

## Analyses of the June 16, 2003 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 June 2003 shipment samples.

### Overall Summary of Results

Method	Total # of laboratories	Total # of results	Positive Donors	Negative Donors	Overall Performance
			False-negative results	False-positive results	
Gen-Probe MTD	64	320	1/128 (0.8%)	2/192 (1.0%)	99.0%
Roche Amplikor	19	94	None	3/56 (5.4%)	97.0%
In-house/Other	7	35	None	1/19 (5.3%)	97.0%

### New Findings

- We included negative samples containing *M. avium* and respiratory pathogens other than *M. tuberculosis*, i.e., *P. aeruginosa* TB03-06-2 and *K. pneumoniae* TB03-06-4. Incorrect results were reported for 2.6% (7/269) of the tests run on samples containing no *M. tuberculosis*.
- The three false positive interpretations reported by laboratories using either Roche or In-house methods for the *K. pneumoniae* (TB03-06-4) represents an overall error rate for this sample of 12% (3/26). However, the sample sizes for these methods in our data set are too low to verify this as a trend.
- Forty-eight of eighty-eight (54.5%) 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, 44% (26/59) indicated that they inquire about the sample submission buffer.

### Findings of note that also have been reported previously

- Of participant laboratories, 16% (14/90) indicated they process *M.tb* specimens in the same biosafety cabinet that is used for *M.tb* NAA testing. Twenty-eight percent (25/90) of participants indicated “Other” uses for the *M.tb* NAA testing BSC.
- It is a concern that 13% (11/88) 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 containing organisms other than *M. tuberculosis* shipped in June 2003. Responses were received from 91 of 92 (99%) laboratories participating in this shipment. (One laboratory sent demographic data, but no testing results.) 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 88 participants. Participants consisted of 37 hospitals, 36 health departments, 12 independents, and 3 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 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<sup>th</sup> 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% (14/90) of participant laboratories indicated that they process *M.tb* specimens in the same BSC that is used for *M.tb* NAA testing. Among the 28% (25/90) of participants that indicated "Other" uses for the *M.tb* NAA testing BSC, 6 performed *M.tb* culture work (biochemicals, drug susceptibility testing, Accuprobe<sup>®</sup> identification, etc.), 7 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<sup>®</sup> and Gen-Probe<sup>®</sup> MTD) recommend the use of unidirectional workflow. It is a concern that 13% (11/88) 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<sup>®</sup> 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<sup>®</sup>, 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 99% (179/180) for the 2 positive samples: 99% (127/128) sensitivity for Gen-Probe<sup>®</sup> MTD; 100% (38/38) sensitivity for Roche Amplicor<sup>®</sup>; 100% (14/14) sensitivity for In-house methods. The combined analytical specificity of all methods was 97% (262/269) for the 3 negative samples: 99% (190/192) specificity for Gen-Probe<sup>®</sup>; 93% (52/56) specificity for Roche Amplicor<sup>®</sup>; 95% (20/21) specificity for In-house methods. Samples TB03-06-2, TB03-06-4, and TB03-06-5 contained  $2.4 \times 10^6$  theoretical cells/ml of *P. aeruginosa*,  $2.8 \times 10^6$  theoretical cells/ml of *K. pneumoniae* and  $8.27 \times 10^2$  cells/ml of *M. avium* respectively.

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<sup>th</sup> percentile and 75<sup>th</sup> 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, TB03-06-1 and TB03-06-3, the median values of all data were 2,670,608 and 2,617,089 relative light units (RLU), respectively. The median values for the negative samples containing *P. aeruginosa*, TB03-06-2, *K. pneumoniae*, TB03-06-4 and *M. avium*, TB03-06-5 were 2,337, 2,298 and 2,400 relative light units (RLU) 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<sub>450</sub>) for both positive samples, TB03-06-1 and TB03-06-3. The median values for the samples containing *P. aeruginosa*, TB03-06-2, *K. pneumoniae*, TB03-06-4, and *M. avium*, TB03-06-5 were 0.056 (A<sub>450</sub>), 0.052 (A<sub>450</sub>) and 0.053 (A<sub>450</sub>) respectively.

In response to a question regarding inhibition testing, 55% (48/88) 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. The only way to distinguish between a truly negative NAA-TB specimen and one which is negative due to the presence of inhibitors and therefore of no diagnostic help, is to test negative specimens for inhibitors.

Since specimen suspension 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, 44% (26/59) indicated that they did inquire about the sample submission buffer. Resuspension fluids containing very high molarity phosphate concentrations may be incompatible with molecular amplification tests due to inhibition of amplification. Thus, laboratories receiving processed specimen sediments for NAA-TB testing should be aware of the buffer that was used to process the specimen.

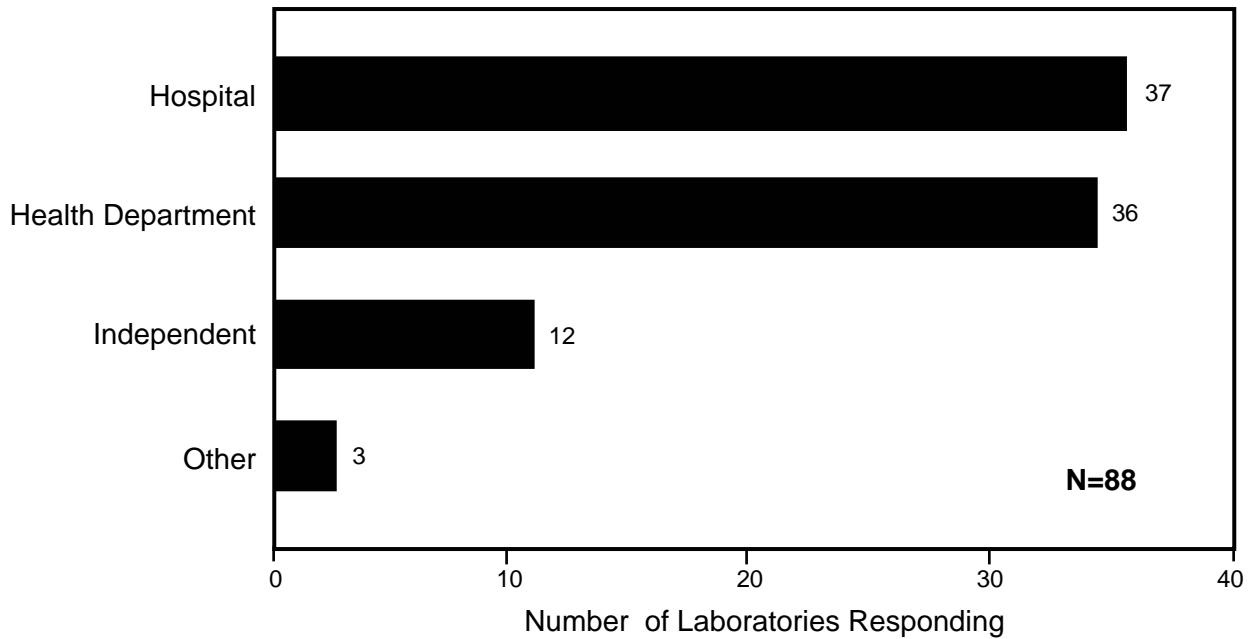
Tables 1-5 provide the qualitative results reported for individual samples by participants. In most instances the laboratories used the manufacturer’s recommended interpretations of quantitative test results; however, there were exceptions. In this shipment, in addition to *M. avium* samples, we included samples containing other potential respiratory pathogens, i.e., *P. aeruginosa* (TB03-06-2) and *K. pneumoniae* (TB03-06-4). One false positive result was reported using the Roche Amplicor® method for *P. aeruginosa*. Two false positive results were reported by laboratories using the Roche method and one false positive result was reported using an In-house method for *K. pneumoniae*. There were two false positive results reported by laboratories using the Gen-Probe MTD method for the *M. avium* sample (TB03-06-5). (One of these was due

to incorrect interpretation for which quantitative results were in the negative range.) Thus incorrect results were reported for 2.6% (7/269) of the tests run on samples containing no *M. tuberculosis*. Laboratories producing false-positive results should review their testing protocols to detect potential sources of cross-contamination.

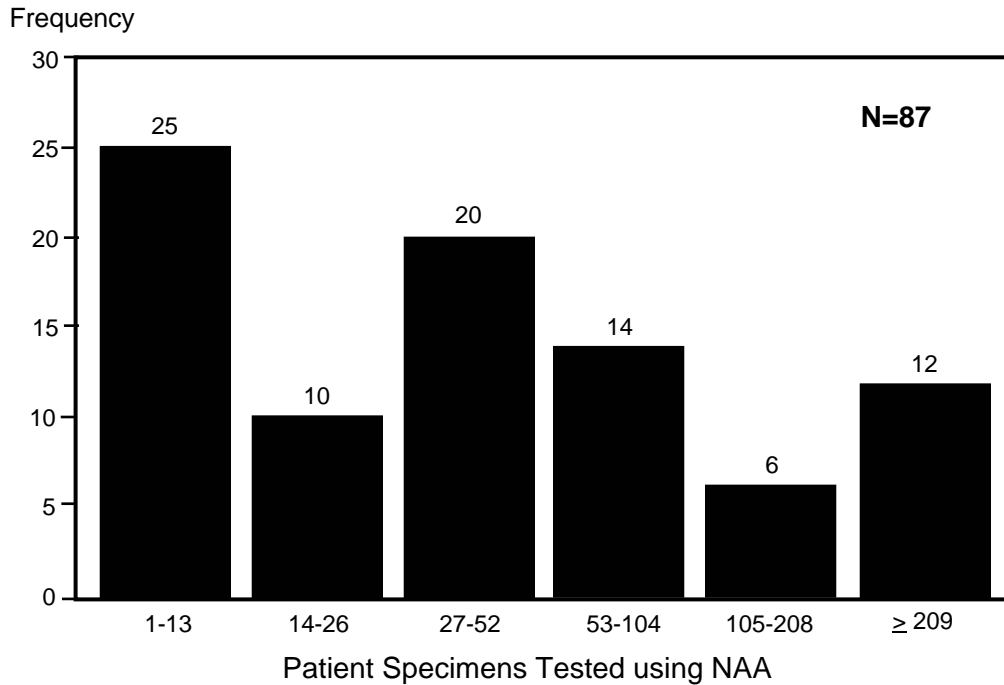
### References

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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.
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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**

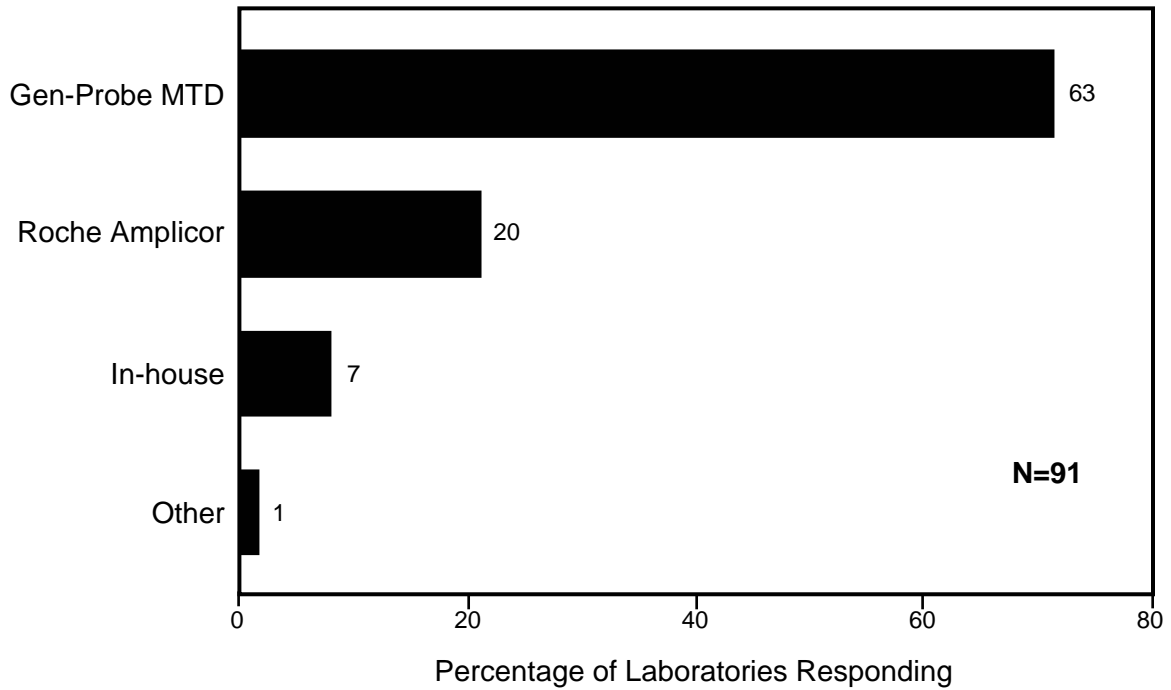


**Figure 2. Number of Patient Specimens Tested for *M.tb* Using TB NAA during the Previous Quarter.\***

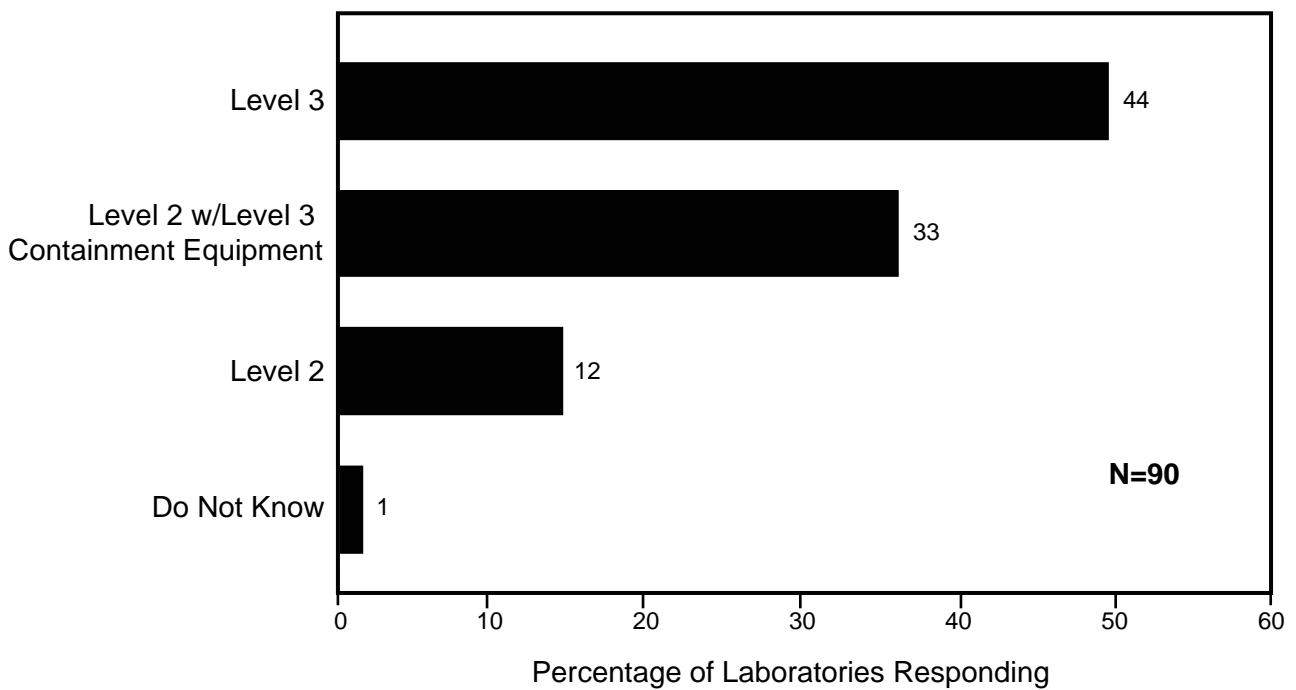


\*See explanation in the analysis.

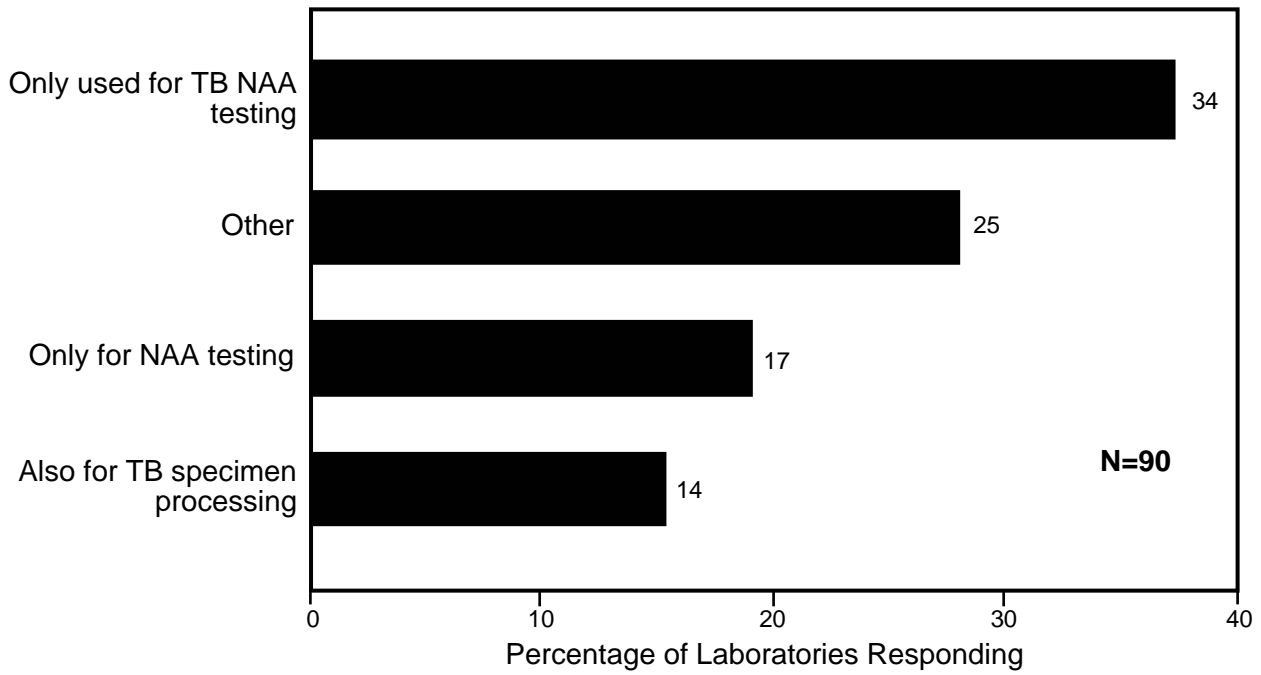
**Figure 3. Amplification Procedure Used for Direct Detection of *M.tb***



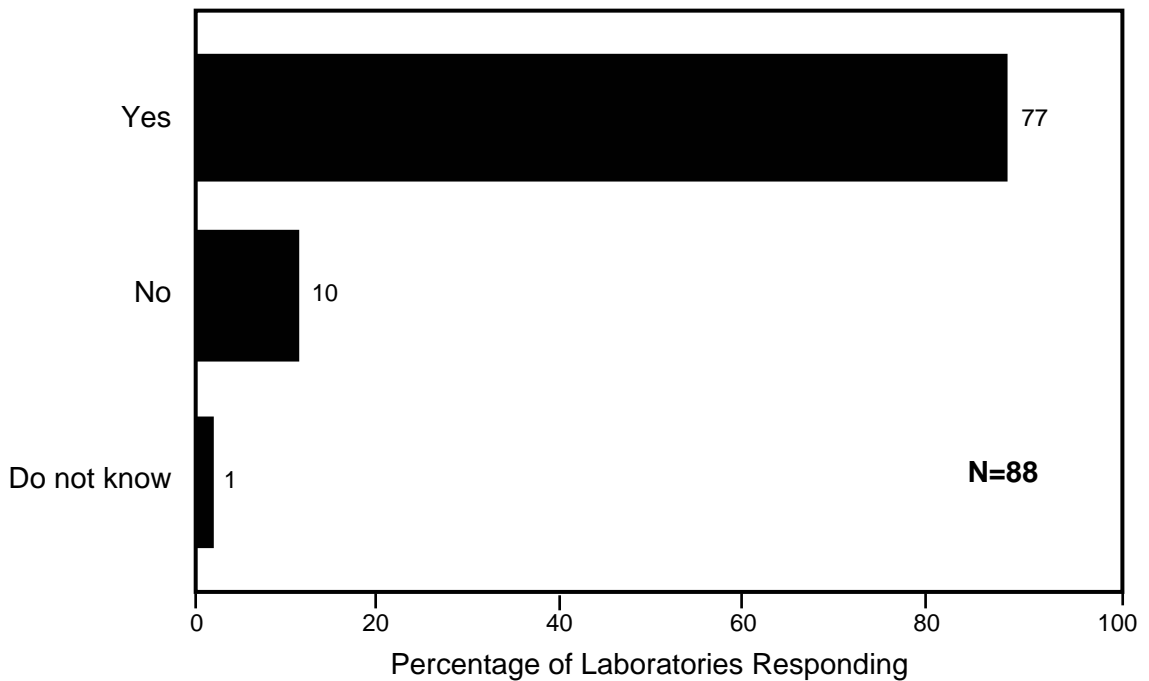
**Figure 4. Biosafety Levels of Participant Laboratories**



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

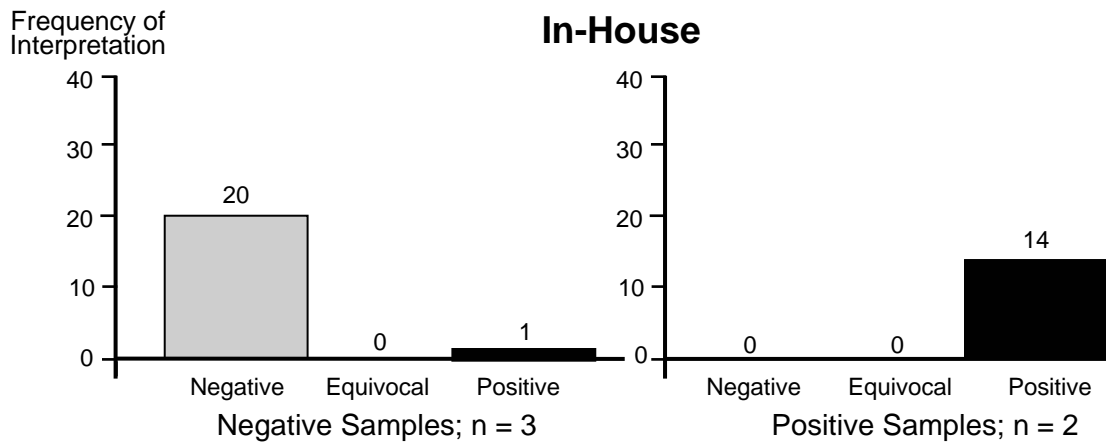
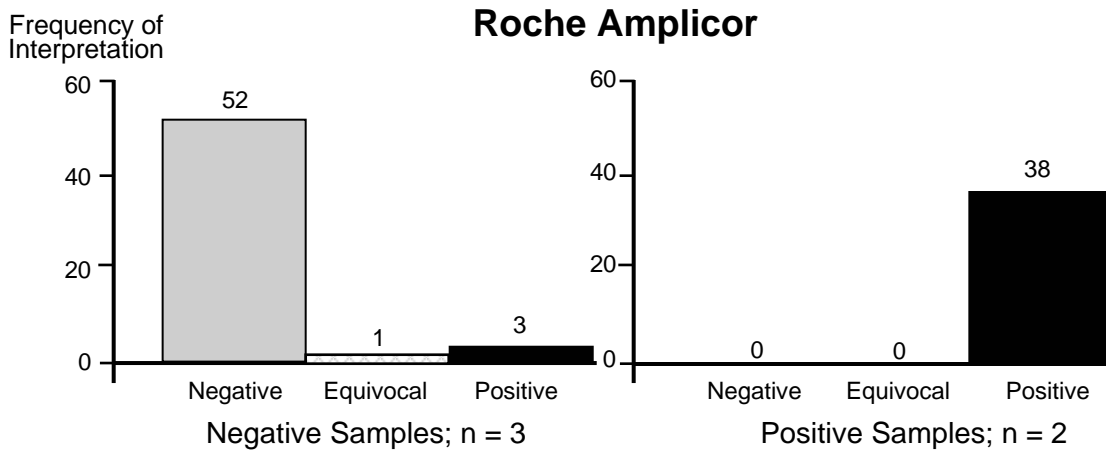
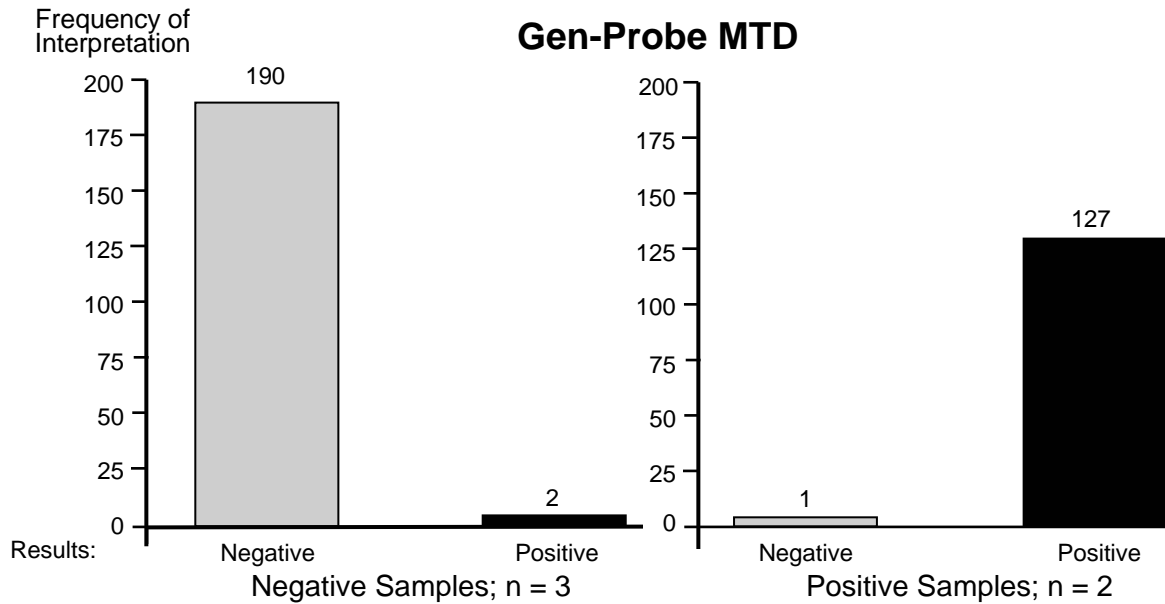


**Figure 6. Use of Uni-directional Workflow by Participating Laboratories**



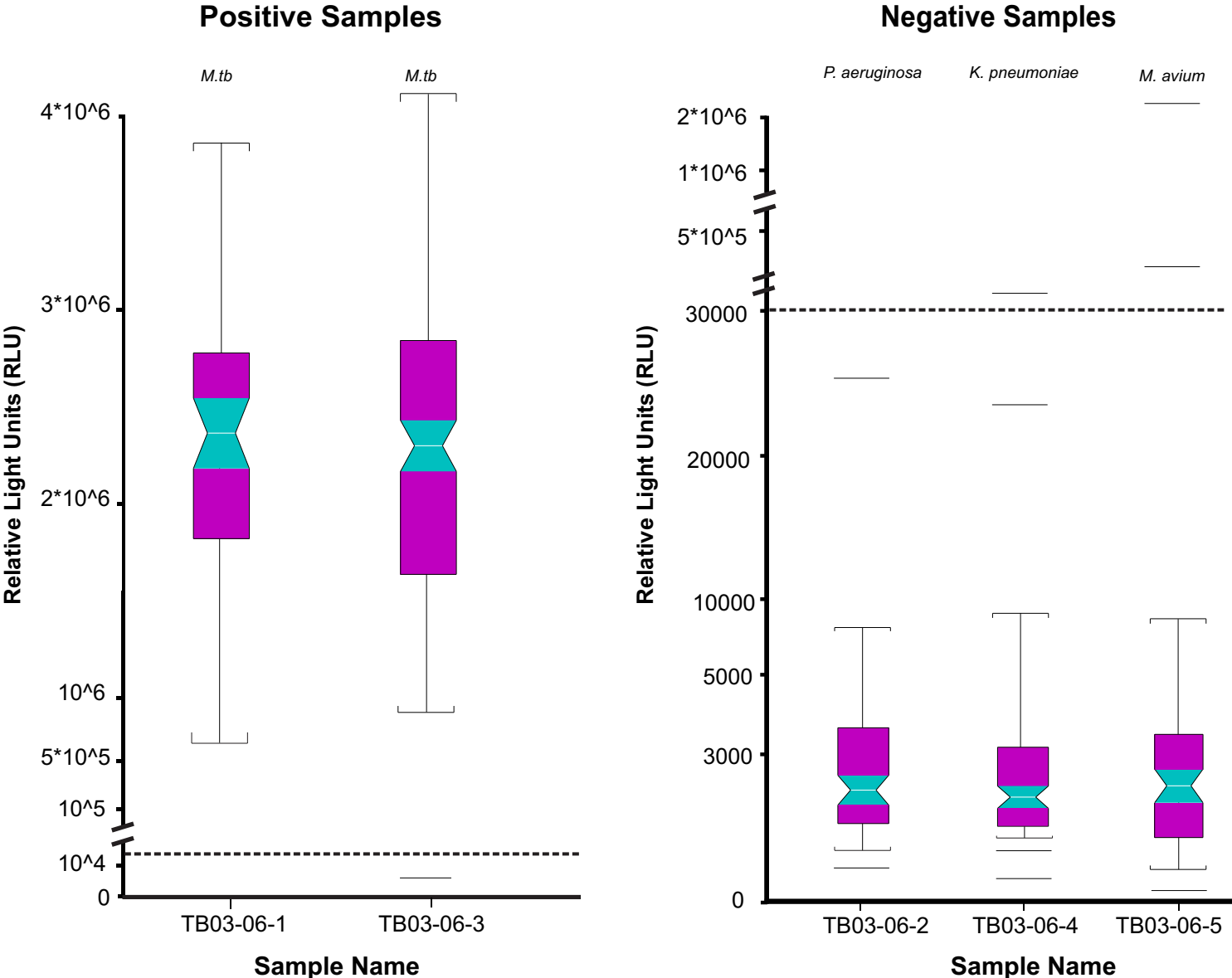


**Figure 7. Frequency of TB NAA Qualitative Test Results by Sample Type for the Gen-Probe MTD, Roche Amplicor, and In-House Methods**



Test Result Interpretations:  Negative  Equivocal  Positive

# Figure 8. Quantitative Results for GenProbe<sup>®</sup> MTD



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



The following tables summarize qualitative results reported by participant laboratories for the June 2003 shipment of samples for the *M. tb* NAA testing performance evaluation program.

Table 1. Sample TB03-06-1 contained *Mycobacterium tuberculosis*

Test Methods	No. Tests Performed	Positive		Equivocal		Negative	
		No.	%	No.	%	No.	%
Gen-Probe	64	64	100.0	0	0.0	0	0.0
In-house	7	7	100.0	0	0.0	0	0.0
Roche	19	19	100.0	0	0.0	0	0.0
All methods	90	90	100.0	0	0.0	0	0.0

Table 2. Sample TB03-06-2 contained *P. aeruginosa*

Test Methods	No. Tests Performed	Positive		Equivocal		Negative	
		No.	%	No.	%	No.	%
Gen-Probe	64	0	0.0	0	0.0	64	100.0
In-house	7	0	0.0	0	0.0	7	100.0
Roche	18	1	5.6	0	0.0	17	94.4
All methods	89	1	1.1	0	0.0	88	98.9

Table 3. Sample TB03-06-3 contained *Mycobacterium tuberculosis*

Test Methods	No. Tests Performed	Positive		Equivocal		Negative	
		No.	%	No.	%	No.	%
Gen-Probe	64	63	98.4	0	0.0	1	1.6
In-house	7	7	100.0	0	0.0	0	0.0
Roche	19	19	100.0	0	0.0	0	0.0
All methods	90	89	98.9	0	0.0	1	1.1

Table 4. Sample TB03-06-4 contained *K. pneumoniae*

Test Methods	No. Tests Performed	Positive		Equivocal		Negative	
		No.	%	No.	%	No.	%
Gen-Probe	64	0	0.0	0	0.0	64	100.0
In-house	7	1	14.3	0	0.0	6	85.7
Roche	19	2	10.5	0	0.0	17	89.5
All methods	90	3	3.3	0	0.0	87	96.7

Table 5. Sample TB03-06-5 contained *Mycobacterium avium* complex

Test Methods	No. Tests Performed	Positive		Equivocal		Negative	
		No.	%	No.	%	No.	%
Gen-Probe	64	2	3.1	0	0.0	62	96.9
In-house	7	0	0.0	0	0.0	7	100.0
Roche	19	0	0.0	1	5.3	18	94.7
All methods	90	2	2.2	1	1.1	87	96.7