

	What happens	Why it happens	Why this is a problem
Digestion and Decontamination 	An equal volume of the NaOH / Sodium Citrate / NALC solution is added to the tube containing the patient sample.	This brings the sample pH to 12.5 to 13.5. This pH level is necessary to kill the oral-pharyngeal bacteria within the specimen. Mycobacterium species are more tolerant to basic pH, but after a brief exposure mycobacteria will begin to die.	After approximately 15 minutes the basic pH begins penetrating the mycolic acids in the cell wall of the mycobacteria. If the sample is not quickly neutralized, as much as 80% of mycobacteria present in the specimen can die.
Alpha Tec's Solution: NAC-PAC™ Red, Alpha Tec's digestion and decontamination solution, is similar to NaOH / Sodium Citrate solution but it contains an integrated pH indicator that will visually signify sample neutralization, indicating that Mycobacteria in the sample are no longer being killed. Alpha Tec's NALC is also unique in that it is sealed under inert gas which extends the mucolytic activity from 24 hours to up to 72 hours.			
Neutralization 	M/15 phosphate buffer or Phosphate Buffered Saline (PBS) is added to the sample after the decontamination period.	M/15 or PBS is added to the sample in an attempt to halt the decontamination process. Unfortunately these reagents are completely unable to neutralize the NaOH used to decontaminate the sample which leaves the sample at a pH above 12, well above the lethal level for <i>M. tuberculosis</i>.	Mycobacteria are susceptible to high pH levels; after a brief exposure the failure to neutralize the decontamination reagents can cause a significant reduction in the number of mycobacteria present in the patient sample. Studies indicate that protocols using M/15 Phosphate Buffer can result in the killing of approximately 80 - 90% of the <i>M. tuberculosis</i> in the patient sample.
Alpha Tec's Solution: NPC-67™ Neutralizing Buffer is Alpha Tec's unique neutralization buffer which, unlike conventional M/15, will immediately neutralize the sample and halt the decontamination process. When used with NAC-PAC™ Red, neutralization is readily apparent as the color of the solution will change from red to clear. NPC-67™ Neutralizing Buffer is formulated for compatibility with solid media, broth based rapid culture and molecular diagnostics.			
Sample Resuspension 	The concentrated specimen pellet is resuspended using incomplete decontamination or M/15 – PBS.	After centrifugation and decantation of the supernatant, the sample must be reconstituted to make it useful for diagnostics. The sample used for diagnostics should be 6.8-7.1 in pH exceeding 3ml in volume with less than 67millimoles of constituent chemicals to optimize conventional solid media, rapid detection broth instruments, and molecular probe diagnostics.	Incomplete decantation or the addition of M/15 or PBS can yield significant variation in the sample used for analytical procedures. This inconsistency can lead to false positives in liquid culture diagnostics, slowed growth in solid culture, and potentially interfere with molecular procedures.
Alpha Tec's Solution: Pellet Resuspension Buffer is the only reagent designed to optimize the specimen for AFB diagnostics. Pellet Resuspension Buffer will provide unmatched control, yielding an ideal final pH of 6.8-7.1. This range provides ideal conditions for solid media and molecular diagnostics, and helps prevent false positives in rapid liquid culture equipment. This tight final pH is especially important for pH sensitive equipment like the MB/BacT Alert which is susceptible to pH fluctuations.			