



Mycobacterium tuberculosis, 2004-2007

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ABSTRACT

CDC conducted a voluntary model performance evaluation (MPEP) program for *M. tuberculosis* nucleic acid amplification testing (NAA-TB). The program provided external quality assessment for U.S. and non-U.S. laboratories performing NAA-TB tests by sending challenge samples to MPEP participants every six months. Participants include hospital, public health, and independent laboratories. One goal of the NAA-TB MPEP was to provide information which would encourage optimal laboratory performance and practices. The current CDC guidelines for NAA-TB testing include recommendations for testing for the presence of inhibitory substances in NAA-TB-negative specimens. (MMWR 2000; 49:593-594) Inhibition testing on NAA-TB-negative specimens is the only way to distinguish between true negative results and results that are falsely-negative due to inhibitors. From 2004 to 2007 three specimens containing inhibitors (high molarity phosphate and heparin) were included in the challenge samples. In January 2004, 32.6% (29/89) of laboratories responded correctly (either "inhibitors detected" or "positive") for the inhibited specimen. In June 2006, 61.0% (50/82) responded correctly, and in June of 2007, the correct response rate increased to 71.6% (58/81). Additionally, participants were asked if they routinely performed inhibition testing on NAA-TB-negative patient specimens. In January 2004, 58.3% (49/84) stated they performed inhibition testing compared with 69.4% (59/85) in June 2006, and 76.8% (63/82) in June 2007. Over the 4 year period there was a significant improvement in the number of laboratories correctly identifying inhibitory samples in the MPEP program and in those stating that they followed the CDC guidelines to test for the presence of inhibitors in NAA-TB-negative specimens.

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INTRODUCTION

The use of nucleic acid amplification tests for *M. tuberculosis* (NAA-TB) has been advocated in the Department of Health and Human Services 2010 initiatives to decrease the time required for laboratory detection and confirmation of *M.tb* infection. Two *M.tb* NAA tests, GenProbe MTD and the Roche Amplicor MTB, are approved by the FDA for use on AFB smear-positive respiratory specimens, and the GenProbe MTD test is also approved for use in smear-negative respiratory specimens. The CDC, through contract with the Wisconsin State Laboratory of Hygiene (WSLH), conducted a voluntary *M.tb* NAA testing performance evaluation program to assess laboratory practices and performance with these tests. This program used samples containing *M.tb* and non-tuberculous mycobacteria to provide an educational challenge for NAA-TB testing methods.

Current CDC guidelines for NAA-TB testing recommend testing negative specimens for inhibitors (1). To test for substances that interfere with amplification, an aliquot of the patient specimen is "spiked" with a known concentration of *M.tb*. Test results for the spiked specimen should be positive unless inhibitors are present. If laboratories are not testing negative specimens for inhibition, there is an increased likelihood of reporting false negative results. In a clinical sample that contains inhibitory substances there is no way of determining that the sample actually contains *M.tb* until the culture becomes positive. In this study we determined how many of the laboratories participating in the NAA-TB MPEP were following the CDC guidelines for the detection of inhibitors in NAA-TB negative clinical specimens.

METHODS

NAA-TB MPEP samples were prepared by WSLH from patient isolates. Cells from *M.tb* cultures grown on Middlebrook 7H10 plates incubated at 37C for 2 weeks were harvested and suspended in 0.067M phosphate buffer (pH6.8) to the equivalent of a 1.0 McFarland standard (theoretical concentration of 3×10^8 cfu/ml). For inhibitory samples further dilutions were made in XPR® buffer, a high molarity phosphate buffer manufactured by Alpha Tec Systems, supplemented with 33.6 USP units/ml of heparin sodium salt (Sigma-Aldrich). The inhibitory samples contained a theoretical concentration of 3×10^5 cfu/ml of *M.tb*. One inhibitory sample was included in each set of 5 MPEP samples shipped in January 2004, June 2006, and June 2007. A questionnaire for collecting laboratory demographic and test method information was included with each shipment. Results of NAA-TB testing and associated demographic and laboratory practice information were collected and entered into a database by WSLH. Results were then forwarded to CDC without identifiers via SAS data sets. Results were analyzed by CDC and interpreted by CDC and WSLH.

Table 1. Primary classification of participating laboratories

Laboratory Classification	2004	2006	2007
Health Department	37	37	39
Hospital	36	36	32
Independent	11	11	11
Other	2	2	2
Total	86	86	84

RESULTS

2004

Table 2. January 2004 NAA-TB test results, sample TB-04-01-3: Intended response was "Inhibition". Acceptable response was "Inhibition" or "Positive".

Test Methods	No. Tests Performed	Positive No. %	Inhibition No. %	Equivocal No. %	Negative No. %
Gen-Probe	67	2 3.0	19 28.4	n/a n/a	46 68.7
In-house	6	2 33.3	2 33.3	1 16.7	1 16.7
Roche	16	0 0.0	4 25.0	1 6.3	11 68.8
All methods	89	4 4.5	25 28.1	2 2.3	58 65.2

2006

Table 3. June 2006 NAA-TB test results, sample TB-06-06-3: Intended response was "Inhibition". Acceptable response was "Inhibition" or "Positive".

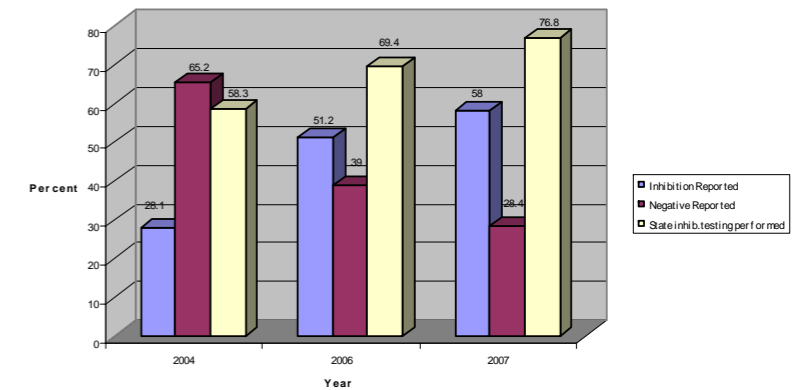
Test Methods	No. Tests Performed	Positive No. %	Inhibition No. %	Equivocal No. %	Negative No. %
Gen-Probe	60	2 3.3	30 50	n/a n/a	28 46.7
In-house	9	6 67	2 22.2	0 0	1 11.1
Roche	13	0 0.0	10 76.9	0 0	3 23.1
All methods	82	8 9.8	42 51.2	0 0	32 39.0

2007

Table 4. June 2007 NAA-TB test results, sample TB-07-06-2: Intended response was "Inhibition". Acceptable response was "Inhibition" or "Positive".

Test Methods	No. Tests Performed	Positive No. %	Inhibition No. %	Equivocal No. %	Negative No. %
Gen-Probe	60	2 3.3	37 61.7	n/a n/a	21 35.0
In-house	9	8 88.9	1 11.1	0 0	0 0.0
Roche	12	1 8.3	9 75.0	0 0	2 16.7
All methods	81	11 13.6	47 58.0	0 0	23 28.4

Figure 1
 Percentage of laboratories reporting inhibition, negative, and stating inhibition testing is performed



CONCLUSIONS AND RECOMMENDATIONS

❖ Over the four year period of the evaluation the percentage of laboratories incorrectly reporting inhibitory samples as negative decreased from 65.2% to 28.4%.

❖ The percentage of laboratories that stated they performed inhibition testing on negative specimens increased from 58.3% to 76%.

❖ Current CDC recommendations for NAA-TB testing recommend testing negative specimens for inhibitors (1). When such a protocol is not routinely followed, 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 was inhibited, until culture results were completed.

❖ 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.

❖ Since fluids containing very high molarity phosphate concentrations may inhibit amplification, laboratories receiving processed specimen sediments for NAA-TB testing should be aware of the buffer that was used to process the specimens.

REFERENCES

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NOTE: The CDC NAA-TB MPEP has been discontinued. A NAA-TB proficiency testing program, "*M.tb* by Molecular Methods", is now available through WSLH PT. Program and enrollment information can be accessed online at www.wslhpt.org.