AlphaTec™
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Directions For Use for the following:

NAC-PAC®

AFB Digestion and Decontamination

PRINCIPLE

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.

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. 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 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, 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™ Pellet Resuspension Buffer 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. NAC-PAC and NALC
2. **Not Provided**
    a. NPC-67 Neutralizing Buffer
    b. XPR-*PLUS* Neutralizing Buffer (for neutralization of digestion/decontamination solutions with ≥ 3% NaOH)
    c. PRB Pellet Resuspension Buffer
    d. Centrifuge
    e. Vortex mixer
    f. Sterile pipettes
    g. Microscope slides
    h. TB media
    i. Centrifuge tubes
    j. 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:** NAC-PAC 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 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.

PROCEDURE

PRECAUTIONS

> The decontamination reagent contains 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.

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". 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 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. Add and equal volume of the NAC-PAC/NALC solution to a sterile 50 ml centrifuge tube containing the specimen to be digested. If the specimens exceeds 8 ml, add a volume of NAC-PAC/NACL 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 five minutes during this step.
7. To complete the AFB diagnostic process, follow the neutralization and diagnostic procedures of your choice. Alpha-Tec 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.

CALCULATIONS

N/A

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. Contact the Alpha-Tec Technical Services for more additional information. NAC-PAC was tested on clinical samples and recovered all culture appropriate *Mycobacterium* spp. when the designated procedures were followed.

LIMITATIONS

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.

NOTES

1. **Procedure Notes**
   - a. Molecular Diagnostics
     NAC-PAC 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. 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.

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.

BIBLIOGRAPHY

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CONTACT

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WARRANTY

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TRADEMARKS

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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
0003465 NAC-PAC (4.0%), 5 x 200 ml, NALC, 5 x 1.0 g
0003466 NAC-PAC (2.5%), 5 x 200 ml, NALC, 5 x 1.0 g
0003469 NAC-PAC (4.0%), 5 x 50 ml, NALC, 5 x 0.25 g
0003472 NAC-PAC (2.5%), 5 x 50 ml, NALC, 5 x 0.25 g
0003499 NAC-PAC (3.0%), 5 x 200 ml, NALC, 5 x 1.0 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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