PRINCIPLE
PRB™ Pellet Resuspension Buffer is a 67 mM phosphate buffer (pH 6.8) that is used to neutralize the centrifuged pellet of respiratory specimens following the digestion and decontamination procedure with basic pH reagents used in the N-acetyl-L-cysteine (NALC) procedure for the recovery of *Mycobacterium* spp.

CLINICAL SIGNIFICANCE
The decontamination and digestion procedure, utilizing the compound N-acetyl-L-cysteine (NALC) combined with sodium hydroxide and sodium citrate (trisodium citrate) solution, results in increased yields of tubercle bacilli. The NALC procedure utilizes N-acetyl-L-cysteine as a mucolytic compound by disrupting chemical bonds in mucus. The sodium hydroxide acts as a bacterial decontaminate and the sodium citrate (trisodium citrate) solution stabilizes the NALC by chelating (binding) any heavy metal ions present in the specimen. Since the sodium hydroxide has a pH of approximately 13.00, it will kill bacteria (including mycobacteria after 15-20 minutes of exposure). As such, timing of the decontamination is critical to limit the amount of *Mycobacterium* spp. killed by the basic pH. A pH indicator is incorporated into the decontamination reagent to monitor the pH throughout the decontamination and buffering procedure, allowing the laboratory technologist to visually see when neutralization has been achieved. Bringing the pH to a neutral range can stop the decontamination process. The NPC-67® Neutralizing Buffer or XPR-*PLUS*® Neutralizing Buffer is used to neutralize the NaOH following the appropriate digestion and decontamination time, resulting in a pH of below 8.10. Adding conventional M/15 phosphate buffer or phosphate buffered saline will result in a pH range of 9.40-12.20, requiring a titration to a neutral pH with 1N HCL, or continued decontamination of *Mycobacterium* spp. will occur. Studies have documented that pH values above 8.10 are toxic to *Mycobacterium* spp., including *Mycobacterium tuberculosis*. Following the decanting step, PRB is added to achieve a tight neutral pH value (6.80-7.10) in the specimen sediment, optimizing mycobacteria recovery.

SPECIMEN COLLECTION AND PREPARATION
Appropriate specimens for the detection of *Mycobacterium* spp. should be collected according to prescribed standards and delivered to the laboratory in a safe and timely manner. Refer to local procedural guidelines for this information. **FOR IN VITRO DIAGNOSTIC USE ONLY.**

REAGENTS AND MATERIALS
1. **Provided**
    a. PRB Pellet Resuspension Buffer
2. **Not Provided**
    a. NAC-PAC® *RED* decontamination reagent with NALC
    b. NPC-67 Neutralizing Buffer or XPR-*PLUS* Neutralizing Buffer
    c. Centrifuge
    d. Vortex mixer
    e. Sterile pipettes
    f. Microscope slides
    g. TB media
    h. Centrifuge tubes
    i. CELL-BOND® Slides.
3. **Storage:** Prior to opening, store at room temperature (15-30º C). After opening, store between 2-8º C. Do not freeze or heat above 30º C. Allow the product to come to room temperature prior to use.
4. **Stability:** PRB is stable to the stated expiration date when stored at the required temperature.

CALIBRATION
N/A

QUALITY CONTROL
Any product showing cloudiness, turbidity, precipitation, or discoloration should be discarded. Quality controlled microorganisms should be utilized to verify procedures, media, and reagents as appropriate for your laboratory's applicable regulatory agency or local procedural guidelines.

PROCEDURE

PRECAUTIONS

All clinical specimens submitted for the diagnosis of tuberculosis and other *Mycobacterium* spp. must be treated with appropriate care so as not to contaminate other specimens or laboratory personnel. Use all approved and regulated equipment for processing and detection procedures.

1. Line up specimens (in centrifuge tubes) in a biosafety hood.
2. Loosen specimen container caps. Work in sets equivalent to a centrifuge load.
3. Open the bottle labeled "NAC-PAC *RED*". Add the NALC powder to the NAC-PAC *RED* bottle. Shake well to dissolve the NALC powder. **NOTE:** Some residual NALC powder may remain in the vial. It is not necessary to liquefy the portion remaining in the vial. *THIS SOLUTION WILL BE GOOD FOR ONLY 72 HOURS AFTER MIXED*. Discard the mixed solution after 72 hours.
4. To the sterile 50 ml centrifuge tube containing the specimen to be digested, add the NAC-PAC *RED* / NALC solution in the following amounts:
    a. For specimens **1-5 ml add a volume of NAC-PAC *RED* / NALC equal to that of the specimen volume.**
    b. For specimens **6-7 ml add 5 ml of NAC-PAC *RED* / NALC.**
    c. For specimens **8-10 ml add an equal volume of NAC-PAC *RED* / NALC and split the specimen after step 6** equally into two centrifuge tubes, proceed with steps 7-9 and then combine the sediments from both tubes into one centrifuge tube and proceed with step #10.

    Following this protocol will help achieve effective decontamination while also allowing for proper neutralization. If you routinely encounter specimens greater than 10 ml in volume, please contact Alpha-Tec Systems Technical Services for special instructions.
5. Tighten the caps on the centrifuge tubes. Mix each specimen on a vortex until liquefied (30 seconds per specimen).
6. Allow each specimen to stand for 15-20 minutes. Vortex every five minutes during this step.
7. Fill each tube with NPC-67 or XPR-*PLUS* until effective base pH neutralization is indicated by a color change from red / pink to colorless. Once a colorless point has been reached, do not continue to add NPC-67 or XPR-*PLUS* to the sample. Tighten cap and swirl by hand to mix. **NOTE:** NPC-67 will achieve a neutral pH (colorless solution) when added to NAC-PAC *RED* with a NaOH concentration of 3% or lower. For NAC-PAC *RED* containing 4% NaOH, use XPR-*PLUS.*
8. Centrifuge the specimen tubes at 3000 xg for 15 minutes. It is recommended but not required to use a refrigerated centrifuge. Each laboratory must check the centrifuge head radius and use an appropriate nomogram for proper speed selection [rpm] to achieve the desired relative cen*trifugal* field of 3000 xg.
9. Working in a biosafety hood, pour off **all** supernatant into a splash-proof container holding an appropriate disinfectant. Use an appropriate disinfectant to disinfect any contamination on the lip of the specimen tube. Do not allow the disinfectant to run down inside the specimen tube.
10. Resuspend the pellet with 0.5 ml-1.0 ml of PRB. Do not resuspend the pellet with NPC-67, XPR-*PLUS*, water, or saline. **NOTE:** To maximize time to detection for rapid growth automated detection systems, resuspend the pellet with 1.0 ml of PRB. Depending on the needs of your laboratory, the pellet may be resuspended in 0.5 ml of PRB to create a more concentrated sample for increased acid-fast smear sensitivity. Once the smears have been made, add an additional 1.0 ml of PRB to inoculate rapid broth detection systems and other media.
11. Mix the sediment and buffer well and inoculate the liquid broth for your automated detection equipment per the manufacturer's instructions.
12. Place two drops of the sediment onto the surface of each of the TB media used. **NOTE:** A contamination control plate (BAP or TSA) can be inoculated at this point and incubated at 35-37º C for 48 hours.
13. Make smears for acid-fast staining. Use adhesive CELL-BOND Slides or appropriate sterile albumin adhesive solutions to attach the specimen to the slide. Dry the smears and proceed with acid-fast staining per the manufacturer's directions. **NOTE:** An acid-fast stain control slide should be stained in conjunction with the patient smears to verify the staining technique and components. Call Alpha-Tec Systems, Inc. for a complete list of acid-fast stains and control slides.
14. To the unused portion of the specimen, add the balance of the PRB and refrigerate at 2-8º C to save for future diagnostic procedures or reprocessing if necessary.

CALCULATIONS

N/A

RESULTS

If *Mycobacterium* spp. are present in the clinical specimen and processed according to the procedures listed within this document, the recovery of cultivable, viable, and clinically significant *Mycobacterium* spp. can be expected. PRB was tested on clinical samples and recovered all culture appropriate *Mycobacterium* spp. when the designated procedures were followed.

LIMITATIONS

Timing of the decontamination step, proper buffering, speed and timing of the centrifugation step, proper decanting and addition of the PRB to the pellet are vital to the recovery of *Mycobacterium* spp. Failure to follow the listed procedures may result in decreased numbers of *Mycobacterium* spp. or total loss of *Mycobacterium* spp. resulting in an inaccurate culture report.

NOTES

1. **Procedure Notes**
    a. Molecular Diagnostics
        The NAC-PAC *RED* system has been validated for use with multiple molecular diagnostic methods and systems. For more information regarding compatibility with specific methods or systems, contact Alpha-Tec Technical Services.
    b. Small Volume Specimens
        Small volume specimens with correspondingly low post neutralization volumes can make centrifuge balancing difficult. If your laboratory frequently encounters small volume specimens, it is acceptable to add **sterile** saline to the sample to reach a combined volume of 5 ml prior to the addition of NAC-PAC *RED* / NALC solution. In this case, the sample should be decontaminated with 5 ml of NAC-PAC *RED* / NALC solution. This will increase the final post neutralization specimen volume making centrifuge balancing easier.
    c. Specimens contaminated with *Pseudomonas* spp.
        Specimens contaminated with *Pseudomonas* spp. will need additional treatment with 5% Oxalic Acid (OxA® Oxalic Acid Reagent Kit #0004805). Refer to the Oxalic Acid Directions For Use for complete instructions, or call Alpha-Tec Systems, Inc. Technical Services for information on the pH effects of the Oxalic Acid procedure and the appropriate buffering requirements.
    d. Bloody Specimens
        Following the decontamination of the specimen with NAC-PAC *RED*, bloody specimens may remain pink after the addition of the NPC-67 or XPR-*PLUS* due to the residual hemoglobin in the specimen. If the color change cannot be visualized due to hemoglobin, add the NPC-67 or XPR-*PLUS* up to the 50 ml mark to ensure complete neutralization. For additional information, contact Alpha-Tec Technical Services.

2. **Summary of Technology**
    a. Decontamination and Digestion
        i. Sodium Hydroxide
            1. Digests bacteria (including *Mycobacterium* spp.) utilizing a high, basic pH.
            2. Mucolytic compound that disrupts chemical bonds in mucus resulting in total specimen digestion.
        ii. N-acetyl-L-cysteine (NALC)
            1. Mucolytic compound that disrupts chemical bonds in mucus resulting in total specimen digestion.
            2. Combines with sodium hydroxide and trisodium citrate resulting in increased yields of tubercle bacilli.
        iii. Timing
            1. Timing is critical so as not to limit the die-off of *Mycobacterium* species present in the patient specimen by the basic pH.
    b. Neutralization
        i. NPC-67 Neutralizing Buffer or XPR-*PLUS* Neutralizing Buffer
            1. Used to neutralize the NALC reagents following the appropriate digestion decontamination time, resulting in a pH ≤ 8.10.
            2. Following the decanting step, PRB is added to achieve a tight neutral pH value (6.8-7.1) in the specimen sediment, optimizing Mycobacteria recovery.
            3. Studies have documented that pH values above 8.1 are toxic to *Mycobacterium* spp., including *Mycobacterium tuberculosis*. Bringing the pH to a neutral range can stop the digestion procedure.
            4. A pH indicator is incorporated in the digestion decontamination reagents to monitor the pH throughout the decontamination and buffering procedure, allowing the laboratory technologist to visually see when neutralization has been achieved.

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CONTACT

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PRODUCT CODES

0004510 PRB Pellet Resuspension Buffer, 50 x 3 ml
0004512 PRB Pellet Resuspension Buffer, 10 x 50 ml

Manufactured by Alpha-Tec Systems, Inc.
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GLOSSARY OF SYMBOLS

LOT Batch code / Numéro de lot / Número de Lote / Numero di lotto / Lot Nummer / Lotnummer / Lotnummer / Šaržna številka / Número de lote

REF Catalog number / Référence du catalogue / Número de catálogo / Numero di catalogo / Katalognummer / Catalog nummer / Het aantal van de catalogus / Kataloška številka / Número de catálogo

IVD In vitro diagnostic medical device / Pour usage diagnostique in vitro / Para uso diagnóstico in vitro solamente / Solo per uso diagnostico in vitro / Nur zur Verwendung als in vitro-Diagnostikum / Alleen voor in vitro diagnostisch gebruik / För invitrodiagnostik enbart / Samo za invitro diagnostiko / Apenas para uso em diagnóstico in vitro

EC REP Authorized representative in the European Community / Représentant européen autorisé / Representante Europeo Autorizado / Rappresentante europeo autorizzato / Autorisierter Europäischer Repräsentant / Germachtigde Europese vertegenwoordiger / Auktoriserad europeisk representant / Pooblaščen evropski predstavnik / Representante Europeu Autorizado

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