

July 18, 2006

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 2006 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 2006 shipment of samples for the CDC *Mycobacterium tuberculosis* Nucleic Acid Amplification (*M.tb* NAA) Testing Performance Evaluation Program. Participant laboratories received five individual samples. Responses were received from 84 of 92 (91.3%) 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 report includes the results from supplemental questions regarding the use of biosafety cabinets for various steps in the *M.tb* NAA testing process. This data was analyzed and interpreted in collaboration with Dr. David Warshauer, Ms. Judy Nichols, Ms. Julie Tans-Kersten and the WSLH staff.

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 (404) 718-1047.

Sincerely yours,

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Enclosures

Analyses of the January 24, 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:**

Method	Total # of laboratories	Total # of results	2 Negative Samples	3 Positive Samples	Overall Performance
			TB06-01-2 TB06-01-5	TB06-01-1 TB06-01-3 TB06-01-4	
Gen-Probe MTD	64	319*	4/127 (3.1%)	4/192 (2.1%)	97.5%
Roche Amplicor	11	55	1/22 (4.5%)	1/33 (3.0%)	96.4%
In-house/Other	9	45	1/18 (5.6%)	2/27 (7.4%)	93.3%

* One laboratory did not report results for one sample.

Results of supplemental questions regarding biological safety cabinet (BSC) use:

Questions		Yes	No	Do Not Know	No Response
A.	Does your laboratory have a biological safety cabinet?	100% (84/84)			
Indicate which of the following procedures are performed in the BSC:					
B.	Decontamination of samples and set-up of mycobacterial culture?	76% (64/84)	24% (20/84)		
C.	The lysis step of the NAA procedure?	88% (74/84)	12% (10/84)		
D.	The amplification step of the NAA procedure?	37% (31/84)	63% (53/84)		
E.	The hybridization step of the NAA procedure?	13% (11/84)	83% (70/84)	1% (1/84)	2% (2/84)
F.	The selection step of the NAA procedure?	13% (11/84)	83% (70/84)	1% (1/84)	2% (2/84)
G.	The detection step of the NAA procedure?	10% (8/84)	90% (76/84)		
H.	Do you have a copy of the Biosafety in Microbiological and Biomedical Laboratories (BMBL, 4 th edition, published by CDC) document available in your lab as a reference guide, either as an on-site hard copy or readily accessible to bench staff via computer (intranet or internet)?	81% (68/84)	12% (10/84)	5% (4/84)	2% (2/84)

New Findings

- Overall accuracy for this shipment was 96.9% (406/419).
- One laboratory reported “inhibition” for sample TB06-01-1, *Mycobacterium tuberculosis* (1.0×10^6 theoretical cells/ml) and for sample TB06-01-5, *Mycobacterium kansasii* (1.0×10^3 theoretical cells/ml). This shipment contained no inhibitory samples. These answers were considered incorrect for analysis purposes.
- Two laboratories using In-house methods for sample TB06-01-4, *Mycobacterium tuberculosis*, reported false negative results. One laboratory using an In-house method reported a false positive result for sample TB06-01-5, *Mycobacterium kansasii*.
- Of the participating laboratories, 9.5% (8/84) reported that they do not use or don’t know if they use uni-directional workflow. Since the June 2005 shipment this number has increased from 6.9% (6/87). This is due to two laboratories changing their response from “yes” to “no”.
- One laboratory using the GenProbe MTD® method didn’t report an interpretation for sample TB06-01-2, containing *Mycobacterium avium* complex (1.0×10^3 theoretical cells/ml).

Note:

- Eleven of eighty-three (13.3%) participants reported using Biosafety level 2. (One laboratory reported that they did not know their biosafety level.) 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](http://whqlibdoc.who.int/hq/1998/WHO_TB_98.258_(part1).pdf) for more information.

January 2006 *M.tb* NAA Shipment Report

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 2006. Responses were received from 84 of 92 (91.3%) 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 TB06-01-1, TB06-01-3 and TB06-01-4 were all *M. tuberculosis*. The negative samples in this shipment were *M. avium* complex (1.0×10^3 theoretical cells/ml) and *M. kansasii* (1.0×10^3 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. The samples were also tested by five reference laboratories before shipping.

Results

Figure 1 shows the laboratory classification represented by 82 participants. Participants consisted of 37 health departments, 34 hospitals, 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, 9.6% (8/83) indicated that they process *M.tb* specimens in the same BSC that is used for *M.tb* NAA testing. Among the 27.7% (23/83) of participants that indicated other uses for the *M.tb* NAA testing BSC, 26 performed *M.tb* testing procedures or culture work (biochemicals, drug susceptibility testing, Accuprobe® identification, etc.), 8 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 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 9.5% (8/84) of responding laboratories reported that unidirectional workflow is not being used or that they do not know if it is 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 3 positive and 2 negative samples. The combined analytical sensitivity of all methods was 97.2% (245/252) for TB06-01-1 (1.0×10^6 theoretical cells/ml), TB06-01-3 (1.0×10^5 theoretical cells/ml) and TB06-01-4 (1.0×10^4 theoretical cells/ml): 97.9% (188/192) sensitivity for Gen-Probe® MTD; 97.0% (32/33) sensitivity for Roche Amplicor®; 92.6% (25/27) sensitivity for In-house methods. The combined analytical specificity of all methods was 96.4% (161/167) for the 2 negative samples, *M. avium* complex, TB06-01-2, (1.0×10^3 theoretical cells/ml) and *M. kansasii*, TB06-01-5, (1.0×10^3 theoretical cells/ml): 96.9% (123/127) specificity for Gen-Probe®; 95.5% (21/22) specificity for Roche Amplicor®; 94.4% (17/18) specificity 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 indentation 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. For the positive samples, TB06-01-1, TB06-01-3 and TB06-01-4 the median values of all data were 3,353,459, 3,383,319 and 3,465,838 relative light units (RLU), respectively. The median values for the negative samples containing *M. avium* complex, TB06-01-2, and *M. kansasii*, TB06-01-5, were 3,134 and 3,552 relative light units (RLU) respectively, similar to median values for other negative samples previously used in the program.

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, TB06-01-1, TB06-01-3 and TB06-01-4 were 3.328 (A_{450}), 3.685 (A_{450}) and 3.073 (A_{450}) respectively. The median values for the negative samples containing *M. avium* complex, TB06-01-2, and *M. kansasii*, TB06-01-5, were 0.041 (A_{450}), and 0.042 (A_{450}) respectively. These median values are similar to results for other negative samples previously used in the program.

Discussion

In this shipment, false negative errors tended to occur when testing the lower concentration *M. tuberculosis* sample (TB06-01-4) containing 1.0×10^4 theoretical cells/ml. False positive errors tended to occur when testing the *M. kansasii* sample (TB06-01-5) containing 1.0×10^3 theoretical cells/ml. One laboratory reported “inhibition” for sample TB06-01-1 containing *M. tuberculosis*, and for sample TB06-01-5, *M. kansasii*. Since there were no inhibitory samples in this shipment, the “inhibition” response was considered an incorrect interpretation in both cases.

Twelve of eighty-three (14.5%) participants reported using biosafety Level 2 for *M.tb* NAA testing or that they did not know their biosafety level. This has been a consistent observation throughout the program. To more completely assess biosafety practices among participants, we included several supplemental questions regarding biosafety cabinet (BSC) use.

All eighty-four responding laboratories have a BSC. Twenty of eighty-four (24%) laboratories reported that they do not use the BSC for decontamination of clinical samples and set-up of mycobacterial cultures. 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.

Twelve percent (10/84) of the laboratories did not perform specimen lysis for the NAA procedure in a BSC. Again, the BMBL guidelines state that this activity must be performed in a BSC. Performing this step in the BSC not only provides increased safety to the technologist, but also provides additional protection against cross contamination of specimens.

Post-lysis steps in the NAA procedures can safely be performed outside a BSC as eighty percent of laboratories (269/336) indicated in their responses.

We acknowledge the contribution of the WSLH staff in writing this report.

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