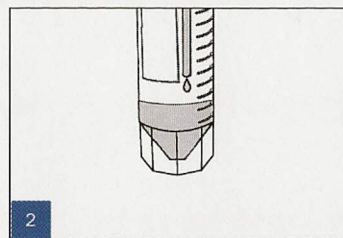


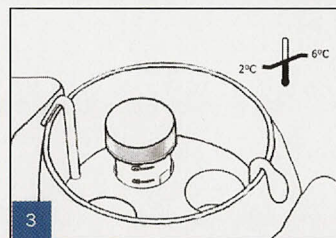
NAC-PACTM N30 SPECIMEN PROCESSING PROTOCOL



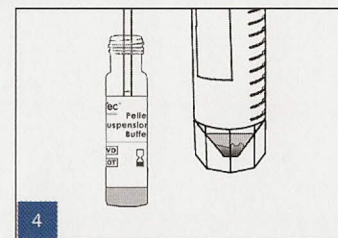
1 Break the ampule containing N-Acetyl-L-Cysteine (NALC) and add the powdered NALC to the diluent provided.



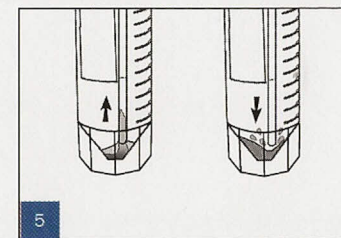
2 Add NALC/Diluent to the specimen in a volume equal to that of the specimen (up to 10ml). Recap the centrifuge tube containing the specimen and NALC/Diluent solution mixture and vortex for 60 seconds. Let stand for 5 minutes until it is liquefied.



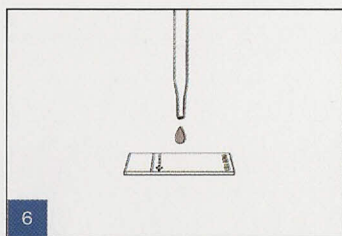
3 Centrifuge the specimen at 3000xg for 15 minutes (use a nomograph to calculate xg for your centrifuge). Following centrifugation, decant all of the supernatant, being careful to retain the pellet.



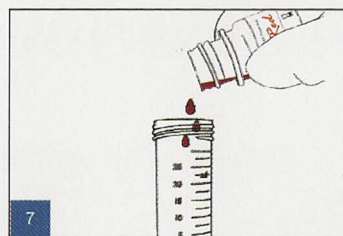
4 **Pellet Resuspension:** Aspirate 1 ml of Pellet Resuspension Buffer and add to the specimen pellet. This minimal volume will ensure smear concentration consistency.



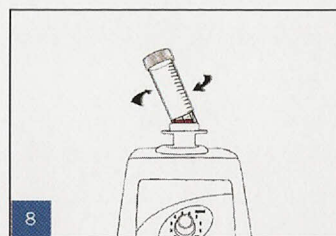
5 Using a pipette carefully mix the specimen through gentle aspiration and discharge. Draw up a small amount of the mixed pellet (~0.2ml) into the pipette.



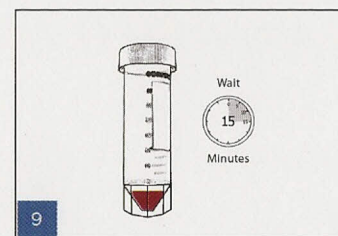
6 Place a drop of the mixed pellet from the pipette onto a clean microscope slide to stain for AFB. **Do not touch the slide with the pipette.** Return unused sample in the pipette to the specimen container.



7 Add the contents of one NAC-PACTM Red bottle to the specimen.



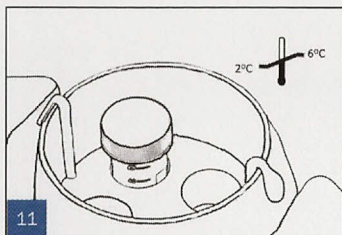
8 Recap the centrifuge tube containing the specimen and NAC-PACTM Red. Vortex the specimen for at least 30 seconds to combine the decontamination solution and sample.



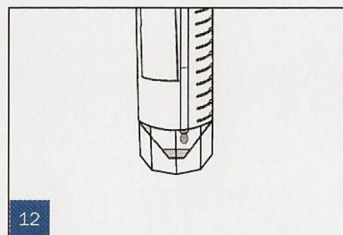
9 Place the specimen upright to decontaminate for 15 minutes. **Vortex the specimen at least two more times during the decontamination period.** Do not allow the decontamination step to exceed 20 minutes.



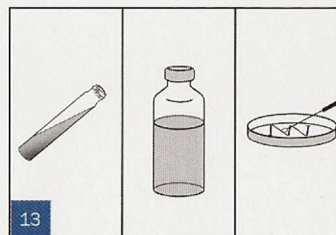
10 **Neutralization:** Add the contents of one bottle of NPC-67TM AFB Neutralizing Buffer to the specimen tube. Effective neutralization will be indicated by a color change from red/pink to colorless.



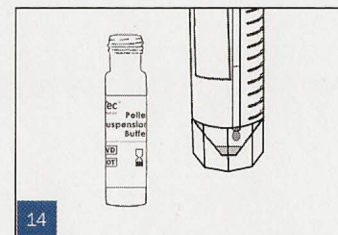
11 Centrifuge the specimen at 3000xg for 15 minutes (use a nomograph to calculate xg for your centrifuge). Following centrifugation, decant all of the supernatant, being careful to retain the pellet.



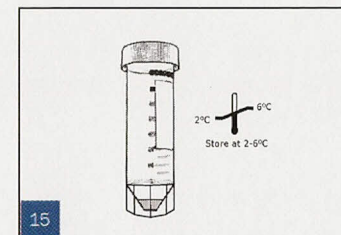
12 Add an additional 1.0-2.0ml of Pellet Resuspension Buffer from the same vial used prior to smear preparation to the remaining sample mixture.¹



13 Inoculate solid media and automated detection broth according to manufacturer's instructions; inoculate a TSA or 5% SBA plate to monitor for breakthrough contamination.



14 Add the balance of the Pellet Resuspension Buffer to any remaining specimen pellet. Cap the specimen tube.



15 Store the specimen (in a smaller tube if necessary) at 2°-6°C so that it is available for molecular diagnostics or reprocessing if required.

¹ In order to maximize the relative concentration of organisms present, the volume of the buffer added should only be enough to accommodate the diagnostic procedures being performed.