Introduction

TB is primarily caused by *Mycobacterium tuberculosis*, a member of the *Mycobacterium tuberculosis* complex, a set of genetically similar *Mycobacterium* spp. that can cause respiratory disease in humans. In humans, *M. tuberculosis* infection is typically caused by the inhalation of infected aerosols that are expelled during coughing by people with active pulmonary disease. Only HIV/AIDS surpasses TB as the greatest killer worldwide due to a single infectious agent; in 2011 alone, 8.7 million people fell ill with TB and 1.4 million people died from the disease. The scientific and medical communities in collaboration with the World Health Organization are currently in a global health initiative to reverse the incidence of TB by 2015; however, incidence is only declining slowly—especially among underserved and vulnerable populations, notably in Africa. It is currently recommended that all persons with symptoms of tuberculosis, a positive tuberculin skin test, or an interferon-gamma release assay, which are indicative of *M. tuberculosis* infection, should have sputum samples collected for diagnostic evaluation. The digestion and decontamination method used to process these patient specimens can have a direct effect on TB diagnosis and patient outcome.

Importance of pH in the Digestion/Decontamination of Patient Specimens

Currently, there are several commonly used TB diagnostic techniques that benefit from specimen pre-processing. These methods include culture examination, acid-fast (AFB) microscopy, and nucleic acid amplification (NAA). Regardless of the downstream diagnostic method, the digestion/decontamination procedure is absolutely critical for liberation and concentration of any mycobacteria present within the sample and elimination of contaminating normal flora organisms that could otherwise outgrow/outcompete the mycobacterial colonies when cultured, or make visualization of mycobacteria difficult with AFB microscopy. Kubica et al. described a sputum processing technique in which 0.5% N-acetyl-L-cysteine and 2% sodium hydroxide (NALC-NaOH) in citrate buffer was used to effectively digest and decontaminate sputum sample specimens. This processing step is accomplished by liquefying the sample with the use of the NALC reagent and then decontaminating the sample by generating alkaline conditions, through the use of an agent that modifies the pH of the sample, such as sodium hydroxide (NaOH). Unfortunately, mycobacteria cells are also negatively impacted by prolonged exposure to elevated pH. Approximately 30% of mycobacteria are killed during NaOH processing following the methods of Kubica et al. Additionally, the overkill of mycobacteria can continue during centrifugation if the NaOH decontamination step is not neutralized efficiently (Figure 1).
Figure 2 - pH levels and its effect during the different stages of sample pre-processing. The blue MycoDDR™ NaOH Reagent A is added to patient samples to elevate the pH and kill contaminating normal flora. Obtaining a neutral pH is critical for the survival of mycobacteria, so unlike the traditional method of specimen pre-processing, the MycoDDR™ NaOH Reagent A contains a pH indicator that turns the solution from blue to colorless, at near neutral pH, with the addition of Neutralization Buffer B. This solution is then subjected to centrifugation and decanted. Failure to adequately neutralize the solution will result in additional death of mycobacteria during the centrifugation and resuspension steps.
Consequently, the solution pH should be tightly regulated and monitored during this pre-processing procedure to prevent excessive loss of mycobacteria viability due to chemical processing (Figure 1). In order to address this concern, the MycoDDR™ reagents were developed to facilitate the recovery of viable mycobacteria throughout the pre-processing procedure by providing greater neutralization efficiency and a visual indication that the NALC-NaOH solution has been completely neutralized by changing the color of the solution from blue to colorless (Figure 2 and 3).

**MycoDDR™: Design, Technology, and Advantages**

The MycoDDR™ product line is designed based on the NALC-NaOH procedure published by Kubica et al. The mucolytic compound, NALC, is combined with a NaOH in a sodium citrate solution to digest the mucus while the high pH of the NaOH kills any contaminating bacteria. As previously mentioned, the high pH of this solution can also kill *Mycobacterium* spp. after 15-20 minutes, which makes the timing of the digestion/decontamination process critical and equally critical that the solution be brought back to a neutral pH as quickly as possible in order to facilitate the recovery of viable mycobacteria. The MycoDDR™ NaOH Reagent A includes a pH indicating reagent that changes from blue at basic pH, to colorless at near neutral pH (Figure 3). This allows the laboratory technologist to visually titrate the solution using the included Neutralization Buffer B. This visual indicator of neutralization, in addition to the increased buffering capacity of the Neutralization Buffer B, facilitates the critical timing needed for survival of the mycobacteria present in the sample. This solution is then subjected to centrifugation and decanted. The resulting uniform specimen pellet is then re-suspended in Resuspension Buffer C and is a pH of between 6.8 and 7.2; making it optimal for automated growth detection systems.

- **Supports Samples up to 10 mL**
  The MycoDDR™ is able to support specimens up to 10 mL due to the advanced buffering capacity of the neutralization buffer. This is a substantial advantage compared to other products on the market that have a maximum sample size limit of 6-7 milliliters. When a sample is above this lower sample size limit, labs must split the sample and digest/decontaminate both of the resulting samples. Splitting samples doubles the amount of reagents used, and forces labs to perform an extra step in the treatment process—this drastically increases the material costs and labor costs associated with the digestion/decontamination process. Therefore, a higher maximum sample size limit is extremely advantageous to laboratories, as it will limit the number of samples that need to be split.

- **Easy to Process Specimens Containing Blood**
  Treating bloody specimens with the MycoDDR™ is easy because of the unique, blue color of the NALC-NaOH solution. When the NALC-NaOH is added to a sample specimen containing blood, the solution turns purple (Figure 4). Then as the neutralization buffer is added, it is easy to see the color change from purple to light pink. At this point, the lab technician knows the solution has been properly neutralized.

![Figure 4 - MycoDDR™ facilitates processing of bloody specimen samples.](image)

Other digestion/decontamination products on the market that utilize an integrated pH indicator have a pinkish-red NALC-NaOH reagent. This is problematic when dealing with bloody specimens as the NALC-NaOH reagent turns the color of the solution to pink/red, which is the same color that is achieved when the neutralization buffer is added. A video demonstrating this comparison can be found at: http://www.immy.com/products/myco-ddr/

“Color Comparison of NaOH” on Videos tab.
Table 1: Comparison of digestion/decontamination products currently on the market.

<table>
<thead>
<tr>
<th>Features and Benefits</th>
<th>Competitor #1</th>
<th>Competitor #2</th>
<th>MycoDDR™</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH Indicator</td>
<td>Pink to Red</td>
<td>None</td>
<td>Blue</td>
</tr>
<tr>
<td>Indicates Neutralization</td>
<td>Yes</td>
<td>----</td>
<td>Yes</td>
</tr>
<tr>
<td>Bloody Samples</td>
<td>Potential Interference</td>
<td>----</td>
<td>No Interference</td>
</tr>
<tr>
<td>Max Sample Size</td>
<td>6-7 mL</td>
<td>10 mL</td>
<td>10 mL</td>
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<tr>
<td>Compatible with Molecular Assays</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Gamma Irradiation of Packaged Buffers</td>
<td>No</td>
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<td>Yes</td>
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</tbody>
</table>

Conclusions

Tuberculosis (TB) is a serious disease that affects a significant portion of the world’s population and is one of the leading causes of death in developing countries. Diagnosis of TB is important not only for correct treatment of the disease but also for containment of the highly contagious infected individual. Processing of the patient sample is a critical step in facilitating the diagnosis of TB. The MycoDDR™ product line was developed as an exceptionally reliable specimen processing system that can be used to digest and decontaminate patient specimens in an easy and affordable manner. It provides a number of benefits over other products on the market including improved visualization of sample pH, ability to process higher volume samples, and decreased time to positive positive culture.

Additional Materials


PrepBLUE™ vs. Trident™

To give all types of labs the opportunity to use the highest quality digestion/decontamination reagents, IMMY has developed two packaging options for MycoDDR™:

- MycoDDR™ Trident™ - Convenient packaging with the least amount of work on the lab
- MycoDDR™ PrepBLUE™ – The economical choice, but preparation work is required

Table 2: PrepBLUE™ vs. Trident™.

<table>
<thead>
<tr>
<th>Features</th>
<th>PrepBLUE™</th>
<th>Trident™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capable of rigid pH Control</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Individually Packaged Buffers</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>Dried chemical packets for Neutralization Buffer B and Resuspension Buffer C</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Preparation Work</td>
<td>Moderate</td>
<td>None</td>
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<tr>
<td>Digestant/Decontaminate</td>
<td>NaOH Reagent A and NALC</td>
<td>NaOH Reagent A and NALC</td>
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<td>Neutralization</td>
<td>Neutralization Buffer B</td>
<td>Neutralization Buffer B</td>
</tr>
<tr>
<td>Resuspension</td>
<td>Resuspension Buffer C</td>
<td>Resuspension Buffer C</td>
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</tbody>
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Reference List


