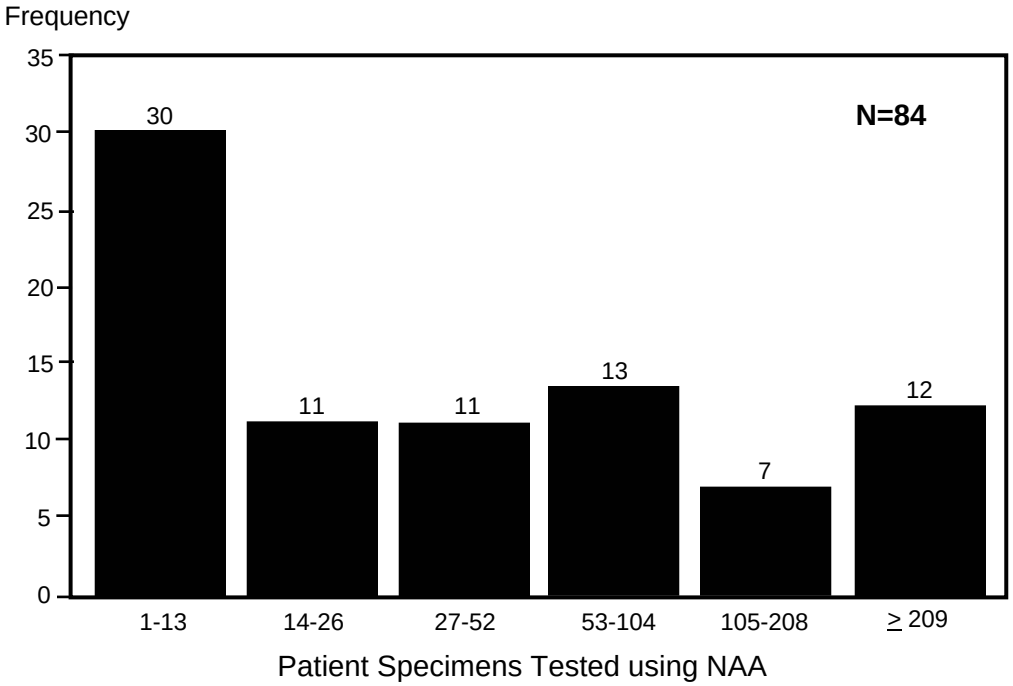


Figure 2. Number of Patient Specimens Tested for *M.tb* Using TB NAA during the Previous Quarter.*



*See explanation in the analysis.

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

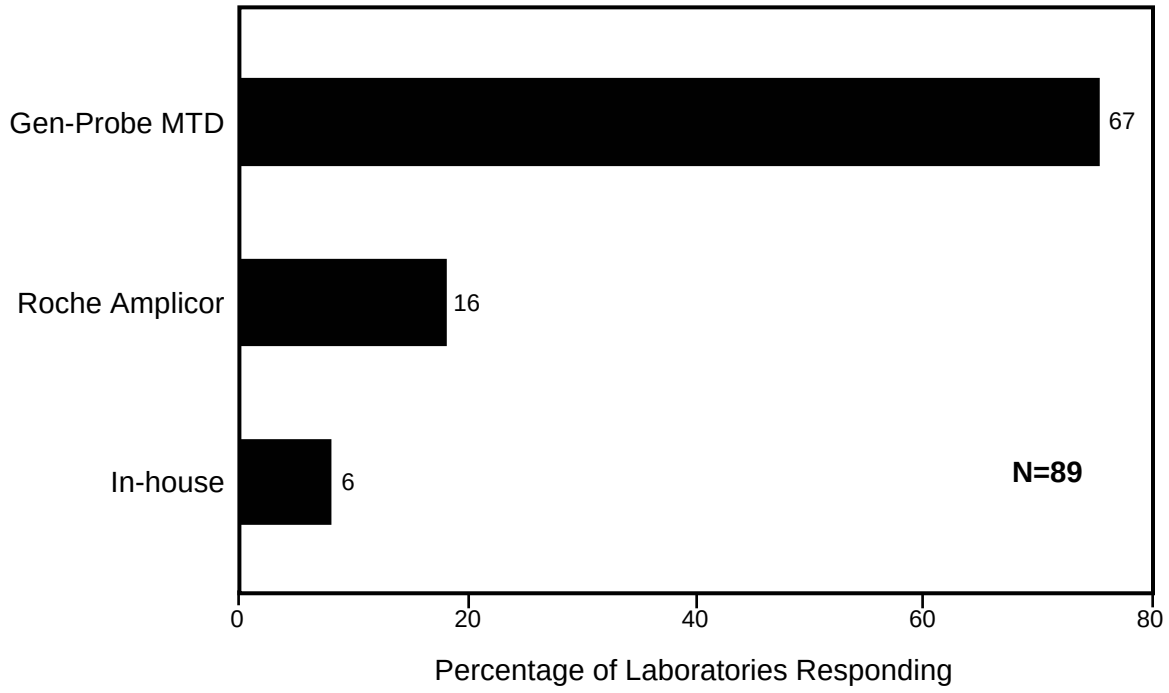


Figure 4. Biosafety Levels of Participant Laboratories

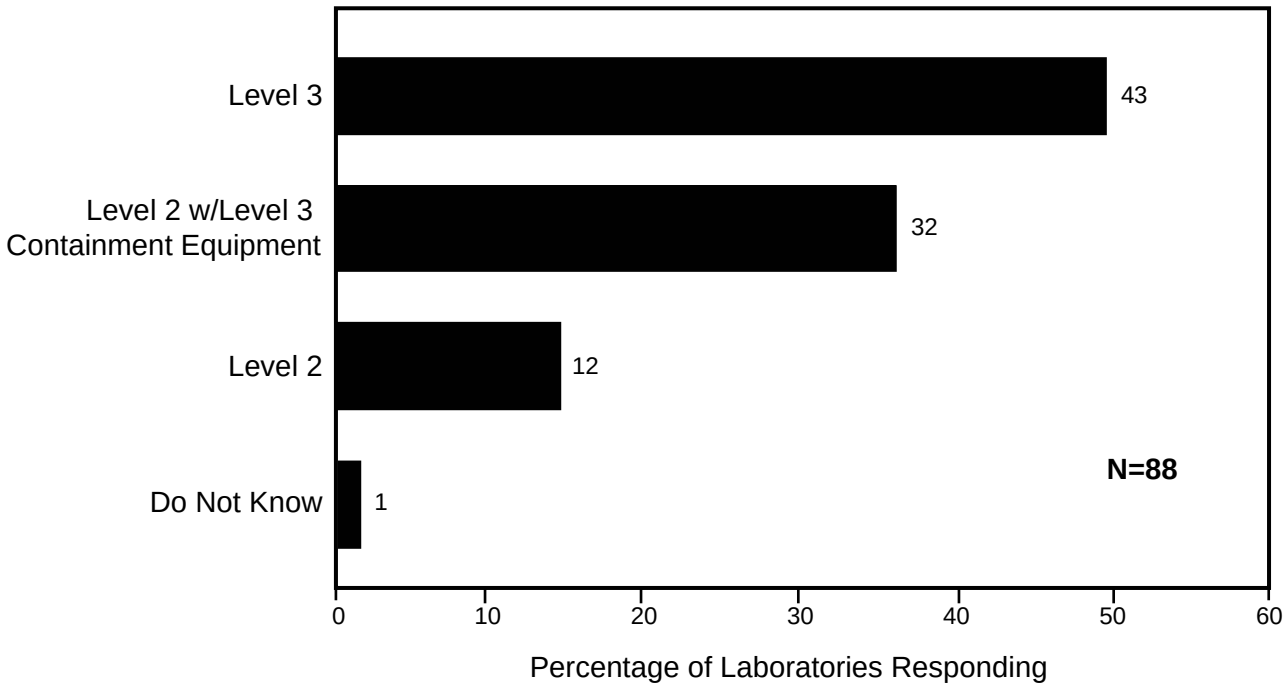


Figure 5. Is the Biological Safety Cabinet that is Used for TB NAA Testing Used for Other Purposes?

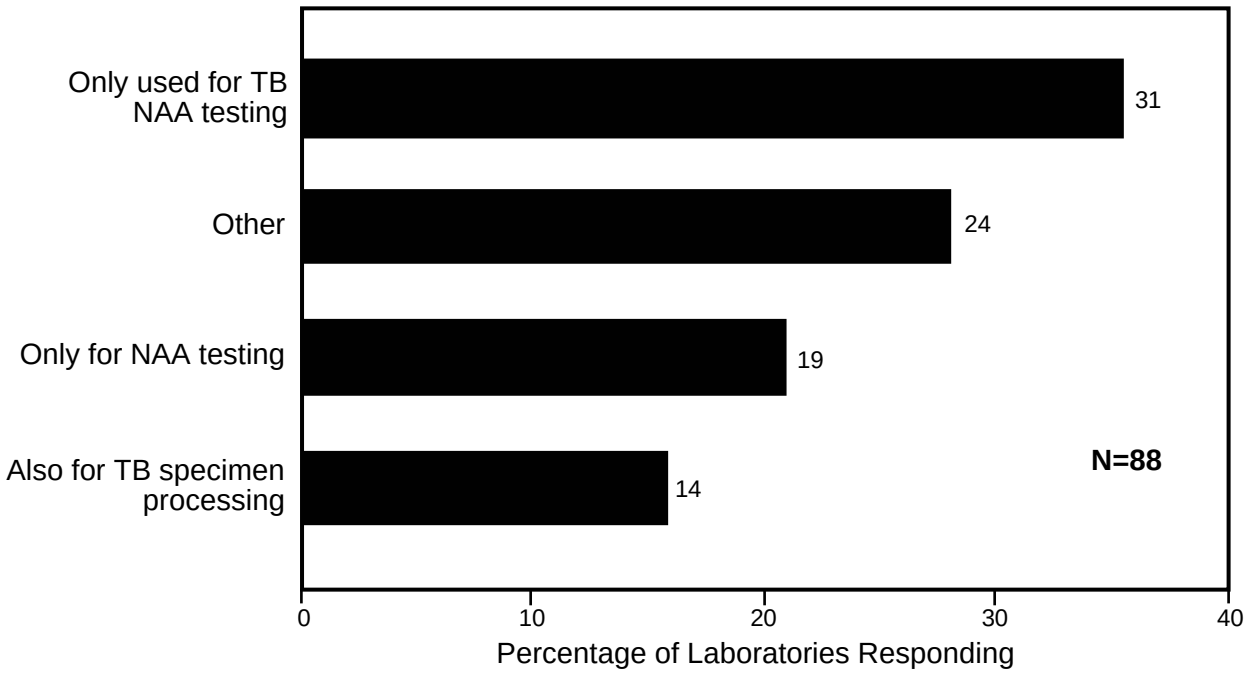


Figure 6. Use of Uni-directional Workflow by Participating Laboratories

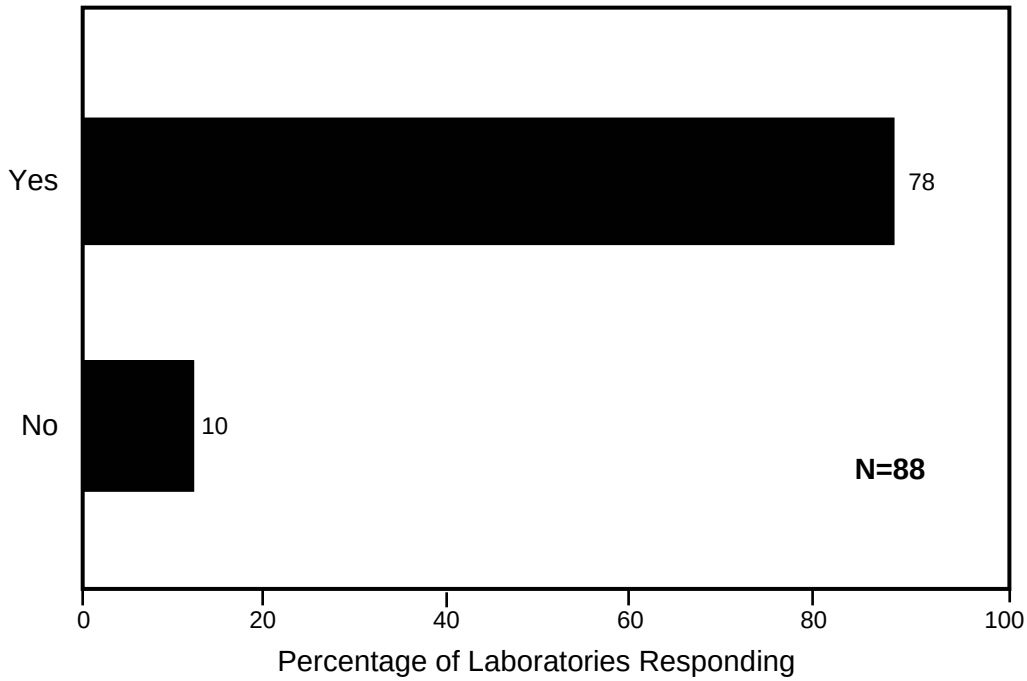


Figure 7. Frequency of TB NAA Qualitative Test Results by Sample Type for the Gen-Probe MTD, Roche Amplicor, and In-House Methods

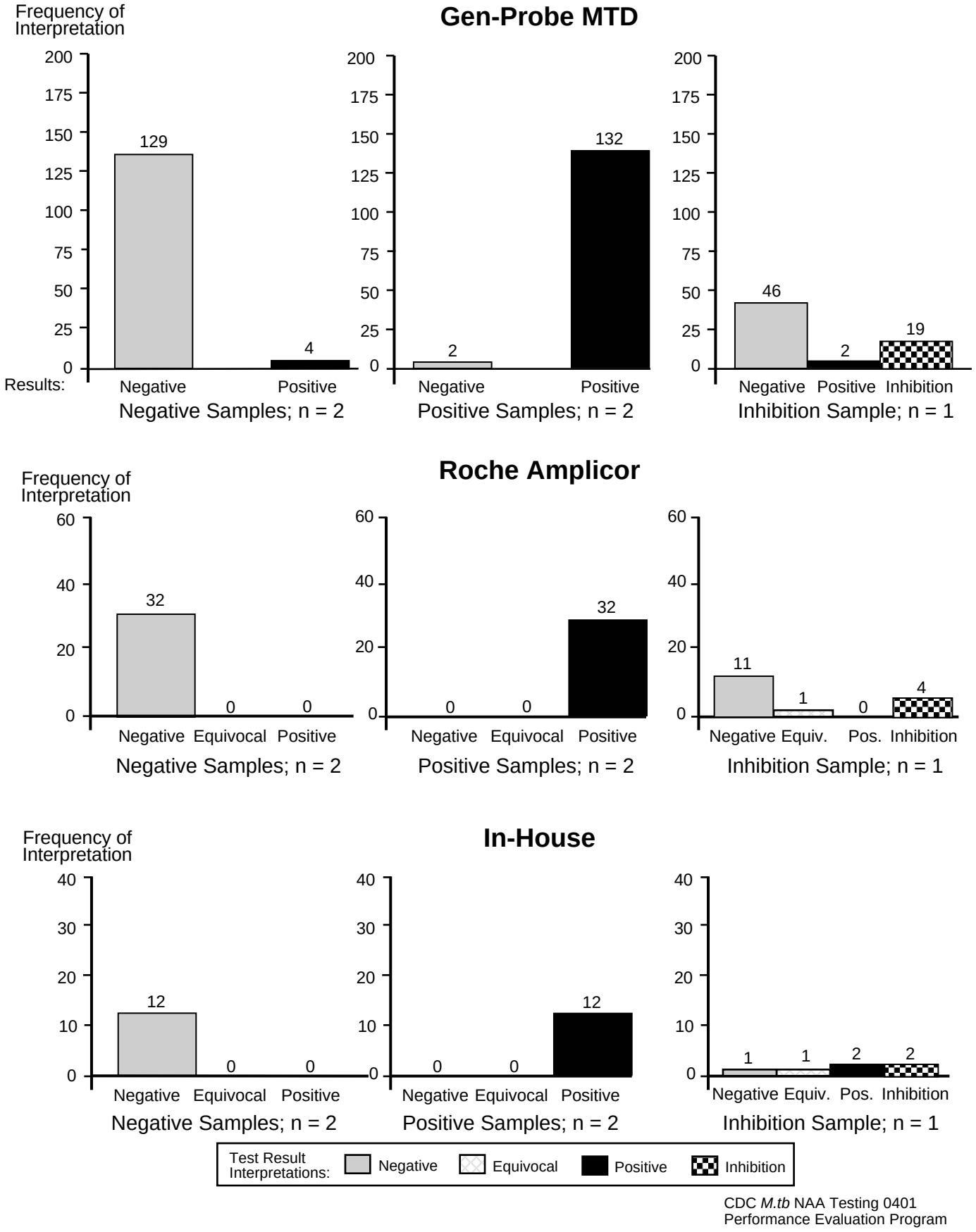


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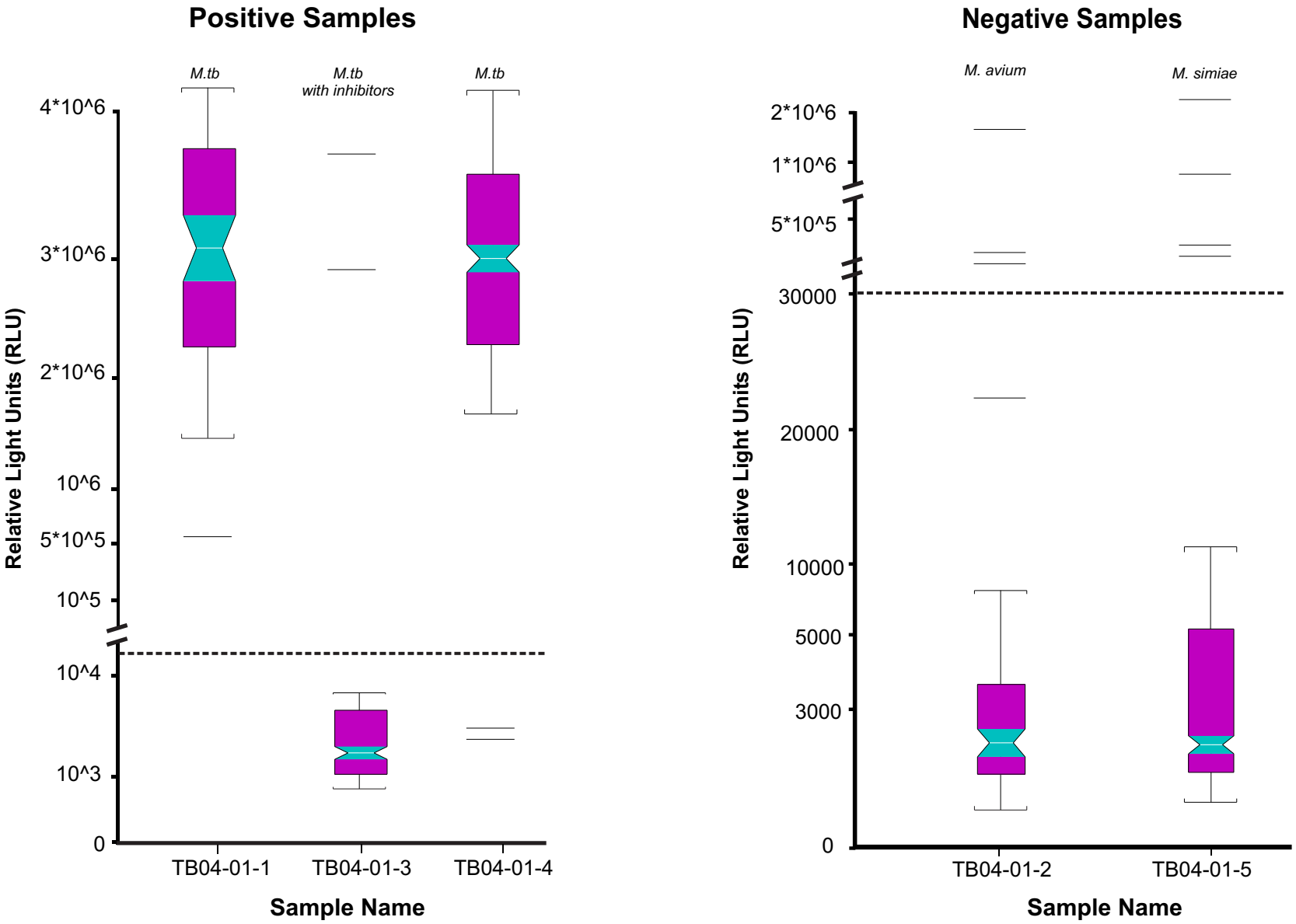
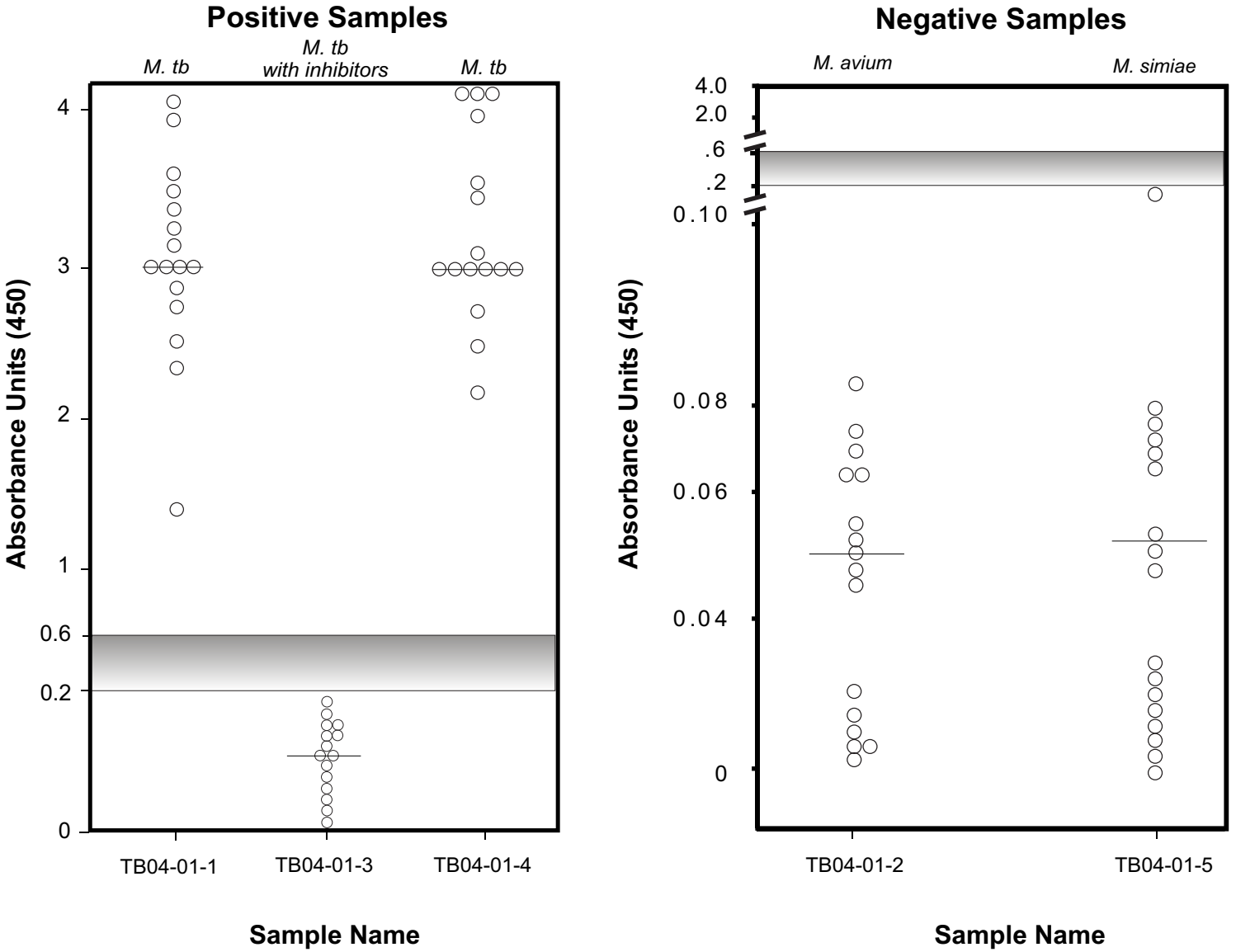
Figure 8. Quantitative Results for GenProbe® MTD



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 2004 shipment of samples for the *M. tb* NAA testing performance evaluation program.

Table 1. Sample TB04-01-1 contained *Mycobacterium tuberculosis*

Test Methods	No. Tests Performed	Positive		Inhibition		Equivocal		Negative	
		No.	%	No.	%	No.	%	No.	%
Gen-Probe	67	67	100.0	0	0.0	n/a	n/a	0	0.0
In-house	6	6	100.0	0	0.0	0	0.0	0	0.0
Roche	16	16	100.0	0	0.0	0	0.0	0	0.0
All methods	89	89	100.0	0	0.0	0	0.0	0	0.0

Table 2. Sample TB04-01-2 contained *Mycobacterium avium* complex

Test Methods	No. Tests Performed	Positive		Inhibition		Equivocal		Negative	
		No.	%	No.	%	No.	%	No.	%
Gen-Probe	67	1	1.5	1	1.5	n/a	n/a	65	97.0
In-house	6	0	0.0	0	0.0	0	0.0	6	100.0
Roche	16	0	0.0	0	0.0	0	0.0	16	100.0
All methods	89	1	1.1	1	1.1	0	0.0	87	97.8

Table 3. Sample TB04-01-3 contained inhibited *Mycobacterium tuberculosis*

Test Methods	No. Tests Performed	Positive		Inhibition		Equivocal		Negative	
		No.	%	No.	%	No.	%	No.	%
Gen-Probe	67	2	3.0	19	28.4	n/a	n/a	46	68.7
In-house	6	2	33.3	2	33.3	1	16.7	1	16.7
Roche	16	0	0.0	4	25.0	1	6.3	11	68.8
All methods	89	4	4.5	25	28.1	2	2.3	58	65.2

Table 4. Sample TB04-01-4 contained *Mycobacterium tuberculosis*

Test Methods	No. Tests Performed	Positive		Inhibition		Equivocal		Negative	
		No.	%	No.	%	No.	%	No.	%
Gen-Probe	67	65	97.0	0	0.0	n/a	n/a	2	3.0
In-house	6	6	100.0	0	0.0	0	0.0	0	0.0
Roche	16	16	100.0	0	0.0	0	0.0	0	0.0
All methods	89	87	97.8	0	0.0	0	0.0	2	2.2

Table 5. Sample TB04-01-5 contained *Mycobacterium simiae*

Test Methods	No. Tests Performed	Positive		Inhibition		Equivocal		Negative	
		No.	%	No.	%	No.	%	No.	%
Gen-Probe	67	3	4.5	0	0.0	n/a	n/a	64	95.5
In-house	6	0	0.0	0	0.0	0	0.0	6	100.0
Roche	16	0	0.0	0	0.0	0	0.0	16	100.0
All methods	89	3	3.4	0	0.0	0	0.0	86	96.6