Analyses of the June 12, 2006 Performance Evaluation Results for *M. tuberculosis* Nucleic Acid Amplification Testing Reported to the Centers for Disease Control and Prevention

Overall Summary of Results

***M.tb* positive and negative samples:**

			1 Negative Sample TB06-06-2	3 Positive Samples TB06-06-1 TB06-06-4 TB06-06-5	
Method	**Total # of laboratories**	**Total # of results**	**False-positive results**	**False-negative results**	**Overall Performance**
Gen-Probe MTD	63	252	1/63 (1.6%)	0/189 (0.0%)	99.6%
Roche Amplicor	13	52	0/13 (0.0%)	1/39 (2.6%)	98.1%
In-house/Other	9	36	0/9 (0.0%)	2/27 (7.4%)	94.4%

***M.tb* positive inhibitory sample:**

			Positive Donor with Inhibitors TB06-06-3			
Method	**Total # of Laboratories**	**Total # of Results**	**Total # Inhibited**	**Total # Positive**	**Total # False-negative Results**	**Overall Performance**
Gen-Probe MTD	63	60*	30/60 (50.0%)	2/60 (3.3%)	28/60 (46.7%)	53.30%
Roche Amplicor	13	13	10/13 (76.9%)	0/13 (0%)	3/13 (23.1%)	76.90%
In-house/Other	9	9	2/9 (22.2%)	6/9 (66.7%)	1/9 (11.1%)	88.90%

*Three laboratories using the GenProbe MTD® method didn't report an interpretation for sample TB06-06-3, containing *Mycobacterium tuberculosis* with inhibitors (3.0×10^5 theoretical cells/ml).

New Findings

- Overall accuracy for this shipment was 91.0% (384/422).

- Of the participating laboratories, 5.9% (5/85) reported that they do not use or don't know if they use uni-directional workflow (compared with 9.5% [8/84] for the January 2006) shipment which represents an improvement in this practice.

- In this shipment sample TB06-06-3 contained 3×10^5 theoretical cells/ml of *M. tuberculosis* and inhibitors (a high molarity phosphate buffer and heparin). Of all participants, 39.0% (32/82) reported an interpretation of negative which is considered to be incorrect. This is an improvement from the January 2004 shipment in which 67.4% (60/89) of laboratories reported incorrect interpretations for an inhibitory specimen.

Note:

Thirteen of eighty-five (15.3%) participants reported using Biosafety level 2; last shipment (2006-01), 11 of 83 (13.3%) participants reported using Biosafety level 2. Biosafety level 2 practices and precautions are required for non-aerosol producing manipulations of clinical specimens that may contain *M. tuberculosis*. All aerosol-generating activities must be conducted in a Class I or Class II biological safety cabinet. We recommend that Biosafety level 2 with Biosafety level 3 precautions (respirator, gown, gloves) be used when working with patient samples that may contain *M. tuberculosis*. Biosafety level 3 practices, containment equipment, and facilities are required for laboratory activities in the propagation and manipulation of cultures of *M. tuberculosis*. Please refer to the CDC/NIH manual, Biosafety in the Microbiological and Biomedical Laboratories (4th edition), www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm. Non-U.S. laboratories refer to http://whqlibdoc.who.int/hq/1998/WHO_TB_98.258_(part1).pdf for more information.

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 non-tuberculous mycobacteria shipped in June 2006. Responses were received from 85 of 88 (96.6%) laboratories participating in this shipment. The *M.tb* NAA Performance Evaluation Program 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 (WSLH).

Challenge Samples

Participant laboratories received five individual samples. Positive samples TB06-06-1, TB06-06-4 and TB06-06-5 contained *M. tuberculosis*. Sample TB06-06-3 also contained *M. tuberculosis* (3.0×10^5 theoretical cells/ml), but was inhibited with high molarity phosphate buffer and heparin. The negative sample in this shipment, TB06-06-2, contained *M. peregrinum* (3.0×10^5 theoretical cells/ml). 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 results. Lastly, samples were analyzed by five reference laboratories and all results were reviewed for correlation prior to release of the scheduled shipment.

Results

Figure 1 shows the laboratory classification represented by 84 participants. Participants consisted of 38 hospitals, 35 health departments, 10 independents, and 1 other type of laboratory.

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 (9/9) 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 (4th edition), www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm for recommendations and for determining their correct biosafety level.

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.

Of the participating laboratories, 15.5% (13/84) indicated that they process *M.tb* specimens in the same BSC that is used for *M.tb* NAA testing. Among the 25.0% (21/84) of participants that indicated other uses for the *M.tb* NAA testing BSC, 12 performed *M.tb* testing procedures or culture work (biochemicals, drug susceptibility testing, Accuprobe® identification, etc.), 6 performed mycology, and 5 performed other microbiology or clinical specimen work. One laboratory 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 unidirectional 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. For this shipment the number of laboratories reporting use of unidirectional workflow was an improvement over previous shipments. Only 5.9% of laboratories (5/85) reported that it was not being used or that they did not know if it was 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" to reflect the manufacturer's recommendation for reporting indeterminate quantitative test results by Roche Amplicor®.

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 1 negative, 1 inhibited, and 3 positive samples. The combined analytical specificity of all methods was 97.6% (83/85) for the negative sample, TB06-06-2 (*M. peregrinum*, 3.0×10^5 theoretical cells/ml): 98.4% (62/63) specificity for

Gen-Probe®; 92.3% (12/13) specificity for Roche Amplicor®; 100.0% (9/9) specificity for In-house methods. For the positive samples, the combined analytical sensitivity of all methods was 98.4% (251/255) for TB06-06-1 (3.0 x 10^5 theoretical cells/ml), TB06-06-4 (3.0 x 10^4 theoretical cells/ml) and TB06-06-5 (3.0 x 10^4 theoretical cells/ml): 99.5% (188/189) sensitivity for Gen-Probe® MTD; 97.4% (38/39) sensitivity for Roche Amplicor®; 92.6% (25/27) sensitivity for In-house methods.

For sample TB06-06-3 (3.0 x 10^5 theoretical cells/ml, *M. tuberculosis*) 61.0% (50/82) of laboratories reported acceptable interpretations of either positive or inhibition: 53.3% (32/60) for Gen-Probe® MTD; 76.9% (10/13) for Roche Amplicor®; 88.9% (8/9) for In-house methods.

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 Gen-Probe® MTD value reported which was outside these ranges is signified by one of the solid lines drawn outside the brackets. Median values for the positive samples were 3,493,965 for TB06-06-1, 3,528,971 for TB06-06-4 and 3,627,647 relative light units (RLU) for TB06-06-5. The median value for the negative sample containing *M. peregrinum*, TB06-06-2, was 3,161 relative light units (RLU), similar to median values for other negative samples previously used in the program. The median value for sample TB06-06-3 (3.0 x 10^5 theoretical cells/ml) by Gen-Probe® MTD was 3,166, similar to the median value for the negative sample.

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 for positive samples, TB06-06-1, TB06-06-4 and TB06-06-5 were 3.675 (A_{450}), 3.000 (A_{450}) and 3.660 (A_{450}) respectively. The median value for sample TB06-06-3, containing *M. tuberculosis* and inhibitors was 0.039 (A_{450}), similar to the median for negative samples. The median value for the negative sample containing *M. peregrinum*, TB06-06-2, was 0.043 (A_{450}).

In response to a question regarding inhibition testing, 69.4% (59/85) of participants performed inhibition testing on the *M.tb*-inhibited specimen sent in this shipment. 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® test contain procedures for the testing of inhibitors in preliminarily negative specimens.

Discussion

In this shipment, we included an *M.tb*-positive sample, TB06-06-3, containing a high molarity phosphate buffer (analogous to some currently available *M.tb* specimen resuspension buffers) and other inhibitors. There were 32 false negative interpretations reported for this sample (28/60 by laboratories using Gen-Probe MTD®; 3/13 using Roche Amplicor®; 1/9 using In-house methods). Fifty-nine of 85 laboratories, 69.4%, reported that they performed inhibition testing on negative specimens. This was an increase from 58.3% of laboratories in 2004. 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 for the inhibited sample improved as compared to the performance for an inhibitory sample in the January 2004 challenge. For the current shipment 61% (50/82) of laboratories responded correctly as compared to only 32.6% (29/89) in 2004. 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 may have been inhibited, until *M. tb* was detected in culture. 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.

In this June 2006 shipment, there was a decrease in the percentage of laboratories not using unidirectional work flow – 5.9% vs. 9.5% for the January 2006 shipment. This indicates an improving trend with respect to using recommended testing protocols.

Thirteen of eighty-five (15.3%) participants reported using biosafety Level 2 for *M.tb* NAA testing. This has been a consistent observation throughout the program. The CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) states that all aerosol-generating activities must be conducted in a BSC. This would include activities such as the decontamination and concentration of samples and inoculation of culture media. We suggest that laboratories not using a BSC for these activities review their policies and implement procedures as recommended in the BMBL to provide additional safety.

We thank the WSLH staff for contributing to this report.

References

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Figure 1. Primary Classification of Participating Laboratories

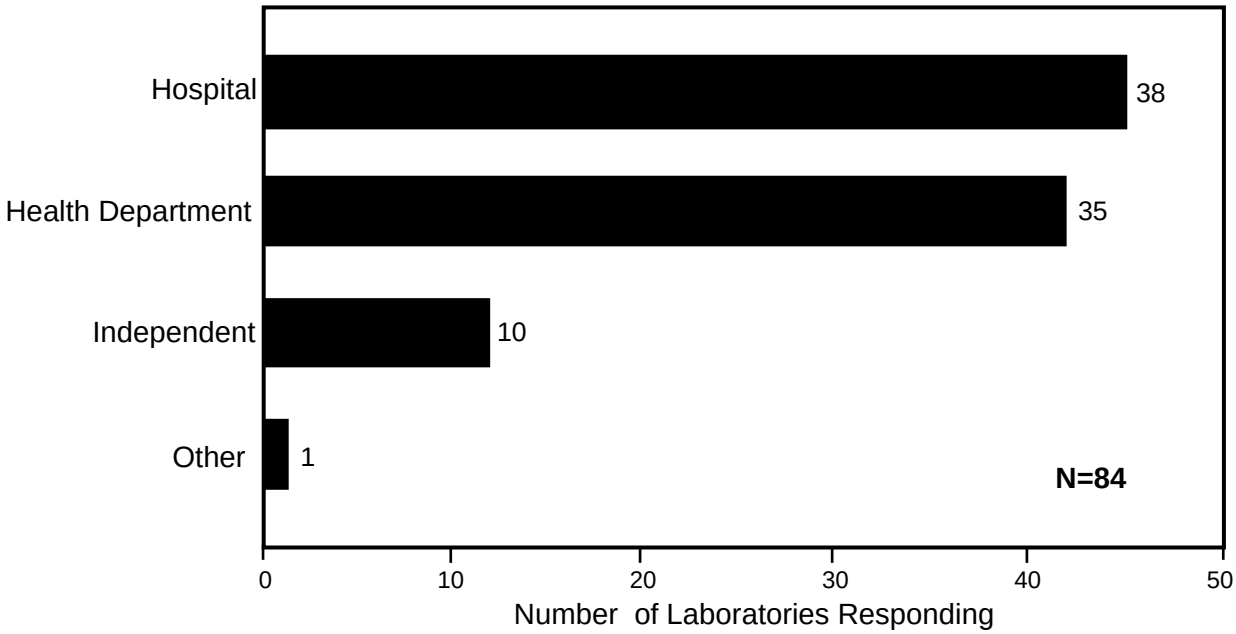
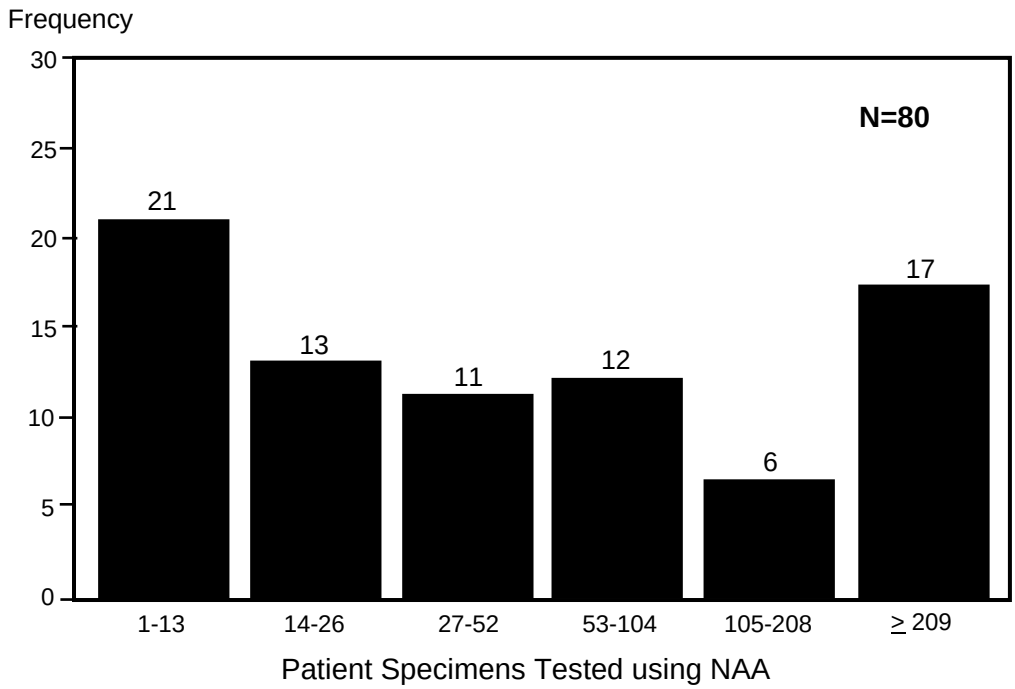


Figure 2. Number of Patient Specimens Tested for *M.tb* Using TB NAA during the Previous Quarter.*



*See explanation in the analysis.

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

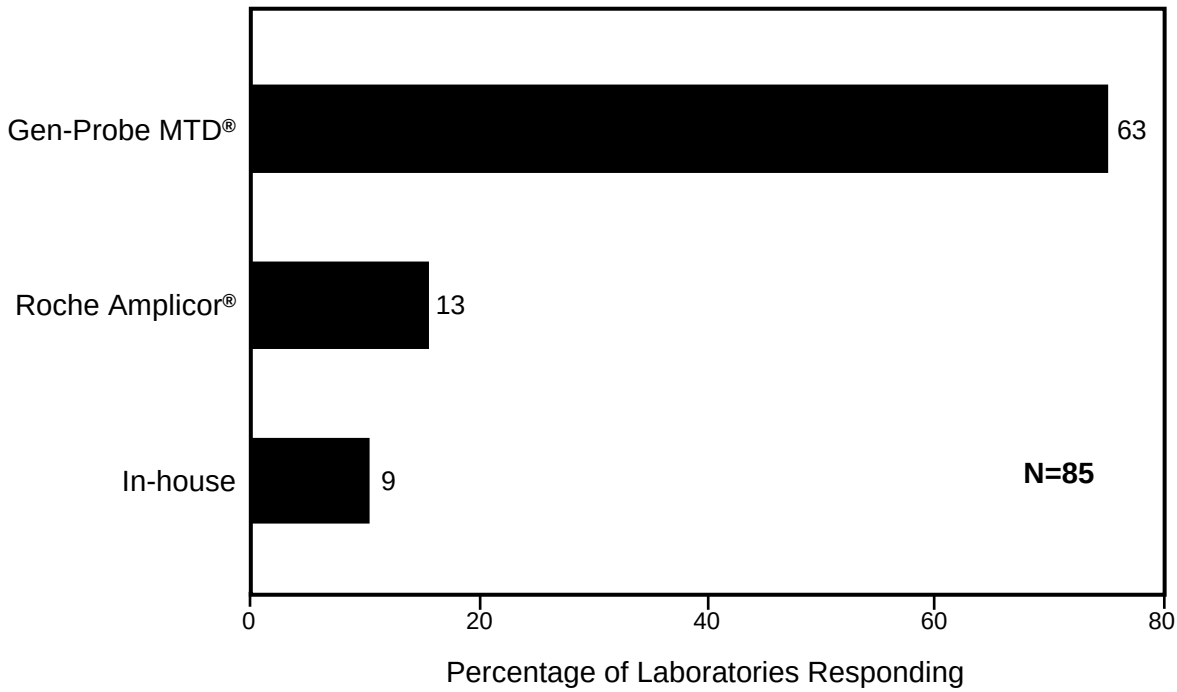


Figure 4. Biosafety Levels of Participant Laboratories

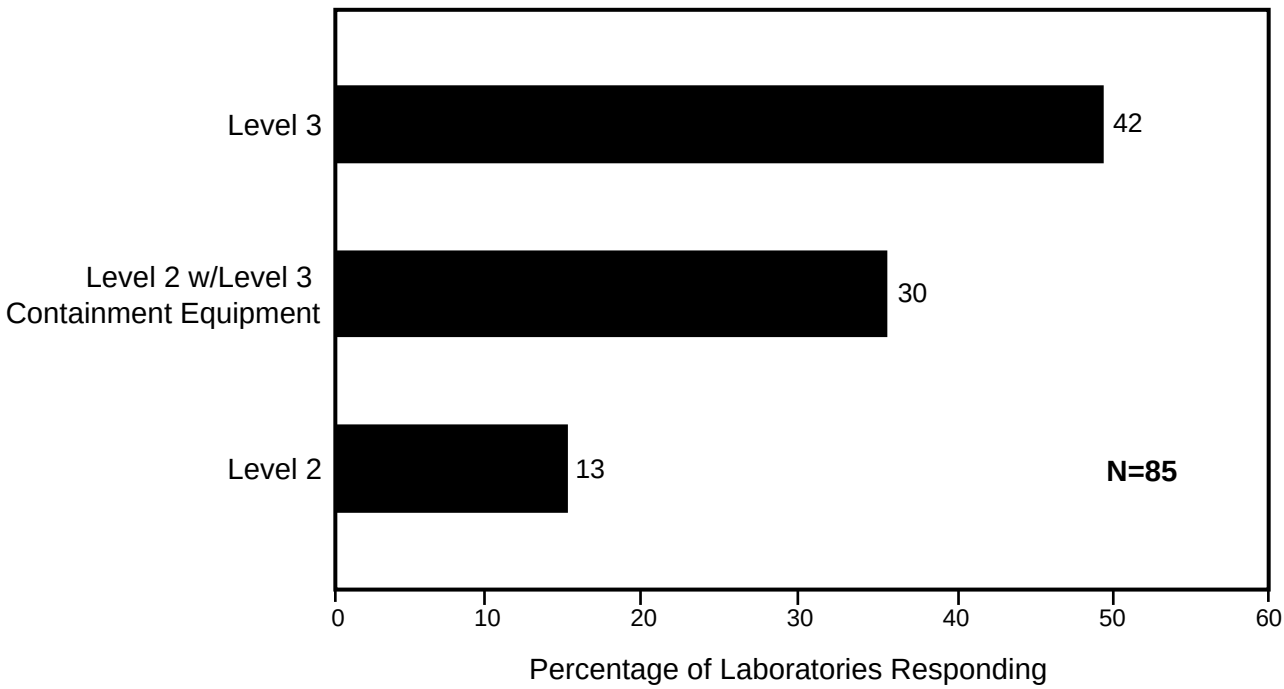


Figure 5. Is the Biological Safety Cabinet that is Used for TB NAA Testing Used for Other Purposes?

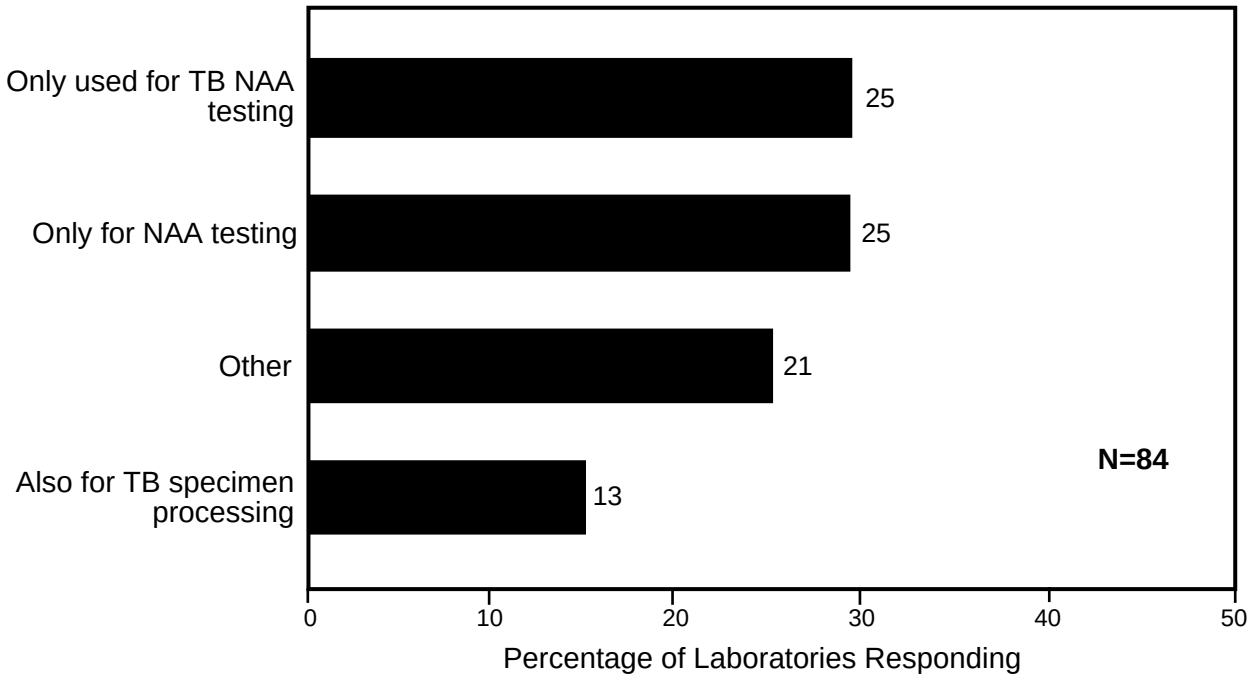


Figure 6. Use of Uni-directional Workflow by Participating Laboratories

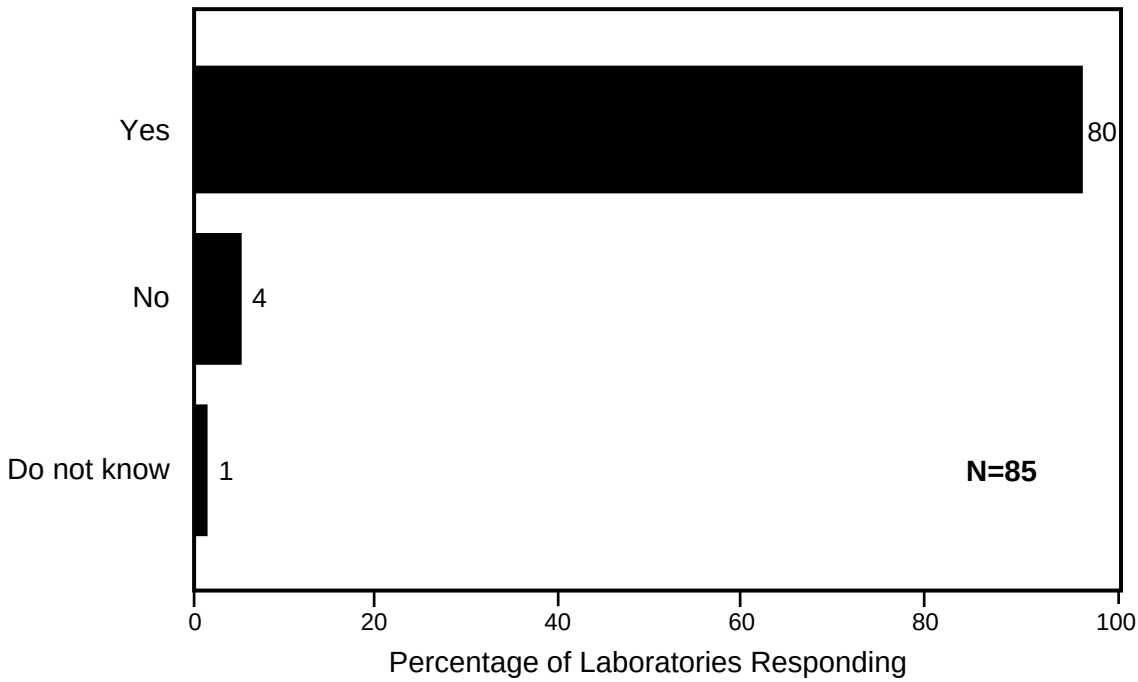


Figure 7. Frequency of TB NAA Qualitative Test Results by Sample Type for the Gen-Probe MTD, Roche Amplicor, and In-House Methods

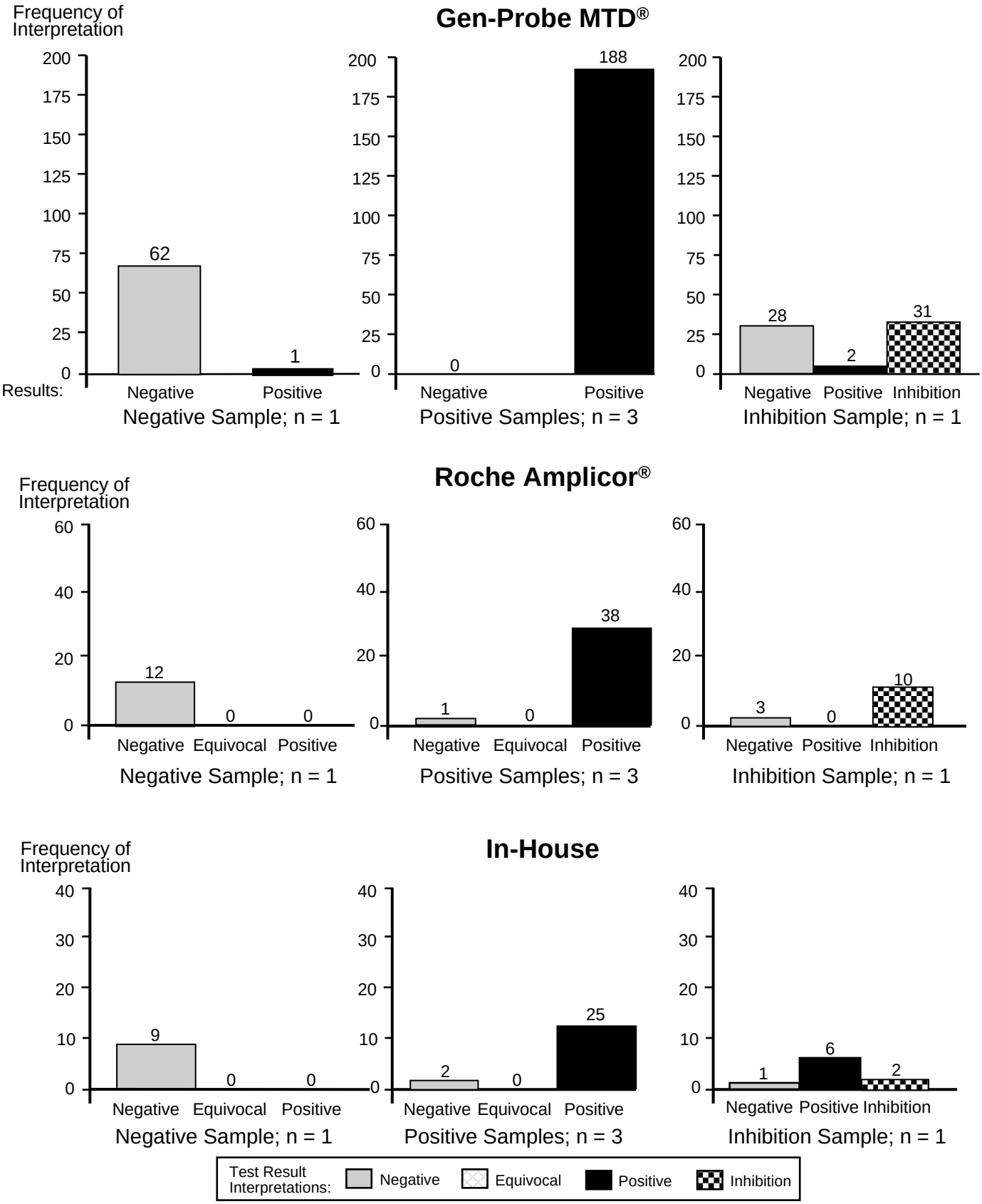


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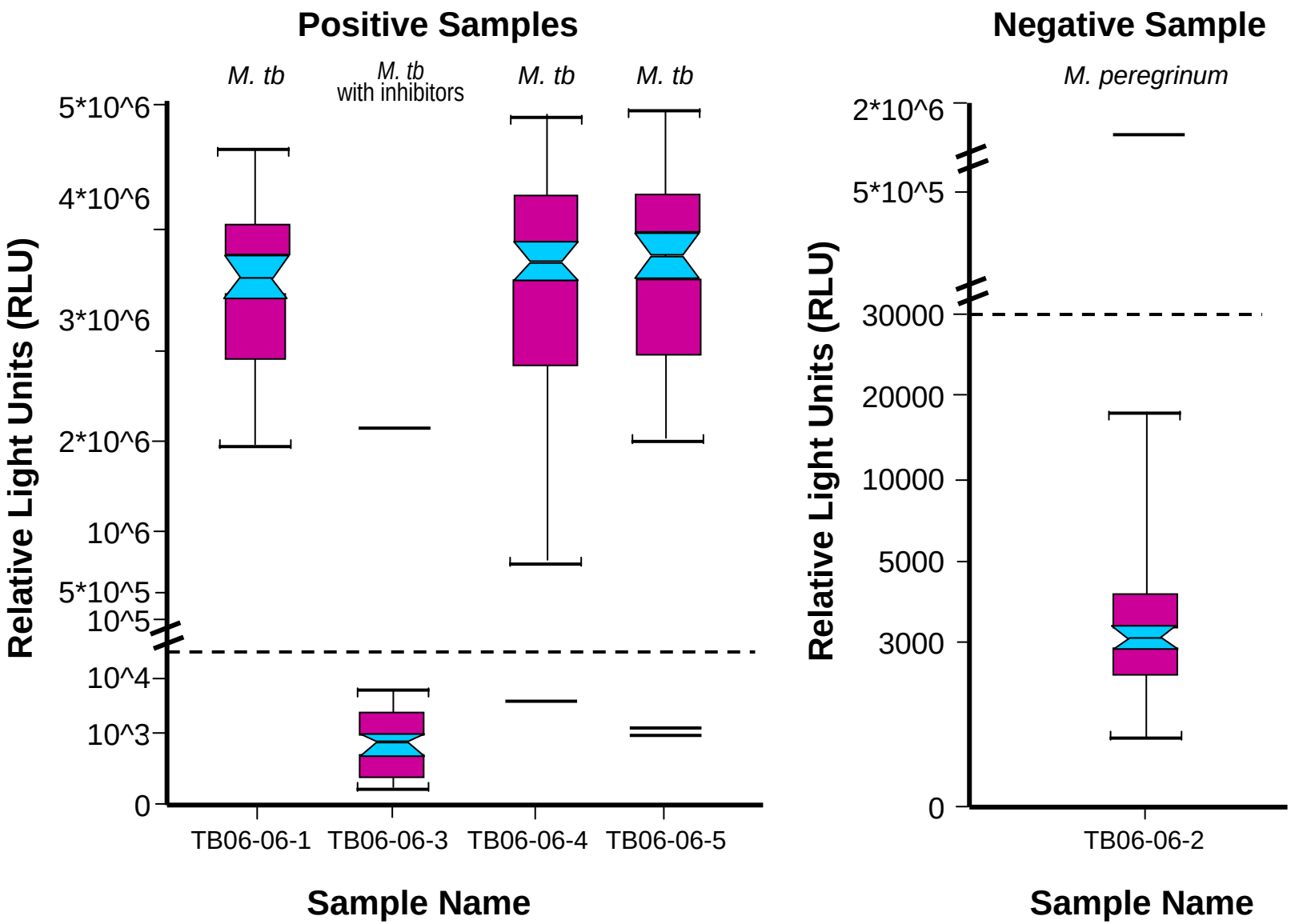
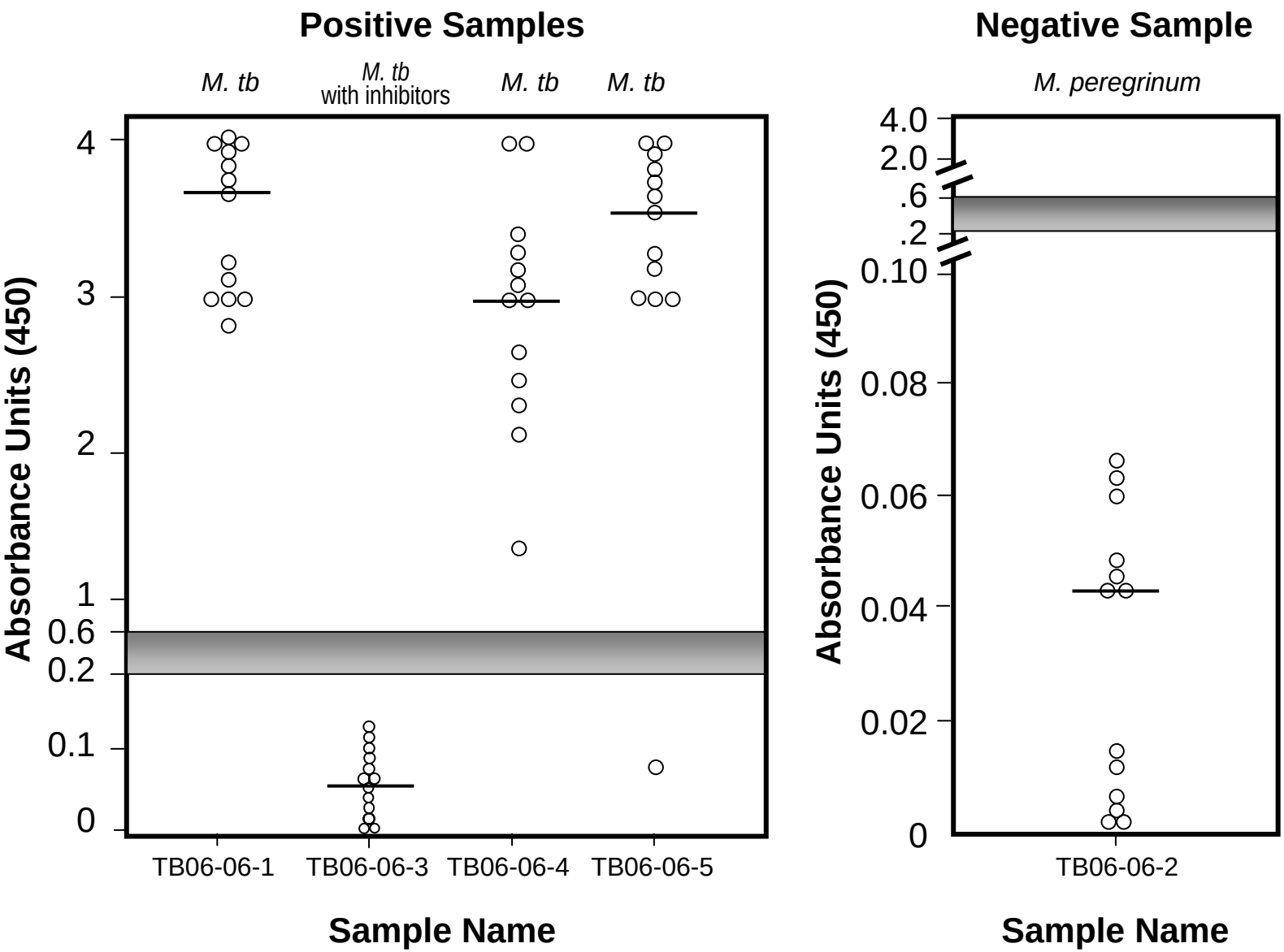
Figure 8. Quantitative Results for GenProbe® MTD



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 June 2006 shipment of samples for the *M. tb* NAA testing performance evaluation program.

Table 1. Sample TB06-06-1 contained *Mycobacterium tuberculosis* (3.0 x 10^5 theoretical cells/ml)

Test Methods	No. Tests Performed	Positive No.	Positive %	Inhibition		Equivocal No.	Equivocal %	Negative No.	Negative %
Gen-Probe	63	63	100.0	-	-	n/a	n/a	0	0.0
In-house	9	9	100.0	-	-	0	0.0	0	0.0
Roche	13	13	100.0	-	-	0	0.0	0	0.0
All methods	85	85	100.0	-	-	0	0.0	0	0.0

Table 2. Sample TB06-06-2 contained *Mycobacterium peregrinum* (3.0 x 10^5 theoretical cells/ml)

Test Methods	No. Tests Performed	Positive No.	Positive %	Inhibition No.	Inhibition %	Equivocal No.	Equivocal %	Negative No.	Negative %
Gen-Probe	63	1	1.6	-	-	n/a	n/a	62	98.4
In-house	9	0	0.0	-	-	0	0.0	9	100.0
Roche	13	0	0.0	1	7.7	0	0.0	12	92.3
All methods	85	1	1.2	1	1.2	0	0.0	83	97.6

Table 3. Sample TB06-06-3 contained *Mycobacterium tuberculosis* (3.0 x 10^5 theoretical cells/ml) with heparin

Test Methods	No. Tests Performed	Positive No.	Positive %	Inhibition No.	Inhibition %	Equivocal No.	Equivocal %	Negative No.	Negative %
Gen-Probe	60	2	3.3	30	50.0	n/a	n/a	28	46.7
In-house	9	6	66.7	2	22.2	0	0.0	1	11.1
Roche	13	0	0.0	10	76.9	0	0.0	3	23.1
All methods	82	8	9.8	42	51.2	0	0.0	32	39.0

Table 4. Sample TB06-06-4 contained *Mycobacterium tuberculosis* (3.0 x 10^4 theoretical cells/ml)

Test Methods	No. Tests Performed	Positive No.	Positive %	Inhibition No.	Inhibition %	Equivocal No.	Equivocal %	Negative No.	Negative %
Gen-Probe	63	62	98.4	1	1.6	n/a	n/a	0	0.0
In-house	9	8	88.9	0	0.0	0	0.0	1	11.1
Roche	13	13	100.0	0	0.0	0	0.0	0	0.0
All methods	85	83	97.6	1	1.2	0	0.0	1	1.2

Table 5. Sample TB06-06-5 contained *Mycobacterium tuberculosis* (3.0 x 10^4 theoretical cells/ml)

Test Methods	No. Tests Performed	Positive No.	Positive %	Inhibition Not applicable		Equivocal No.	Equivocal %	Negative No.	Negative %
Gen-Probe	63	63	100.0	0	0.0	n/a	n/a	0	0.0
In-house	9	8	88.9	0	0.0	0	0.0	1	11.1
Roche	13	12	92.3	0	0.0	0	0.0	1	7.7
All methods	85	83	97.6	0	0.0	0	0.0	2	2.4