September 16, 1997

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 April 1997 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 April 1997 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 80 of 94 (85%) 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.

This *M.tb* NAA testing program and resulting report represent the contributions of staff from CDC, Wisconsin State Laboratory of Hygiene (WSLH), and the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) including:

CDC/PHPPO - Dr. Laurina Williams, Ron Fehd, Mae Lee, and James Handsfield
CDC/NCID - Dr. Beverly Metchock, Dr. Jack Crawford
WSLH - Sue Legois, Louise Kubista, Neil May, Michelle Bussen, and Dr. Peter Shult
ASTPHLD - Dr. Nancy Warren

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 comment or suggestions on the format selected for the results, or questions regarding this report, you may call me at (770) 488-4674.

Sincerely yours,

John C. Ridderhof, Dr.P.H.
Science Administrator
Division of Laboratory Systems
Public Health Practice Program Office

Enclosures

Analyses of the April 1997 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 analyses 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 April 1997. Testing results were received from 80 of 94 (85%) laboratories participating in this shipment. A number of laboratories enrolled in this NAA testing program, however, either did not perform NAA testing or where not currently performing NAA testing. The participation was 80 of 83 (96.4%) among enrolled laboratories that reported they currently performed *M.tb* NAA testing. 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, WSLH.

The particular characteristics of NAA tests, such as increased sensitivity and concerns about specificity, provide particular challenges to developing reliable and consistent test samples for performance evaluation. Therefore, this report also provides information on test sample content and validation before shipment.

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).

The average number of organisms in test samples was determined through flow cytometry and culture for colony forming units (cfu). Experiments were also 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 five reference laboratories before shipping.

Figure 1 shows the laboratory classification represented by 78 of the 80 participants. Participants consisted of 41 hospitals, 23 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 between January and March 1997. A 3-month period was used since many laboratories have only recently implemented *M.tb* NAA testing, and annual test volumes might be misleading. The volume of specimens tested is represented in ranges that are multiples

of 13 to estimate the average weekly test volume for participant laboratories in this 3-month period.

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. The two participant laboratories that indicated "other" procedures were manufacturers of *M.tb* NAA test systems not yet cleared by the FDA. 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. 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 27.5% (22/80) of participant laboratories indicated that they also use the *M.tb* NAA testing BSC for *M.tb* specimen processing. Among the 23.8% (19/80) of participants that indicated "other" uses for the *M.tb* NAA testing BSC, 8 performed *M.tb* culture work (biochemicals, drug susceptibility testing, Accuprobe® identification, etc.), 5 performed mycology, and 3 performed virology testing.

Figure 6 provides participant responses to a question on the use of uni-directional workflow for *M.tb* NAA testing. Although 65.8% (52/79) of participants responded that they used uni-directional workflow, 24% (19/79) did not use uni-directional workflow and 10.1% (8/79) did not know whether they used uni-directional workflow for *M.tb* NAA testing. In addition to recommendations (3) that emphasize considerations of laboratory design for NAA testing, one of the manufacturer's (Roche Amplicor®) recommends 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 (6 reported using absorbance detection systems, 3 ethidium bromide, and 1 radioactive probe). 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 3 *M.tb*-negative and 2 *M.tb*-positive samples.

Figure 8 is a 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 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, 1-5 and 1-7, the median values of all data were 2,859,731 relative light units (RLU) and 2,846,670 RLU, respectively. The median values for the negative samples, 1-4 and 1-6, were 5,029 RLU and 5,718 RLU, respectively. A broad range of values were reported for sample 1-8, the *M.kansasii* sample (R= 3,390 - 1,547,343 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, 1.5 and 1.7, the median values were 2.80 (A_{450}) and 2.99 (A_{450}), respectively. The median values for both negative samples was 0.06 (A_{450}). The median value for the *M. kansasii* sample, 1-8, was 0.06 (A_{450}).

Tables 1-5 provide the qualitative results reported for individual samples by participants. In some instances the laboratories did not use the manufacturer's recommended interpretations of quantitative test results. Of the 305 test results from 61 participant laboratories using the Gen-probe® MTD test; 5 test results were reported positive, although the quantitative results were below the recommended threshold for positivity (>500,000 RLU); and one test result was reported as inconclusive although the quantitative result was in the recommended range for negative (<30,000 RLU). Of the 45 test results from 9 participant laboratories using the Roche Amplicor® test, 2 test results were reported positive although the quantitative results were below the recommended threshold for positivity [>0.4 Absorbance at wavelength 450 (A_{450})].

The sensitivity in detecting positive samples was good for participants using the Gen-probe® MTD, Roche Amplicor®, and in-house *M.tb* NAA tests. Both of the *M. tuberculosis*-positive samples, 1-5 and 1-7, contained low numbers of organisms (31-48 CFU/50μl as determined by colony counts and flow cytometry) to assess test sensitivity in the absence of the various inhibitory substances present in processed respiratory specimens. False negative, inconclusive/equivocal, and false positive test results tended to be reported by the same laboratories including: 1 participant using Gen-probe® MTD had positive quantitative and qualitative results for all 5 samples; 2 participants (1- Gen-probe® MTD, 1-Roche Amplicor®) had inconclusive quantitative results for both *M. tuberculosis*-positive samples, 2 participants (1- Gen-probe® MTD, 1 in-house *M.tb* NAA) had negative results for all 5 samples.

There were 8.6% (7/81) false positives *M.tb* NAA test results reported for sample 1-6 which contained only *M. gordonae*. It is unlikely that these false positives were introduced during the manufacturing of samples since the samples containing *M. gordonae* were produced in a separate area and 9 randomly selected samples were tested for sample validation. Participant laboratories might have cross-contaminated sample 1-6 from one of the *M. tuberculosis*-positive test samples (6), however, as mentioned previously the positive test samples contained very few organisms. To determine if a false positive test result reported for sample 1-6 correlated with specific testing practices the test results for sample 1-6 were compared with the participant's response on selected questions. A significant association was found between reporting a false positive test result for sample 1-6 and reporting that the biological safety cabinet (BSC) used for *M.tb* NAA testing is also used for TB specimen processing (2-tailed Fisher exact test $P<0.001$). The 22/81 participants that reported "the BSC used for *M.tb* NAA testing is also used for TB specimen processing" accounted for 6/7 false positives for sample 1-6. Participants that perform *M.tb* NAA testing in the same BSC that is used for TB specimen processing should re-examine their practices. Laboratories should be aware of recommendations (3) to perform specimen processing and NAA testing in separate equipment and areas.

Sample 1-8 contained >500 CFU/ml of *M. kansasii*. This organism was chosen because of reports of false positive *M.tb* NAA tests in patients with *M. kansasii* infections (Jorgensen, J. et al., False-positive Gen-Probe direct *M. tuberculosis* amplification tests in patients with pulmonary *M. kansasii* infection, ICAAC 1996 New Orleans) that led Gen-Probe to revise the threshold for positivity in the MTD test from 30,000 RLU to 500,000 RLU. The 61 participants using Gen-Probe® MTD had the following quantitative results; (40/61) negative, (18/61) inconclusive, and (3/61) positive results. One participant reported the qualitative result of positive for a quantitative result in the inconclusive range. One of the three positive results was reported by a participant laboratory that reported positive quantitative and qualitative results for all 5 samples, reflecting probable cross-contamination rather than cross-reactivity between *M. kansasii* and the MTD detection probe. No equivocal results were reported for sample 1-8 by the 9 participants using the Roche Amplicor® test. The prevalence of *M. kansasii* infections in different areas of the country may affect the frequency at which laboratories isolate this species from AFB smear positive respiratory specimens. Laboratories, however, should be aware of the potential for *M.tb* NAA test cross-reactivity with non-tuberculosis mycobacteria when reporting results that do not meet the manufacturer's recommended threshold for positivity.

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

Figure 1. Primary Classification of Participating Laboratories

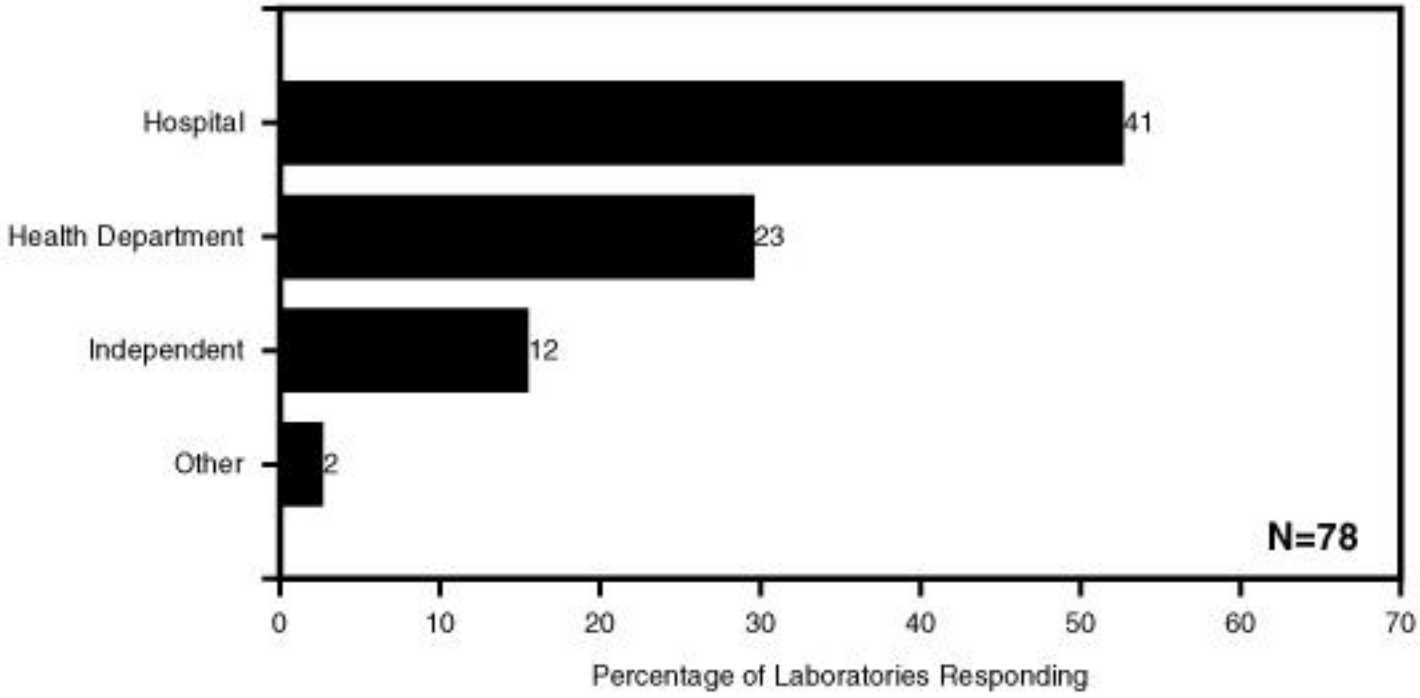


Figure 2: Number of Patient Specimens Tested for M.tb Using TB NAA between January 1, 1997 and March 31, 1997

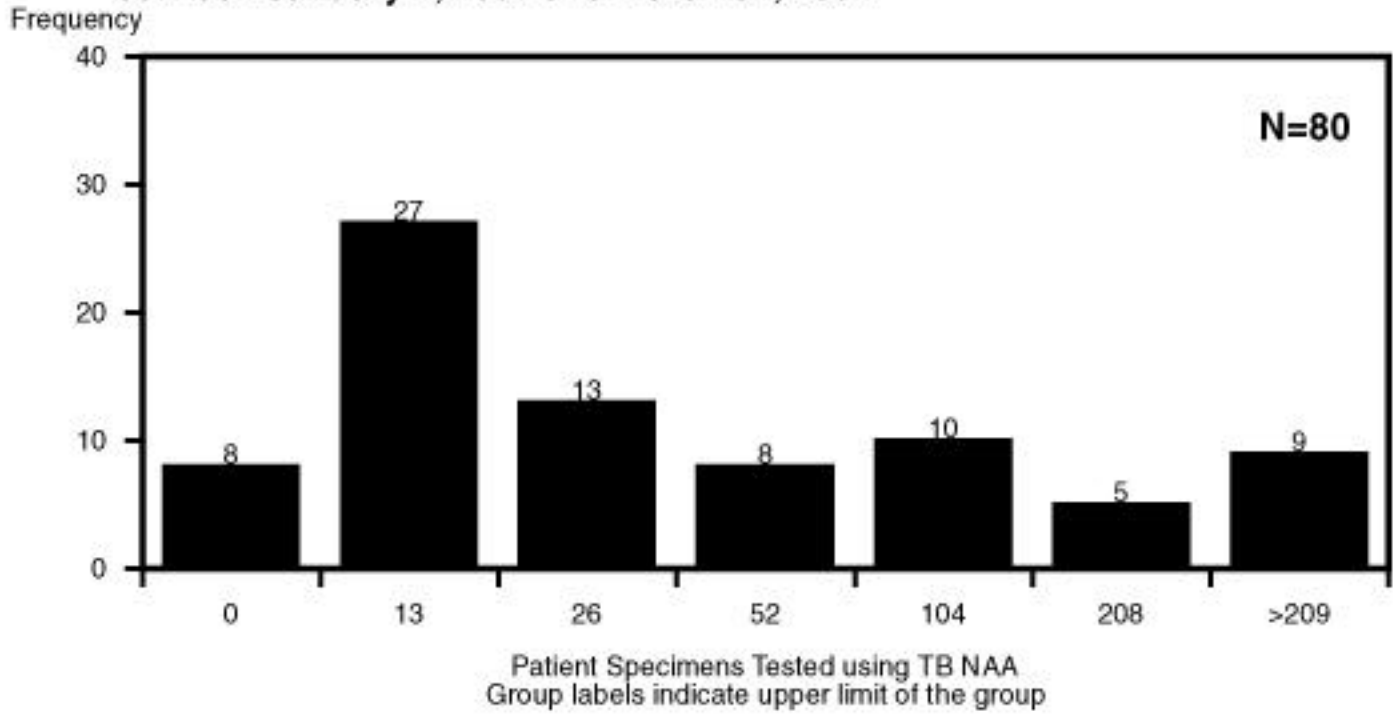


Figure 3: Amplification Procedure Used for Direct Detection of M.tb

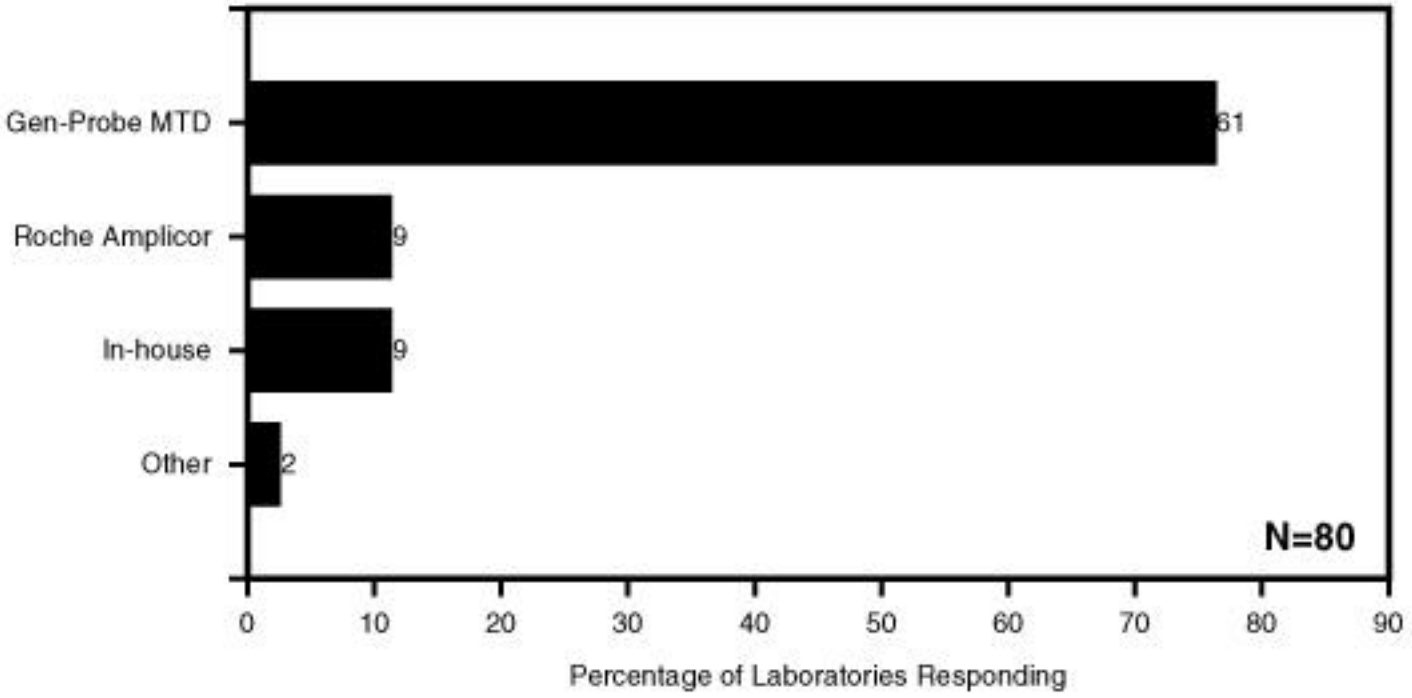


Figure 4: Biosafety Levels of Participating Laboratories

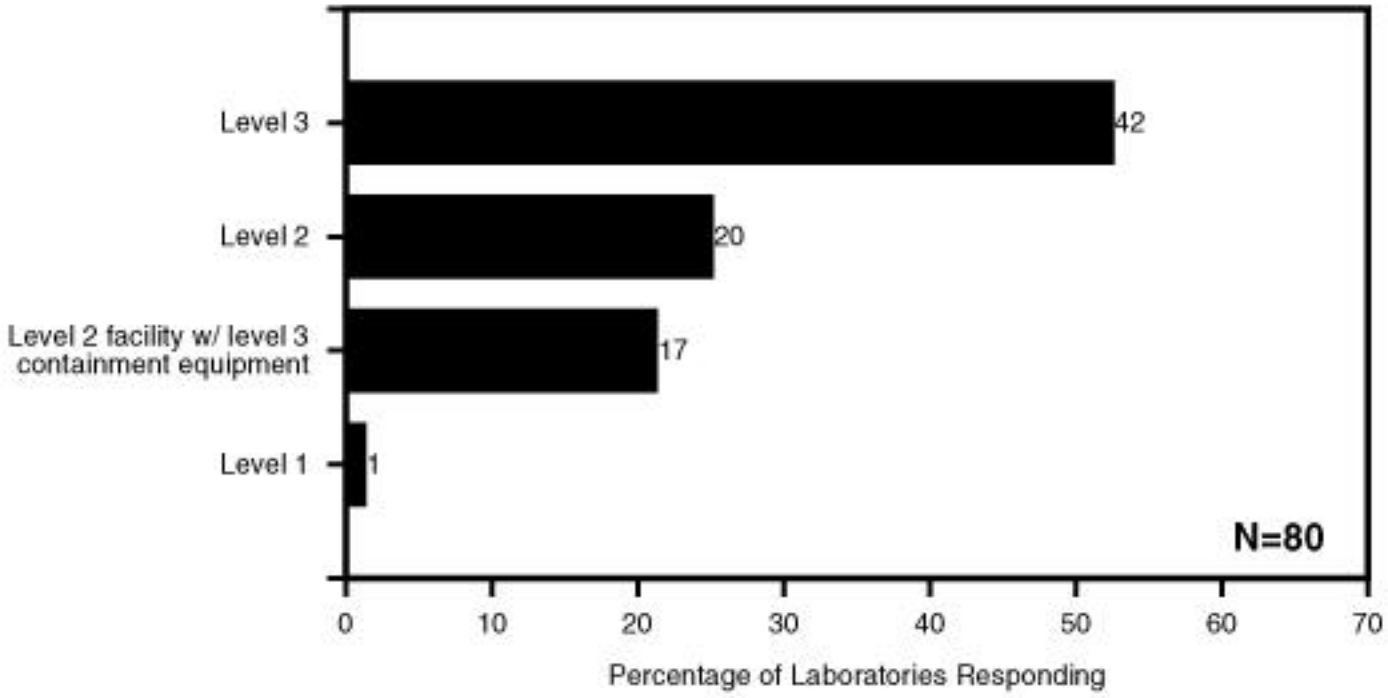


Figure 5: Is the Biological Safety Cabinet that is Used for TB NAA Testing Used for Other Purposes?

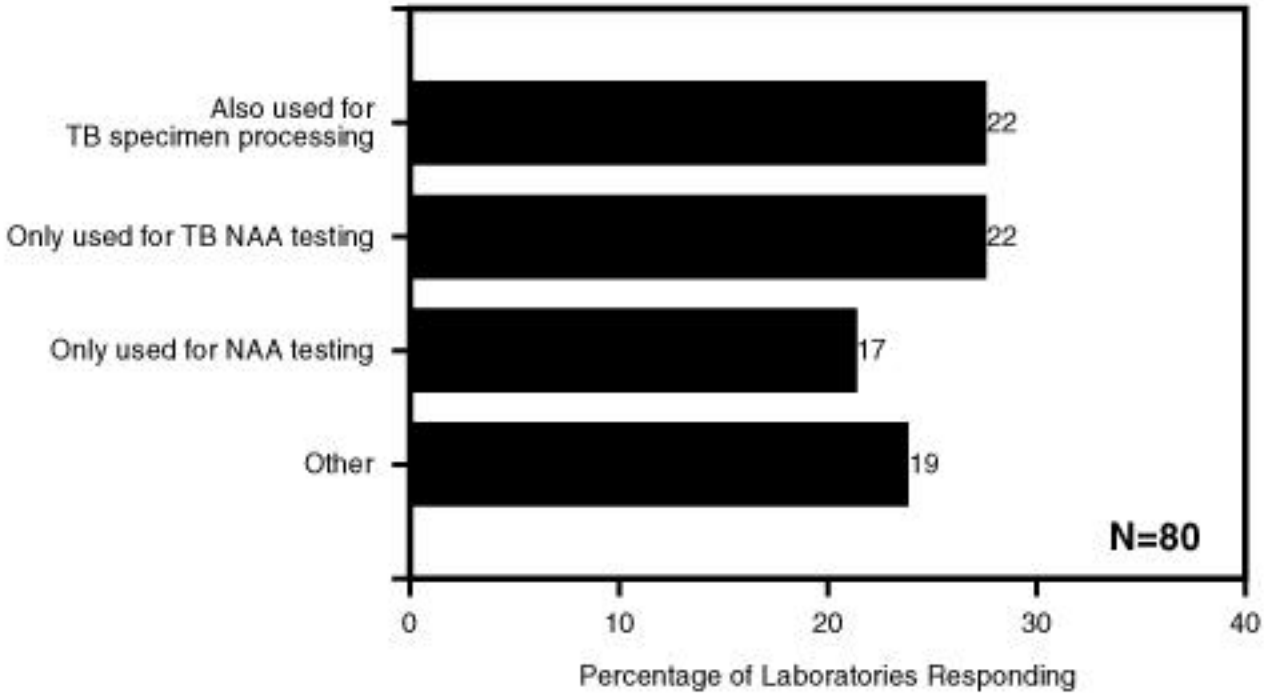


Figure 6: Use of Uni-directional Workflow by Participating Laboratories

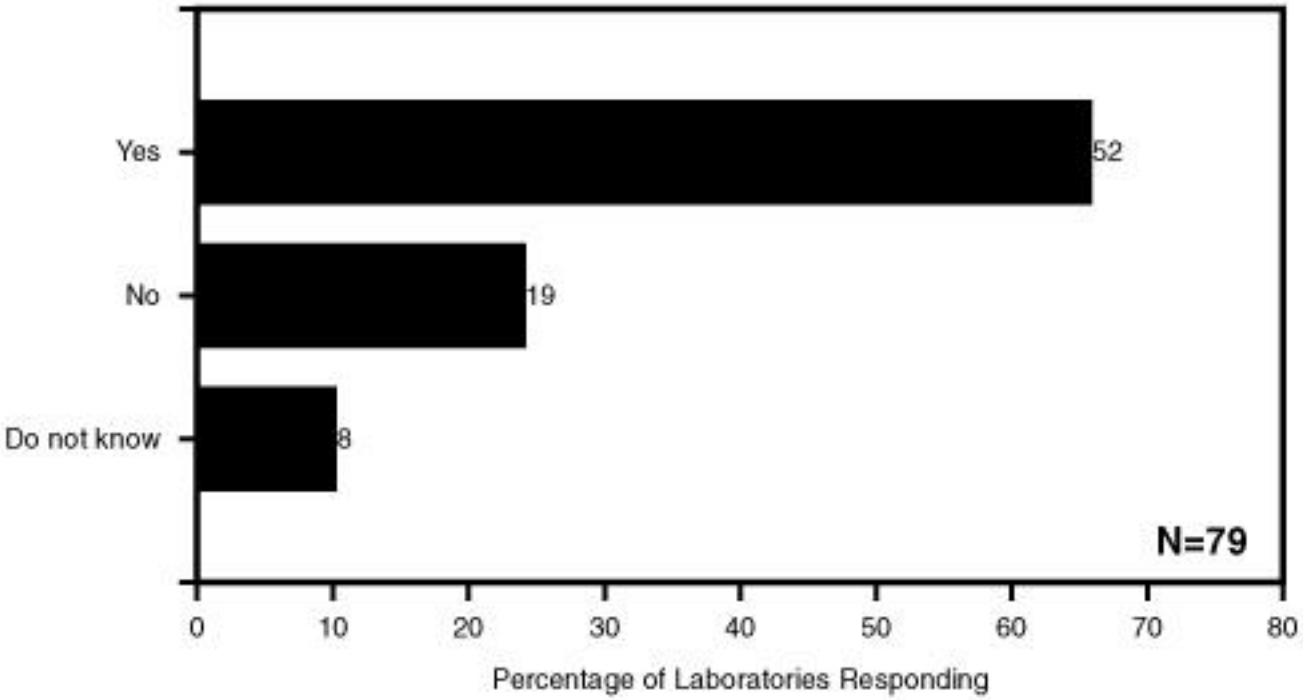


Figure 7: Frequency of TB NAA Qualitative Test Results by Sample Type for the Gen-Probe MTD, Roche Amplicor, and In-house Methods

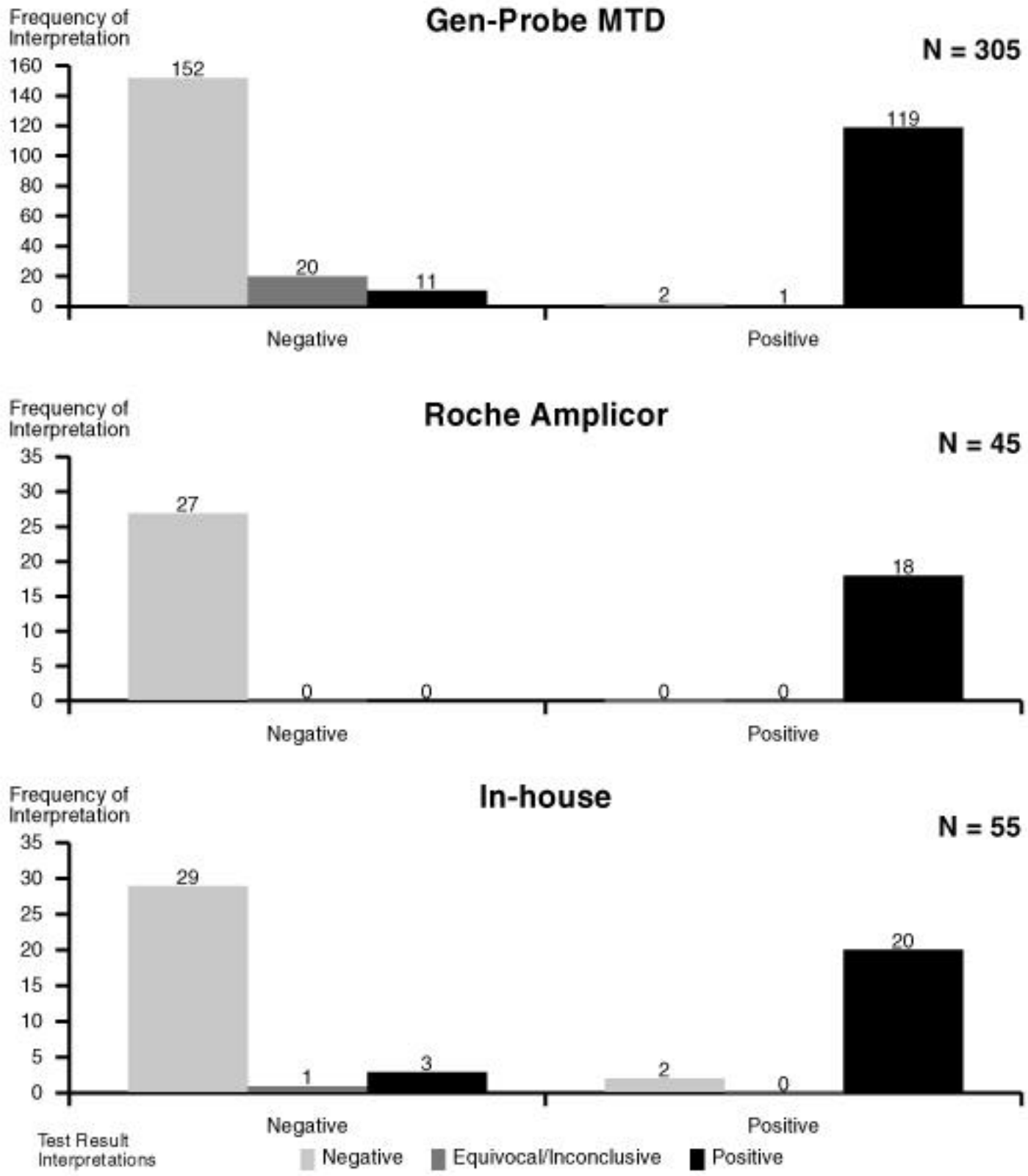
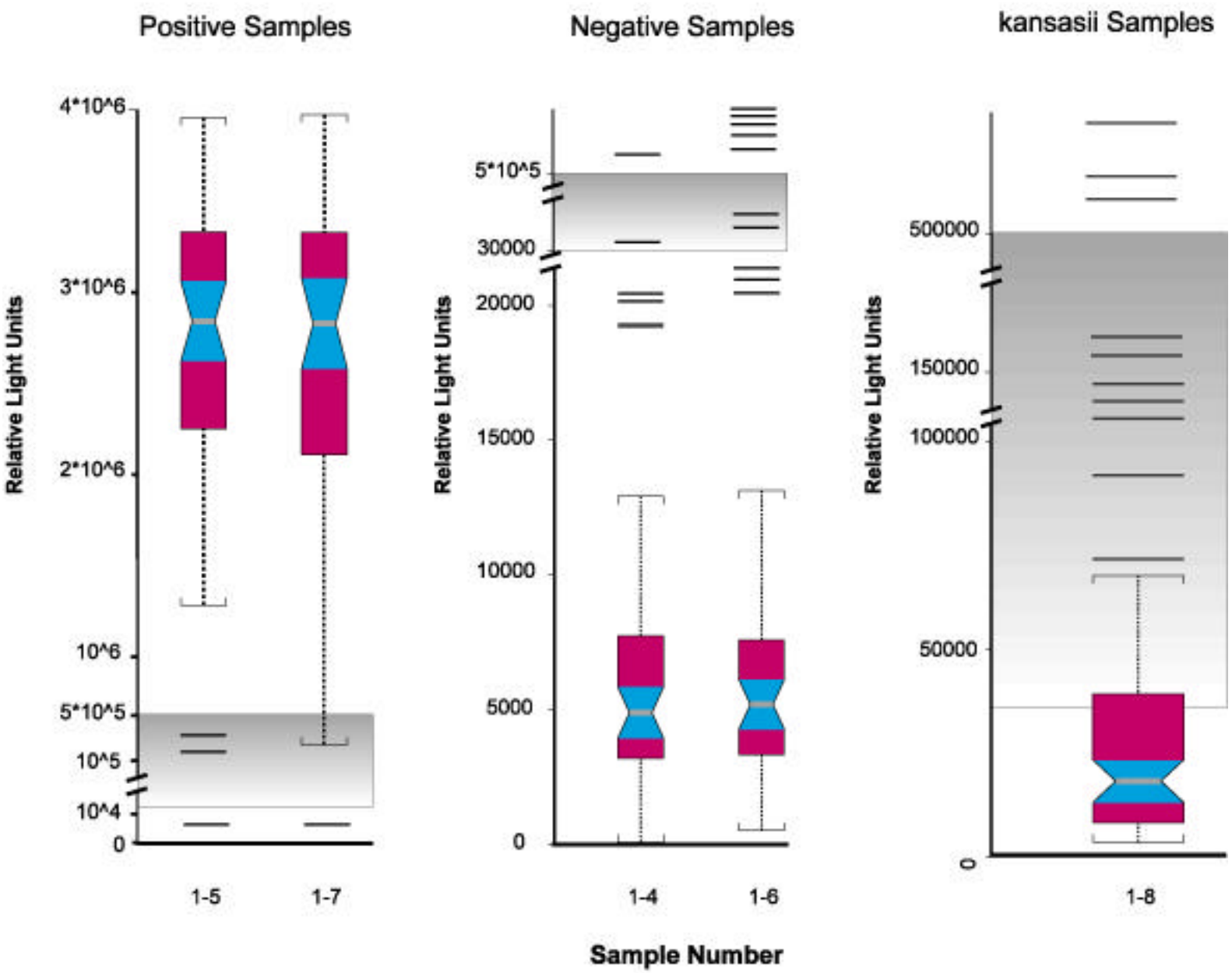
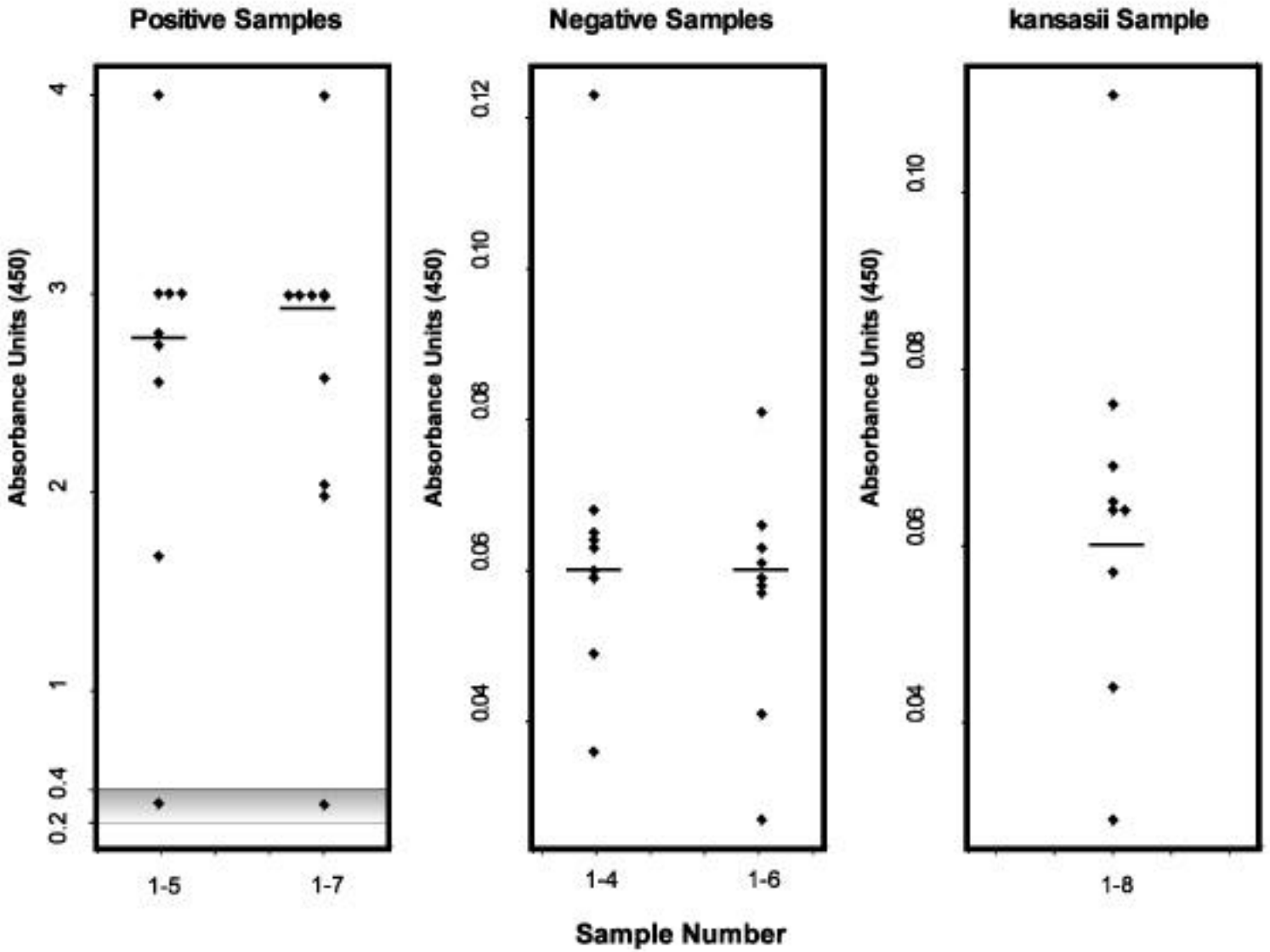


Figure 8. Quantitative Results for GenProbe® MTD



Note: Shaded areas represent inconclusive range.

Figure 9. Quantitative Results for Roche Amplicor®



Note: Shaded areas represent inconclusive range.

The following tables summarize qualitative results reported by participant laboratories for the April 1997 shipment of samples for the *M. tb.* NAA testing performance evaluation program.

Table 1. Sample TB97 1-4 contained only *Mycobacterium gordonae*

Test Methods	No. Tests Performed	Positive No.	Positive %	Inconclusive No.	Inconclusive %	Negative No.	Negative %
Gen-Probe	61	1	1.6	1	1.6	59	96.7
In-house	11	1	9.1	0	0	10	90.9
Roche	9	0	0	0	0	9	100
All methods	81	2	2.5	1	1.2	78	96.3

Table 2. Sample TB97 1-5 contained only *Mycobacterium tuberculosis*

Test Methods	No. Tests Performed	Positive No.	Positive %	Inconclusive No.	Inconclusive %	Negative No.	Negative %
Gen-Probe	61	60	98.4	0	0	1	1.6
In-house	11	10	90.9	0	0	1	9.1
Roche	9	9	100	0	0	0	0
All methods	81	79	97.5	0	0	2	2.5

Table 3. Sample TB97 1-6 contained only *Mycobacterium gordonae*

Test Methods	No. Tests Performed	Positive No.	Positive %	Inconclusive No.	Inconclusive %	Negative No.	Negative %
Gen-Probe	61	6	9.8	1	1.6	54	88.5
In-house	11	1	9.1	1	9.1	9	81.8
Roche	9	0	0	0	0	9	100
All methods	81	7	8.6	2	2.5	72	88.9

Table 4. Sample TB97 1-7 contained only *Mycobacterium tuberculosis*

Test Methods	No. Tests Performed	Positive No.	Positive %	Inconclusive No.	Inconclusive %	Negative No.	Negative %
Gen-Probe	61	59	96.7	1	1.6	1	1.6
In-house	11	10	90.9	0	0	1	9.1
Roche	9	9	100	0	0	0	0
All methods	81	78	96.3	1	1.2	2	2.5

Table 5. Sample TB97 1-8 contained only *Mycobacterium kansasii*

Test Methods	No. Tests Performed	Positive No.	Positive %	Inconclusive No.	Inconclusive %	Negative No.	Negative %
Gen-Probe	61	4	6.6	18	29.5	39	63.9
In-house	11	1	9.1	0	0	10	90.9
Roche	9	0	0	0	0	9	100
All methods	81	5	6.2	18	22.2	58	71.6

OD TB NAA P 97-04
N1FXTSMP: SmplName od * Result summary table

17:48 Monday August 4, 1997 1