Inhibitory Effect of Alpha-Tec XPR-Plus Phosphate Buffer on the Enhanced Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test

Phyllis Della-Latta and Vivian Jonas
J. Clin. Microbiol. **1999, 37(4):1234.**

JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1999, p. 1234–1235 Vol. 37, No. 4
0095-1137/99/$04.00+0

Inhibitory Effect of Alpha-Tec XPR-Plus Phosphate Buffer on the Enhanced Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test

Rapid detection of *Mycobacterium tuberculosis* complex directly from both respiratory and nonrespiratory specimens by transcription-mediated amplification (TMA) is widely used in medical microbiology laboratories to obtain preliminary results of clinical significance within hours of specimen collection rather than weeks to months (1–4, 8). This technology was first marketed by Gen-Probe on December 15, 1995, as the Amplified Mycobacterium Tuberculosis Direct (MTD) test (6), and an enhanced MTD test was introduced on May 15, 1998 (5). The enhanced Amplified MTD assay has incorporated improvements by reducing the time necessary to obtain results from 5 to 3 h and increasing the specimen inoculum from 50 to 450 μl.

Commercially prepared phosphate buffers, such as Alpha-Tec M/15 or the new Alpha-Tec XPR-plus (Alpha-Tec Systems, Inc.), are often used for convenience and standardization purposes to neutralize the harsh digestion-decontamination treatment of specimens with *N*-acetyl-L-cysteine (NALC)–NaOH. However, the impact of differences in phosphate concentration or other components of these buffers can influence nucleic acid amplification assays. We report a deleterious effect of the new XPR-plus buffer (Alpha-Tec Systems, Inc.) on the enhanced Amplified MTD test.

At New York Presbyterian Hospital, Columbia-Presbyterian Medical Center (CPMC), Alpha-Tec M/15 phosphate buffer (pH 6.8) is routinely used during NALC-NaOH treatment and final sediment dilution of clinical specimens for mycobacterial analysis. A new XPR-plus buffer was developed by Alpha-Tec to replace the M/15 phosphate buffer due to manufacturer claims of improved ability to maintain sediment pH near neutrality. During a trial comparison, we split 50 respiratory specimens collected from patients at CPMC and decontaminated and resuspended the pellets in either Alpha-Tec M/15 or Alpha-Tec XPR-plus buffer per established protocol (7). The average pH values of the sediments were 9.4 and 6.3 with M/15 and XPR-plus buffers, respectively, confirming the manufacturer's findings.

All split samples were cultured on both liquid and solid media and tested with the enhanced Gen-Probe Amplified MTD test, as per the manufacturer's instructions. To detect inhibition, all samples were retested after being spiked with 2.5 fg of *M. tuberculosis* rRNA (0.5 cell equivalents) per reaction. The MTD test results, reported as relative light units (RLU), are positive at ≥500,000 RLU and negative at <30,000. Values between 30,000 and 499,999 RLU indicate probable positivity, which is confirmed with a repeat test result of ≥30,000 RLU. Therefore, after spiking of samples with rRNA, specimens with RLU values of ≥30,000 were considered positive and not inhibitory to the MTD test.

Results indicated that the two phosphate buffers were equally effective in promoting mycobacterial growth since equivalent growth was observed on the media from the split positive samples. For 50 respiratory specimens examined, a total of 17 mycobacteria were recovered from both split samples as follows: 7 *M. tuberculosis* strains, 8 *M. avium* complex strains, 1 *M. fortuitum* strain, and 1 *M. neoaurum* strain. However, all 7 *M. tuberculosis* culture-positive specimens treated with the XPR-plus buffer gave false-negative results when tested by the enhanced Gen-Probe Amplified MTD assay. The RLU values for the XPR-plus buffer ranged from 3,038 to 21,621 for unspiked samples and from 2,618 to 18,420 for spiked samples. In contrast, no false-negative reactions occurred when the MTD test was done with *M. tuberculosis* culture-positive samples containing M/15 phosphate buffer. The RLU for these samples ranged from 2,149,021 to 2,981,762 for unspiked samples and from 2,602,358 to 2,857,517 for spiked samples. When all 50 samples with XPR-plus buffer were tested for inhibition, 49 of 50 samples (98%) were inhibitory.

The disparate results might be attributable to different phosphate concentrations in these two buffers. To determine the effect of phosphate molarity on the MTD test, a separate study was conducted with *M. tuberculosis* cells in the presence of NaOH and phosphate buffer concentrations commonly used by clinical laboratories during routine processing. TMA was successfully accomplished in buffers containing 67 mM (M/15) phosphate, as indicated by the observed RLU values. However, the seeded samples in the presence of 134 mM phosphate concentrations exhibited reductions in RLU signals larger than those seen with 67 mM phosphate buffer. A reduction in RLU signals was observed with both buffers as the NaOH concentration was increased from 1 to 1.25 and 1.5%; however, the reduction was significantly greater when 134 mM phosphate buffer was used.

In conclusion, Alpha-Tec XPR-plus phosphate buffer should not be used to prepare samples for testing with the enhanced Gen-Probe Amplified MTD test, due to its inhibitory action. Good amplification results with no inhibition are obtained when samples are resuspended in standard Alpha-Tec M/15 phosphate buffer. Since it was shown that the use of higher-concentration phosphate buffer inhibits MTD performance, it is likely that the higher phosphate concentration of Alpha-Tec XPR-plus (≥134 mM) may be responsible for its deleterious effects on TMA. However, other components in the buffer might also contribute to the inhibition.

We strongly recommend that any new reagent targeted for use in routine processing of mycobacterial specimens be evaluated with respect to its impact on commercially available direct amplification test results before market release.

REFERENCES

1. **Bodmer, T., A. Burtner, K. Schopfer, and L. Maner.** 1994. Screening of respiratory tract specimens for the presence of *Mycobacterium tuberculosis* by using the Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test. J. Clin. Microbiol. **32:**1483–1487.
2. **Della-Latta, P., and S. Whittier.** 1998. A comprehensive evaluation of the performance, laboratory application and clinical utility of two direct amplification technologies for the detection of *M. tuberculosis* complex. Am. J. Clin. Pathol. **110:**301–310.
3. **Della-Latta, P.** 1996. Clinical mycobacteriology: work flow and optional protocols for laboratories in industrialized countries, p. 677–695. *In* L. Heifits (ed.), Clinics in laboratory medicine. W. B. Saunders Co., Philadelphia, Pa.
4. **Ehlers, S., R. Ignatius, T. Regnath, and H. Hahn.** 1996. Diagnosis of extrapulmonary tuberculosis by Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test. J. Clin. Microbiol. **34:**2275–2279.
5. **Gamboa, F., G. Fernandez, E. Padilla, J. M. Manterola, J. Lonca, P. J.**

Cardona, L. Matas, and V. Ausina. 1998. Comparative evaluation of the Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test for direct detection of *Mycobacterium tuberculosis* in respiratory and nonrespiratory specimens. J. Clin. Microbiol. **36:**684–689.
6. **Jonas, V., M. J. Alden, J. I. Curry, K. Kamisango, C. A. Knott, R. Lankford, J. M. Wolfe, and D. F. Moore.** 1993. Detection and identification of *Mycobacterium tuberculosis* directly from sputum sediments by amplification of rRNA. J. Clin. Microbiol. **31:**2410–2416.
7. **Kent, P. T., and G. P. Kubica.** 1985. Public health mycobacteriology. A guide for the level III laboratory. Centers for Disease Control, Atlanta, Ga.
8. **Pfyffer, G. E.** 1994. Amplification techniques: hope or illusion in the direct detection of tuberculosis. Med. Microbiol. Lett. **3:**335–347.

Phyllis Della-Latta
New York Presbyterian Hospital
Columbia-Presbyterian Medical Center
Clinical Microbiology Service
622 West 168th St., BHS-3-325
New York, New York 10032

Vivian Jonas
Gen-Probe Incorporated
10210 Genetic Center Dr.
San Diego, California 92121