INTENDED USE

NAC-PAC® and NALC are used in the N-acetyl-L-cysteine (NALC) digestion and decontamination procedure of clinical specimens for the increased recovery of Mycobacterium species.

SUMMARY

The decontamination and digestion procedure, utilizing the compound N-acetyl-L-cysteine (NALC), and 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. Timing of the decontamination is critical, so as not to kill *Mycobacterium* spp. present in the patient specimen (or to limit the amount of *Mycobacterium* spp. killed by the basic pH). Bringing the pH to a neutral range can stop the decontamination procedure. The NPC-67® Neutralizing Buffer or XPR-*PLUS*® Neutralizing Buffer can be used to neutralize the NaOH reagents following the appropriate digestion and decontamination time, resulting in the desired pH range. Adding conventional M/15 Phosphate Buffer or phosphate buffered saline will result in a pH range of 9.40 to 12.20, requiring titration to 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 mycobacteria, including *Mycobacterium tuberculosis.*

FOR IN VITRO DIAGNOSTIC USE ONLY

PRECAUTIONS

The decontamination reagent contains a caustic chemical (sodium hydroxide). Use appropriate care in the handling of this reagent. All clinical specimens submitted for AFB testing must be treated with appropriate care so as not to contaminate other specimens or laboratory personnel. Use all approved and regulated equipment for AFB processing and detection procedures.

STABILITY AND STORAGE

NAC-PAC is stable to the stated expiration date when stored at the required temperature. Upon receipt, store unopened bottles at room temperature (15–30°C). Do not freeze or heat above 30°C. Refrigerate open bottles at 2–8°C. Allow the product to come to room temperature prior to use.

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: NAC-PAC, NALC powder.
Materials Not Provided: Vortex mixer, pipettes, centrifuge, centrifuge tubes, neutralization buffer, resuspension buffer, 1N HCl.

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 "NAC-PAC Digestion and Decontamination Solution". Add the NALC powder to the NAC-PAC bottle. Shake well to dissolve the NALC powder. (**NOTE:** Some residual NALC powder may remain in the ampule. It is not necessary to liquefy the portion remaining in the ampule. *THIS SOLUTION WILL BE GOOD FOR ONLY 72 HOURS AFTER MIXING.* Discard the mixed solution after 72 hours.
4. Add an equal volume of NAC-PAC / NALC solution to a sterile 50 ml centrifuge tube containing the specimen to be digested. If the specimen exceeds 8 ml, add a volume of NAC-PAC / NALC solution equal to the volume of the patient sample, but split it into two centrifuge tubes prior to the addition of the neutralization buffer. Recombine the sediments after centrifugation and decantation.
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 5 minutes during this step.
7. To complete the AFB diagnostic process, follow the neutralization and diagnostic procedures of your choice. Alpha-Tec Systems strongly recommends the use of either NPC-67 Neutralizing Buffer or XPR-*PLUS* Neutralizing Buffer along with PRB™ Pellet Resuspension Buffer. **NOTE:** Using M/15 Phosphate Buffer will result in a pH range that exceeds the tolerance of *Mycobacterium* spp. and will cause mycobacteria to die off. If M/15 Phosphate Buffer is used, titrate with 1N HCl and an appropriate pH indicator to ensure neutralization. Refer to the manufacturer's Directions For Use for the selected neutralization buffer's appropriate protocol.

PROCEDURE NOTES

1. Specimens contaminated with *Pseudomonas* spp. will need additional treatment with the OxA® Oxalic Acid Reagent Kit (#0004805). Refer to the Oxalic Acid Directions For Use for complete instructions, or call Technical Services for information on the pH effects of the oxalic acid procedure and the appropriate buffering requirements.
2. For improved organism adhesion during the staining procedure, Technical recommends the use of CELL-BOND® Adhesive Slides. Alternately, 0.2% Bovine Albumin Fraction V may be used to adhere specimen pellet material to microscopic slide.

EXPECTED RESULTS

To avoid the loss of any mycobacteria due to extended exposure to an elevated pH, specimens must be neutralized immediately following decontamination. A pH indicator can be added to the solution or NAC-PAC® *RED* can be used in place of NAC-PAC as it contains an integral pH indicator which visually confirms neutralization. Other buffers can be used to avoid the use of 1N HCl acid. Contact Alpha-Tec Technical Services for additional information.

LIMITATIONS OF PROCEDURES

This procedure is designed to be most effective with NPC-67 Neutralizing Buffer or XPR-*PLUS* Neutralizing Buffer. If M/15 Phosphate Buffer is used without proper subsequent neutralization, additional mycobacteria can be lost due to prolonged exposure to pH values above 8.10. To ensure this neutralization occurs, the pH must be measured immediately following the addition of the M/15 Phosphate Buffer and during titration.

SPECIFIC PERFORMANCE CHARACTERISTICS

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

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9. Data on file.

CONTACT

CalibreScientific US, Inc. offers a complete line of QC1™ Quality Control Slides, stains, reagents, and digestion systems for AFB specimen processing. For Technical Assistance email Technical@AlphaTecSystems.com and for Customer Service, email Sales@AlphaTecSystems.com or call [+1] 800.221.6058 or [+1] 360.260.2779 between 8 am and 4 pm Monday through Friday, Pacific Time.

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PRODUCT CODES

0003441 NAC-PAC (2.0%), 5 x 50 ml / NALC, 5 x 0.25 g
0003457 NAC-PAC (2.0%), 5 x 200 ml / NALC, 5 x 1.0 g
0003462 NAC-PAC (3.0%), 5 x 50 ml / NALC, 5 x 0.25 g
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0003472 NAC-PAC (2.5%), 5 x 50 ml / NALC, 5 x 0.25 g

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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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