May 31, 2005

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 2005 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 2005 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 90 of 92 (97.8%) 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 24, 2005 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:**

			3 Positive Samples TB05-01-1 TB05-01-3 TB05-01-5	**2 Negative Samples TB05-01-2 TB05-01-4**	
Method	**Total # of laboratories**	**Total # of results**	**False-negative results**	**False-positive results**	**Overall Performance**
Gen-Probe MTD	69	345	3/207 (1.4%)	2/138 (1.4%)	98.0%
Roche Amplicor	14	70	3/42 (7.1%)	1/28 (3.6%)	94.3%
In-house/Other	7	35	1/21 (4.8%)	1/14 (7.1%)	94.3%

New Findings

- Of all the participating laboratories using the Gen-Probe7 method, 1.5% (2/138) reported inhibition for negative samples TB05-01-2, *M. gordonae* (3.0×10^3 theoretical cells/ml), and TB05-01-4 a phosphate buffer (no theoretical cells/ml). For positive sample, TB05-01-3, *M. tuberculosis* (3.0×10^4 theoretical cells/ml), 0.5% (1/207) reported inhibition. None of the samples in this shipment contained inhibitors.

- A sample containing *M. bovis* (3.0×10^5 theoretical cells/ml), was included as a positive sample in this shipment. One equivocal interpretation (2.4% (1/42)) was given for this sample, using the Roche Amplicor7 method.

- A sample containing only Phosphate Buffer was included in this shipment as a blank. There were two false positive interpretations and one incorrect "inhibited" interpretation reported for this sample.

- Errors were clustered within laboratories. Four laboratories made 67% (8/12) of the errors. Other errors appeared to be random.

- Three new laboratories enrolled in the program for this shipment.

Findings of note that also have been reported previously

- It is a concern that 12.2% (11/90) laboratories reported using Biosafety Level 2. Further, one US laboratory, 1.1% (1/90) reported using Biosafety Level 1 which is inappropriate for TB work. Please refer to CDC/NIH manual, Biosafety in Microbiological and Biomedical Laboratories (4^{th} edition), to determine the correct level of biosafety for your laboratory.

• The number of laboratories that reported not using uni-direction workflow, 10.0% (9/90) has increased from the last shipment. Of 85 laboratories participating in the August 2004 shipment, 6 (7.1%), reported that they were not using uni-directional workflow. The reasons for this are unclear.

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 January 2005. Responses were received from 90 of 92 (97.8%) laboratories participating in this shipment. Three new laboratories enrolled in the program and returned results for 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. 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. The samples were also tested by five reference laboratories before shipping.

Results

Figure 1 shows the laboratory classification represented by 89 participants. Participants consisted of 38 hospitals, 38 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 (7/7) 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), 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 12.2% (11/90) of participant laboratories indicated that they process *M.tb* specimens in the same BSC that is used for *M.tb* NAA testing. Among the 26.7% (24/90) of participants that indicated AOther@ uses for the *M.tb* NAA testing BSC, 14 performed *M.tb* testing procedures or culture work (biochemicals, drug susceptibility testing, Accuprobe7 identification, etc.), 9 performed mycology, and three 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 Amplicor7 and Gen-Probe7 MTD) recommend the use of unidirectional workflow. It is a concern that 10.0% (9/90) 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 Amplicor7 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 Amplicor7, 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 3 positive and 2 negative samples. The combined analytical sensitivity of all methods was 97.4% (263/270) for the TB05-01-1 (3.0×10^5 theoretical cells/ml), TB05-01-3 (3.0×10^4 theoretical cells/ml) and TB05-01-5 (3.0×10^5 theoretical cells/ml): 98.6% (204/207) sensitivity for Gen-Probe7 MTD; 92.9% (39/42) sensitivity for Roche Amplicor7; 95.2% (20/21) sensitivity for In-house methods. The combined analytical specificity of all methods was 97.8% (176/180) for the 2 negative samples TB05-01-2 (3.0×10^3 theoretical cells/ml) and TB05-01-4, which was a phosphate buffer, had no theoretical cells/ml: 98.60% (136/138) specificity for Gen-Probe7; 96.4% (27/28) specificity for Roche Amplicor7; 92.9% (13/14) specificity for In-house methods.

Figure 8 is graphical representation of the quantitative results reported for each sample by participant laboratories using the Gen-Probe7 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-Probe7 value reported which was outside these ranges is signified by one of the solid lines drawn outside the brackets. For the positive samples, TB05-01-1, TB05-01-3 and TB05-01-5 the median values of all data were 3,366,530, 3,368,146 and 3,326,134 relative light units (RLU), respectively. The median values for the negative samples containing *M. gordonae,* TB05-01-2, and Phosphate Buffer, TB05-01-4, were 2,900 and 2,865 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 Amplicor7 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, TB05-01-1, TB05-01-4 and *M. bovis*, TB05-01- 5 were 3.014 (A_{450}), 2.790 (A_{450}) and 3.000 (A_{450}) respectively. The median values for the samples containing *M. gordonae,* TB05-01-2, and Phosphate Buffer, TB05-01-4, were 0.043 (A_{450}), and 0.046 (A_{450}) respectively.

All samples in this shipment were either known positive or negative samples. None contained inhibitors therefore the “inhibited” or “equivocal” interpretations reported for several samples were treated as incorrect results in calculating accuracy. Most of these errors were apparently random. One laboratory using the Gen-Probe method reported an “inhibited” interpretation for sample TB05-01-03, containing M. bovis, although the RLU value reported was well within the positive range. Laboratories are reminded to review test interpretation procedures.

Overall, composite results for all samples were relatively accurate and indicate that laboratories performed very well.

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

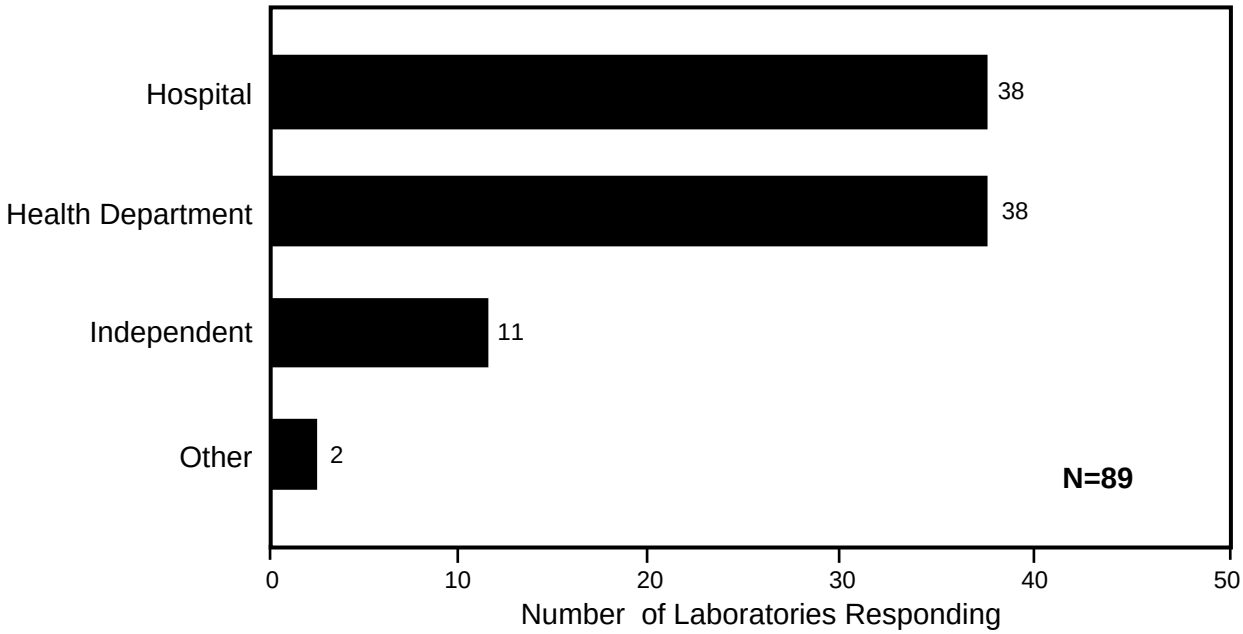
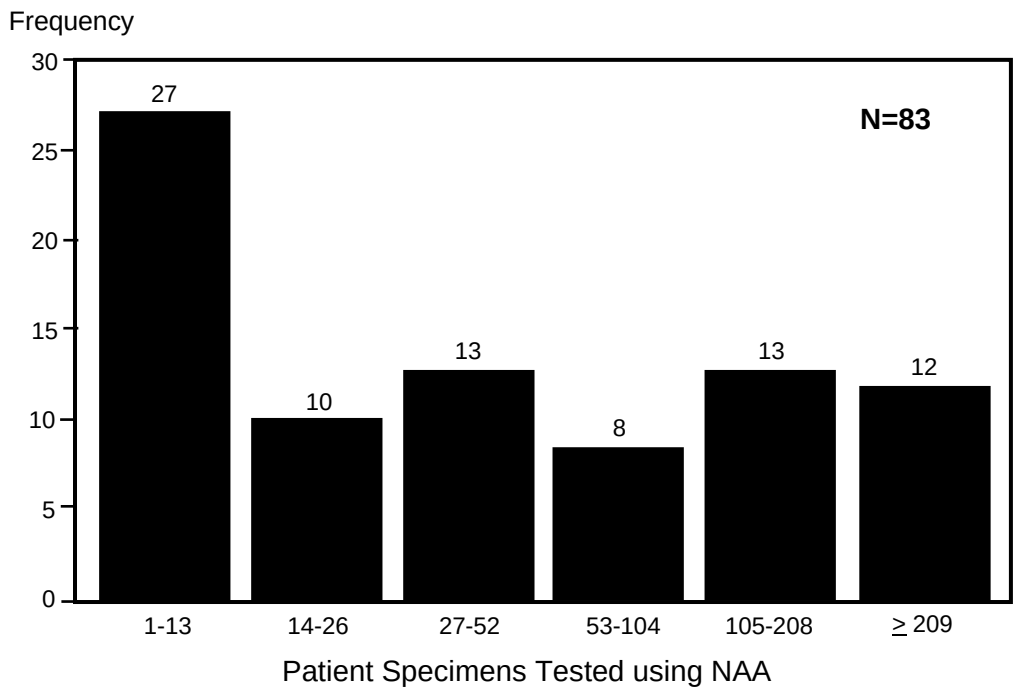


Figure 2. Number of Patient Specimens Tested for *M.tb* Using TB NAA during the Previous Quarter.*



*See explanation in the analysis.

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

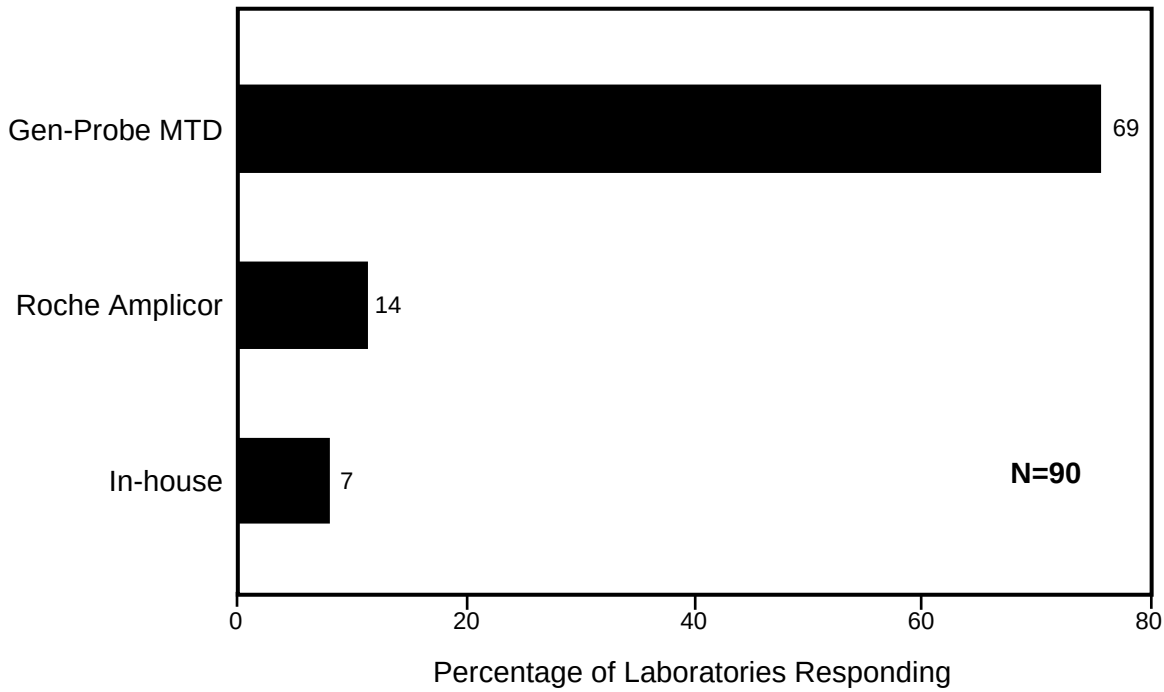


Figure 4. Biosafety Levels of Participant Laboratories

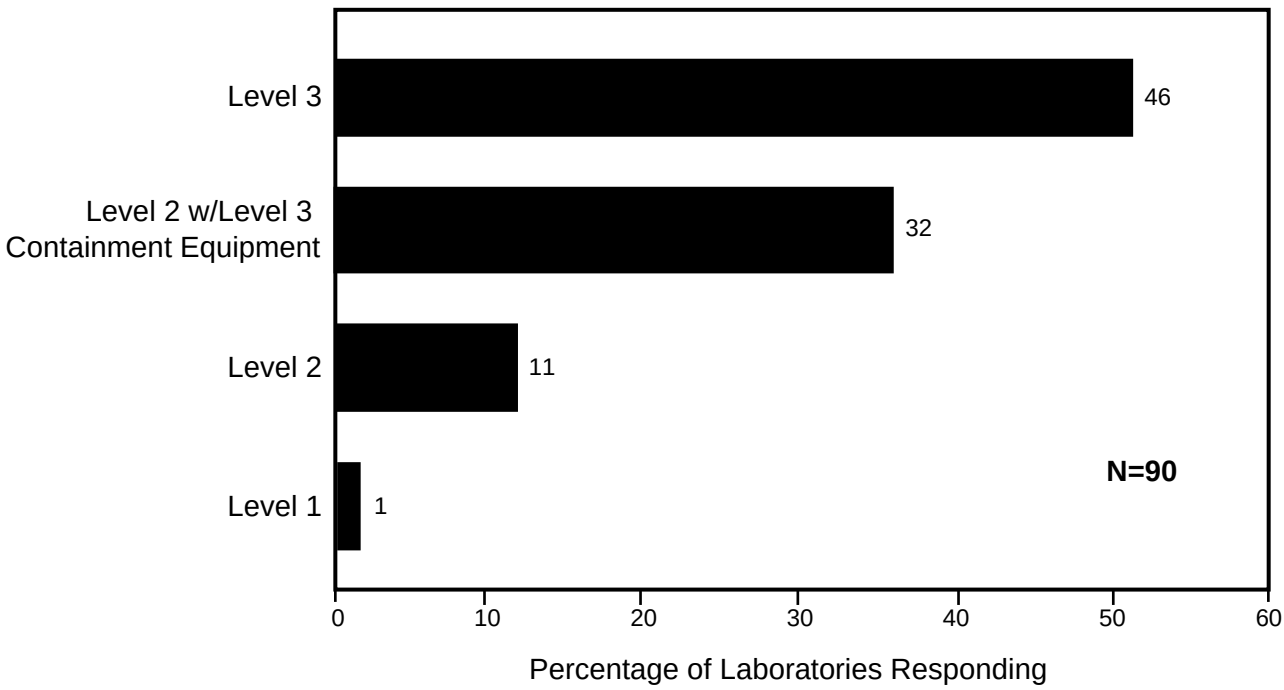


Figure 5. Is the Biological Safety Cabinet that is Used for TB NAA Testing Used for Other Purposes?

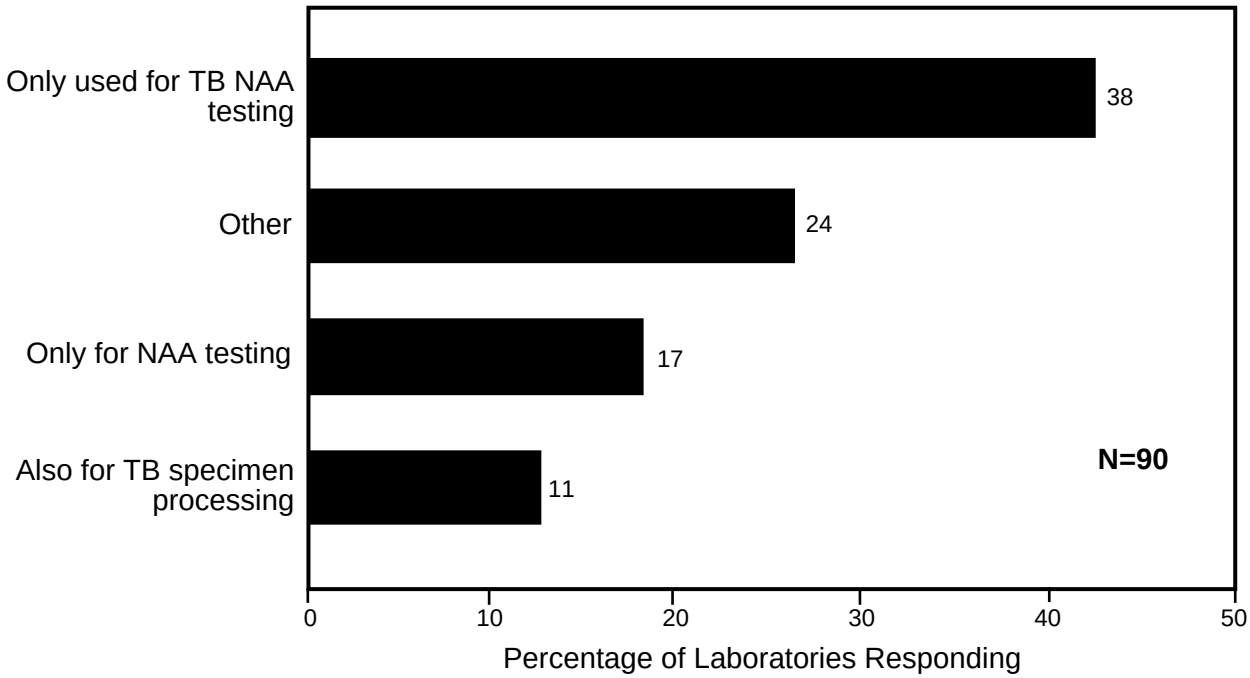


Figure 6. Use of Uni-directional Workflow by Participating Laboratories

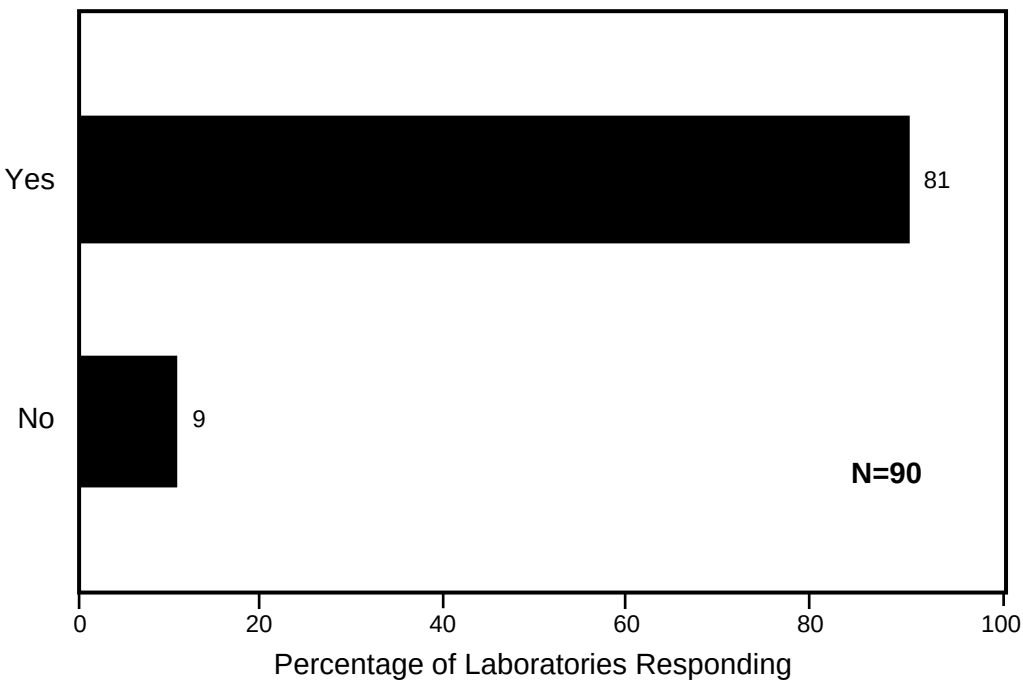


Figure 7. Frequency of TB NAA Qualitative Test Results by Sample Type for the Gen-Probe MTD, Roche Amplicor, and In-House Methods

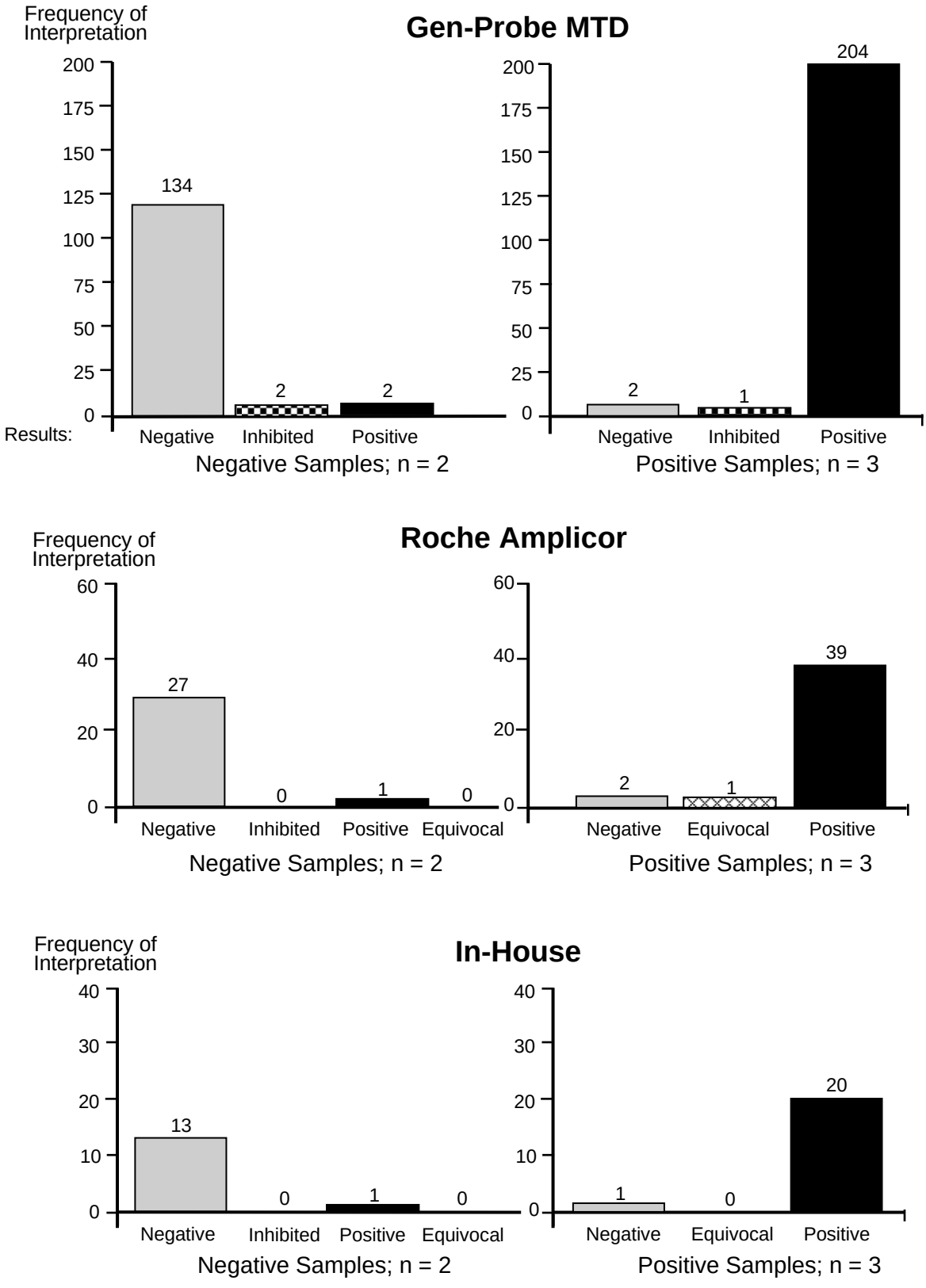
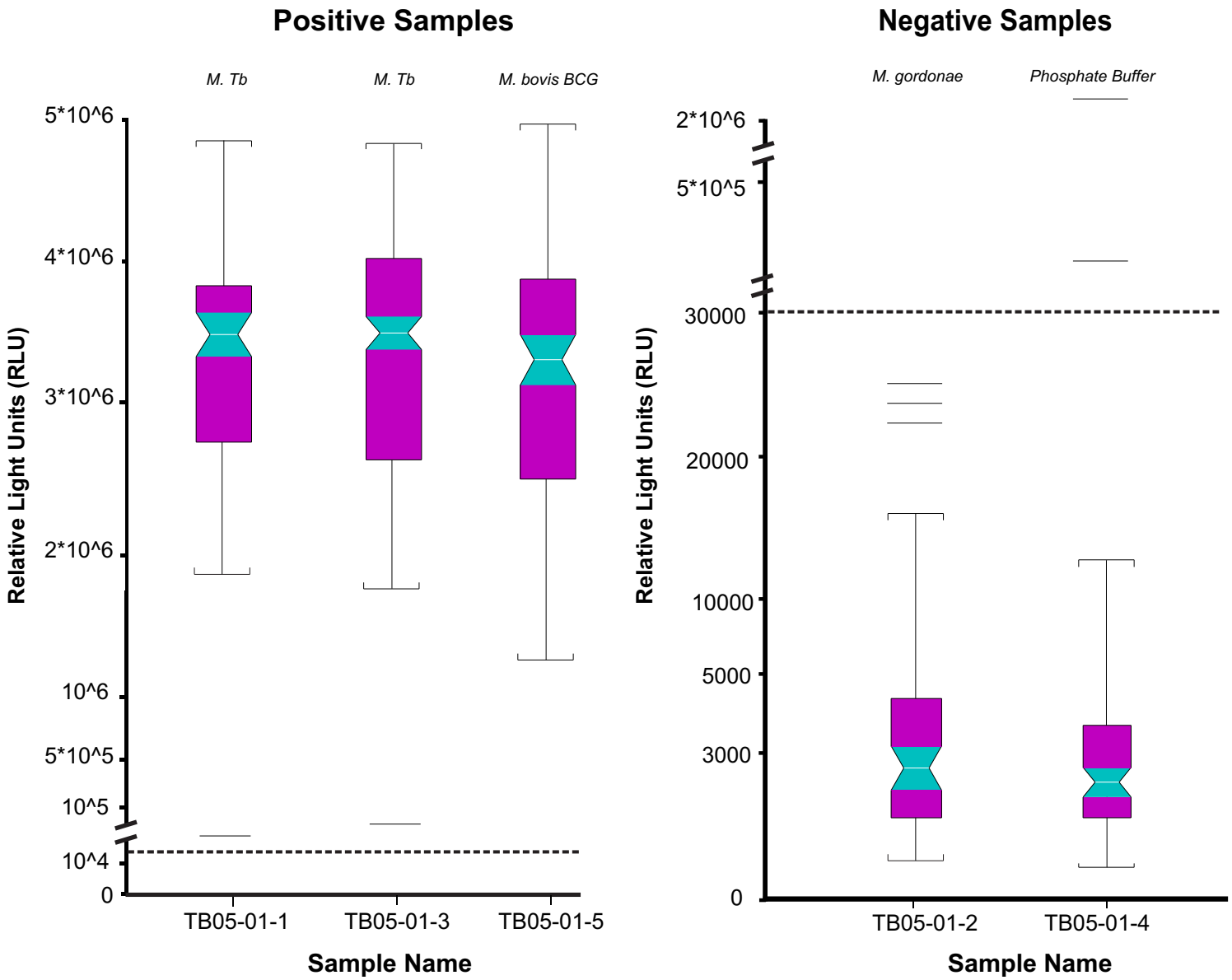
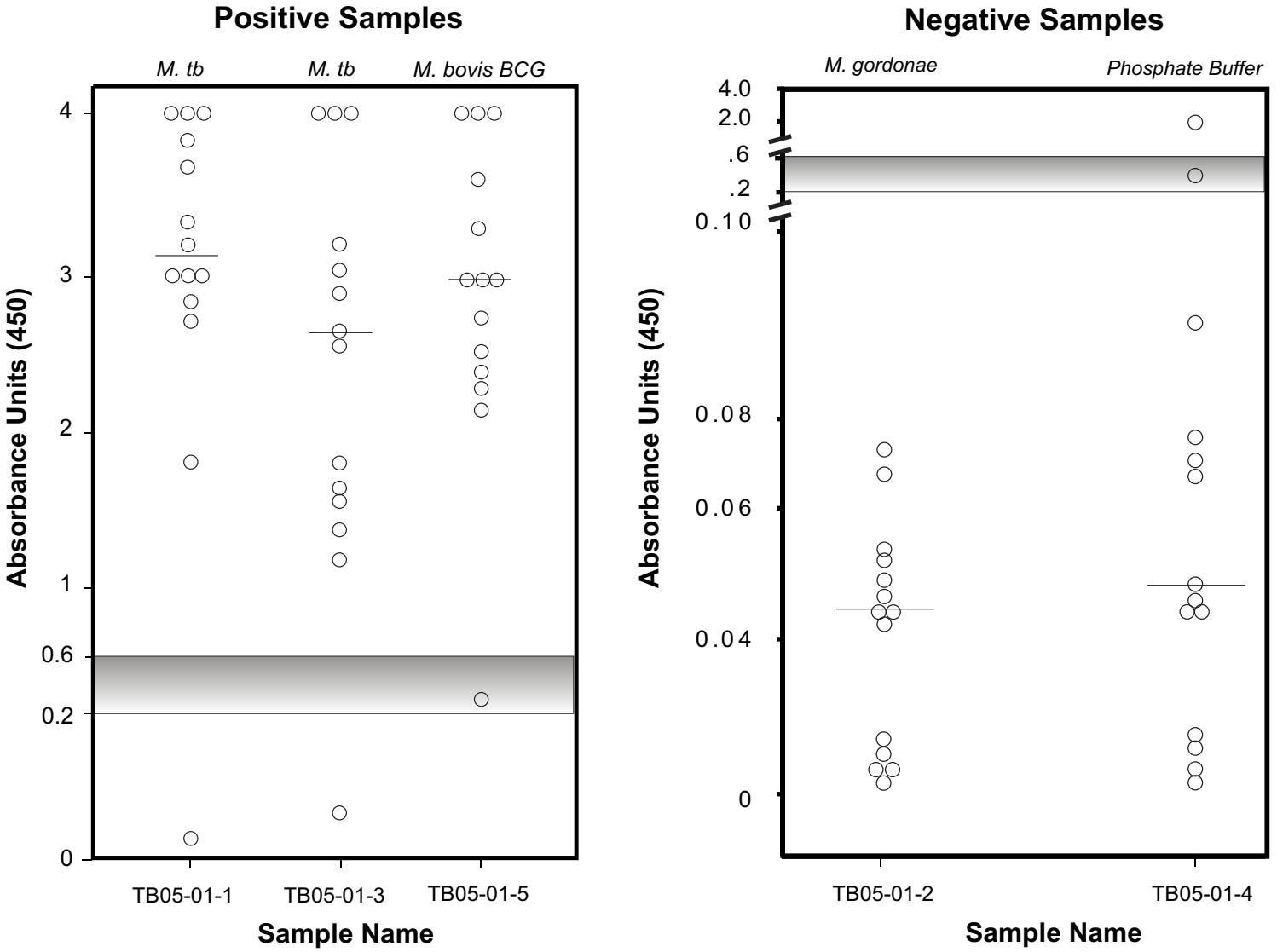


Figure 8. Quantitative Results for GenProbe® MTD



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 2005 shipment of samples for the *M. tb* NAA testing performance evaluation program.

Table 1. Sample TB05-01-1 contained *Mycobacterium tuberculosis*

Test Methods	No. Tests Performed	Positive No.	Positive %	Inhibition Not applicable	Equivocal No.	Equivocal %	Negative No.	Negative %
Gen-Probe	69	68	98.6		n/a	n/a	1	1.4
In-house	7	7	100.0		0	0.0	0	0.0
Roche	14	13	92.9		0	0.0	1	7.1
All methods	90	88	97.8		0	0.0	2	2.2

Table 2. Sample TB05-01-2 contained *Mycobacterium gordonae*

Test Methods	No. Tests Performed	Positive No.	Positive %	Inhibition No.	Inhibition %	Equivocal No.	Equivocal %	Negative No.	Negative %
Gen-Probe	69	0	0.0	1	1.4	n/a	n/a	68	98.6
In-house	7	1	14.3	0	0.0	0	0.0	6	85.7
Roche	14	0	0.0	0	0.0	0	0.0	14	100.0
All methods	90	1	1.1	1	1.1	0	0.0	88	97.8

Table 3. Sample TB05-01-3 contained *Mycobacterium tuberculosis*

Test Methods	No. Tests Performed	Positive No.	Positive %	Inhibition No.	Inhibition %	Equivocal No.	Equivocal %	Negative No.	Negative %
Gen-Probe	69	67	97.1	1	1.4	n/a	n/a	1	1.4
In-house	7	7	100.0	0	0.0	0	0.0	0	0.0
Roche	14	13	92.9	0	0.0	0	0.0	1	7.1
All methods	90	87	96.7	1	1.1	0	0.0	2	2.2

Table 4. Sample TB05-01-4 contained phosphate buffer

Test Methods	No. Tests Performed	Positive No.	Positive %	Inhibition No.	Inhibition %	Equivocal No.	Equivocal %	Negative No.	Negative %
Gen-Probe	69	2	2.9	1	1.4	n/a	n/a	66	95.7
In-house	7	0	0.0	0	0.0	0	0.0	7	100.0
Roche	14	1	7.1	0	0.0	0	0.0	13	92.9
All methods	90	3	3.3	1	1.1	0	0.0	86	95.6

Table 5. Sample TB05-01-5 contained *Mycobacterium bovis*

Test Methods	No. Tests Performed	Positive No.	Positive %	Inhibition Not applicable	Equivocal No.	Equivocal %	Negative No.	Negative %
Gen-Probe	69	69	100.0		n/a	n/a	0	0.0
In-house	7	6	85.7		0	0.0	1	14.3
Roche	14	13	92.9		1	7.1	0	0.0
All methods	90	88	97.8		1	1.1	1	1.1