INTENDED USE
NALC Tablets are used in combination with NAC-PAC® *RED* in the N-acetyl-L-cysteine (NALC) digestion and decontamination procedure of clinical specimens for the increased recovery of *Mycobacterium* spp.

SUMMARY
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. In the NAC-PAC *RED* Digestion and Decontamination Solution, sodium hydroxide acts as a bacterial decontaminate and the sodium 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. Bringing the pH to a neutral range can stop the decontamination process. The NPC-67® Neutralizing Buffer or XPR-*PLUS*® Neutralizing Buffer can be used to neutralize the NALC reagents following the appropriate digestion and decontamination time, resulting in a pH below 8.10. Adding conventional M/15 Phosphate Buffer or phosphate buffered saline will result in a pH range of 9.40 to 12.20, requiring a titration to a neutral pH with 1N HCl, 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™ Pellet Resuspension Buffer is added to achieve a tight neutral pH value (6.80-7.10) in the specimen sediment, optimizing mycobacteria recovery.

FOR IN VITRO DIAGNOSTIC USE ONLY

PRECAUTIONS
Decontamination reagents contain a caustic chemical (sodium hydroxide). Use appropriate care in the handling of this reagent. 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.

STABILITY AND STORAGE
NALC Tablets are stable to the stated expiration date when stored at the required temperature. Store at room temperature (15º-30ºC). After opening, close the cap tightly after each use. Do not freeze or heat above 30°C.

USER QUALITY CONTROL
Any product showing cloudiness, turbidity, precipitation or coloration 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.

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.

PROCEDURE
Materials Provided: NALC Tablets (5 bottles, 10 tablets each).
Materials Not Provided: Centrifuge, vortex mixer, sterile pipettes, centrifuge tubes, TB media, microscope slides, NAC-PAC *RED,* NPC-67 Neutralizing Buffer or XPR-*PLUS* Neutralizing Buffer, PRB Pellet Resuspension Buffer, CELL-BOND® slides.

SPECIMEN PROCESSING
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 "NALC" and place one tablet in each centrifuge tube containing a specimen. Re-cap the NALC bottle tightly. Alternatively, if you are processing 10 specimens at one time, you may add all 10 tablets to 50 ml of NAC-PAC *RED*, tightly cap the bottle, and shake well or vortex to dissolve the tablets. **Once dissolved, the NAC-PAC *RED* / NALC tablet solution will be stable for 72 hours. Store any unused portion at 2°-8° C for up to 72 hours. Allow the refrigerated portion to come to room temperature prior to use.**
    a. To the sterile 50 ml centrifuge tube containing the specimen to be digested, add the NAC-PAC *RED* / NALC solution in the following amounts:
    b. For specimens **1-5 ml add a volume of NAC-PAC *RED* / NALC equal to that of the specimen volume.**
    c. For specimens **6-7 ml add 5 ml of NAC-PAC *RED* / NALC.**
    d. 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.
    e. 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.
4. Tighten the caps on the centrifuge tubes. Mix each specimen on a vortex until liquefied (30 seconds per specimen).
5. Allow each specimen to stand for 15-20 minutes. Vortex every 5 minutes during this step.
6. 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*.
7. 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 centrifugal field of 3000 xg.
8. 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.
9. 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.
10. Mix the sediment and buffer well and inoculate the liquid broth for your automated detection equipment per the manufacturer's instructions.
11. 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 35-37ºC for 48 hours.
12. 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.

13. To the unused portion of the specimen, add the balance of the PRB and refrigerate at 2-8ºC to save for future diagnostic procedures or reprocessing if necessary.

PROCEDURE NOTES

1. NALC Tablets have been validated for use with multiple molecular diagnostic methods and systems. For more information regarding compatibility with specific methods or systems, contact Sales .
2. 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* solution. In this case, the sample should be decontaminated with 5 ml of NAC-PAC *RED* solution. This will increase the final post neutralization specimen volume making centrifuge balancing easier.
3. 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.
4. 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.

EXPECTED 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.

LIMITATIONS OF PROCEDURES

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.

SPECIFIC PERFORMANCE CHARACTERISTICS

NAC-PAC *RED* with NALC Tablets was tested on clinical samples and recovered all culture appropriate *Mycobacterium* spp. when the designated procedures were followed.

BIBLIOGRAPHY

1. Babakhani, F., N. Warren, D. Henderson, and Dalton. 1995. "Effect of Transportation and Acid Neutralization on Recovery of Mycobacteria from Processed Specimens." Am.J. Clin. Pathol. 104(1): 65-68.
2. Chapman J.S., Bernard, J.S. 1962. "The Tolerance of Unclassified Mycobacteria. Limits of pH Tolerance." Am. Rev Respir Dis. 86:582-583.
3. Kent, P., and G. Kubica. 1985. Public Health Mycobacteriology: A Guide for the Level III Laboratory. Centers for the Disease Control and Prevention, Atlanta, GA.
4. Kubica, G.P., et al. 1963. "Sputum Digestion and Decontamination with N-acetyl-L-cysteine-Sodium Hydroxide for Culture of Mycobacteria." Am Rev Respir Dis. 87:775-779.
5. Kubica, G.P., et al. 1964. "Comments on the Use of the New Mucolytic Agent, N-acetyl-L-cysteine, as a Sputum Digestant for the Isolation of Mycobacteria." Am Rev Respir Dis. 89:284-286.
6. Lennette, E.H., et al. 1980. Manual of Clinical Microbiology, American Society for Microbiology Third Edition.
7. Vestal, A.L. 1975. Procedures for the Isolation and Identification of Mycobacteria. C.D.C., Atlanta, GA.
8. Yegian, D., Budd V. 1952. "Toxic Effect of Sodium Hydroxide on Tubercle Bacilli." Am.J. Clinical Pathology. 22:456-460.

CONTACT

CalibreScientific US, Inc. offers a complete line of reagents, stains, and QC1™ Quality Control Slides for AFB, Parasitology, Bacteriology, and Mycology processing, as well as O&P collection systems and concentration devices for Parasitology. For Technical Assistance, email Technical@AlphaTecSystems.com, and for Customer Service, email Sales@AlphaTecSystems.com, or call either [+1] 800.221.6058 (USA) or [+1] 360.260.2779 between 8AM and 4PM Monday through Friday, Pacific Time.

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PRODUCT CODES

0003921 NALC Tablets, 5 x 10 Tablets

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EC REP MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany

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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