

Evaluation of Alpha Tec NAC-PACTM Mycobacteria Digestion and Decontamination System on pulmonary samples*

S. Naidoo and A. Barker / Lancet Laboratories / Johannesburg, South Africa

Introduction and Objectives

The respiratory specimens used to diagnose tuberculosis and other mycobacteria must pass through the oropharyngeal cavity during collection which invariably results in the presence of commensal flora in the sample. If cultured in an unmodified form, this contaminating flora will rapidly mask the presence of slow growing organisms like *Mycobacterium tuberculosis* and must therefore be completely removed prior to analytical procedures. In order to remove this flora, patient samples undergo a pre-analytical digestion and decontamination process, which requires careful control to preserve the viability of mycobacteria while eliminating contaminants and creating a specimen suitable for diagnostic protocols.

Samples collected at regional laboratories for the diagnosis of tuberculosis and other mycobacteria are often subjected only to microscopy when collected and must therefore be transported to centralized laboratories for confirmatory culture, identification, and sensitivity testing. These samples often require extensive transport which increases the time prior to specimen preparation and thus allows for significant overgrowth of contaminant bacteria. With these, the control required to effectively perform the pre-analytical process takes on more significance. Optimal recovery of mycobacteria requires a tightly regulated pH; the basic pH used to eliminate con-taminant bacteria can quickly begin to kill mycobacteria after a brief exposure if it is not properly controlled. Since specimens with high levels of commensal flora growth require treatment with a more concentrated decontaminant or longer exposure to the decontaminant, these samples are especially susceptible to the loss of mycobacteria. The loss of viable mycobacteria that occurs during specimen preparation can have a significant negative impact on final diagnostic accuracy.

The specimen preparation methodology employed by the majority of the world's laboratories relies on a high pH decontaminant mixed with a mucolytic agent to decontaminate and liquefy the sample followed by the addition of M/15 phosphate buffer or phosphate buffered saline (PBS) to dilute the reaction prior to sedimentation

and diagnostics. This conventional procedure is offered with products like the MycoPrepTM produced by Becton Dickinson (Franklin Lakes, NJ USA). Alpha Tec Systems, Inc. (Vancouver, WA USA) offers a reagent system that updates this procedure with reagents that provide better control over pH during this specimen preparation process, and purports to offer improved recovery of mycobacteria while reducing contamination in culture diagnostics. This comparison seeks to determine if the reagent system produced by Alpha Tec can foster greater diagnostic accuracy while maintaining or improving reprocessing caused by overgrowth of contaminant flora.

Materials and Methods

Our laboratory assessed the comparative performance of the Alpha Tec specimen preparation system (NAC-PACTM Red Digestion and Decontamination System, NPC-67TM Neutralizing Buffer, and Pellet Resuspension Buffer) in a high throughput laboratory in Johannesburg, South Africa on 100 suspected mycobacteria positive pulmonary samples. The Alpha Tec reagent system is advertised as the only commercial reagent system designed to control pH and specimen characteristics during the specimen preparation process. This pH control is designed to ensure the sample pH is below 8.1 immediately after buffering to stop the die off of any mycobacteria in the patient sample and maintained between 6.8-7.1 for culturing and diagnosis. These pH levels are designed to minimize contamination and erroneous diagnostic results while optimizing the survival of mycobacteria.

Upon receipt each sample was divided into equal aliquots and an aliquot from each sample was processed using both the Alpha Tec reagent system, and the BD MycoPrepTM Kit. After specimen preparation using each system, each aliquot was cultured using the BD BACTECTM liquid culture system to confirm the presence of mycobacteria. All methods were performed according to the manufacturers' procedures.

* Based upon an abstract published electronically for ECCMID 2010

