AlphaTec™

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Directions For Use for the following:

NAC-PAC® *EA3*

PRINCIPLE

NAC-PAC® *EA3* contains the necessary reagents–NAC-PAC *RED*, NALC tablets, NPC-67® Neutralizing Buffer, and PRB™ Pellet Resuspension Buffer–for use in the qualitative digestion and decontamination procedure of clinical specimens 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 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 stabilizes the NALC by chelating (binding) any heavy metal ions. Since the sodium hydroxide has a pH of approximately 13.00, it will kill bacteria (including mycobacteria after 15–20 minutes of exposure). Therefore, 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 NAC-PAC *RED* decontamination reagent to monitor the pH throughout the decontamination and buffering procedure. Bringing the pH to a neutral range stops the decontamination process. The NPC-67 is used to neutralize the NAC-PAC *RED* following the appropriate digestion and decontamination time, resulting in a pH below 8.10. 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 *Mycobacteria* 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 *RED*
    b. NALC tablets
    c. NPC-67 Neutralizing Buffer.
    d. PRB Pellet Resuspension Buffer.
2. **Not Provided**
    a. Centrifuge
    b. 50 ml centrifuge tubes
    c. Vortex mixer
    d. Sterile pipettes
    e. Microscope slides
    f. TB growth media
    g. Centrifuge tubes
    h. CELL-BOND® Slides.
3. **Storage:** Store at room temperature (15–30ºC).
4. **Stability:** All products in NAC-PAC *EA3* are 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

> 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. Prior to opening the specimen tubes, line up specimens (collected 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. Alternatively, if you are processing 10 specimens at one time, you may add all 10 tablets to the bottle labeled "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.**
4. To the specimen tube containing the NALC tablet, add NAC-PAC *RED* (or the NAC-PAC RED/NALC tablet solution) as follows:
    a. For specimens **1-5 ml add a volume of NAC-PAC *RED* equal to that of the specimen volume**
    b. For specimens **6-7 ml add 5 ml of NAC-PAC *RED***
    c. For specimens **8-10 ml add an equal volume of NAC-PAC *RED* and then 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.
5. Tighten the caps on the centrifuge tubes. Mix each specimen on a vortex until liquefied and the NALC tablet has dissolved (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 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 to the sample. Tighten cap and swirl by hand to mix. **NOTE:** Use each bottle of the NPC-67 on a single specimen only. Using on bottle for multiple specimens can lead to cross-contamination and potentially erroneous results.
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 centrifugal 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 0.5–1.0 ml of PRB. Do not resuspend the pellet with NPC-67, water, or saline. **NOTE:** To maximize time to detection for rapid growth automated detections systems, resuspend the pellet with 1.0 ml of PRB. Depending on the needs of your laboratory, the pellet may be resuspended with 0.5 ml of PRB to create a more concentrated sample for increased acid-fast smear staining 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 35–37°C for 48 hours.
13. Make smears for acid-fast staining. Use adhesive CELL-BOND slides or 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 control slide should be stained in conjunction with the patient smears to verify the staining technique and components. Contact Alpha-Tec 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 further 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. The products within NAC-PAC *EA3* were 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
        Alpha-Tec's NAC-PAC *EA3* 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 the NALC tablet. 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® Kit #0004805). Refer to the OxA Kit 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 due to the residual hemoglobin in the specimen. If the color change cannot be visualized due to hemoglobin, add the NPC-67 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 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
      1. Used to neutralize the NALC reagents following the appropriate digestion decontamination time, resulting in a pH of ≤ 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.

BIBLIOGRAPHY

1. Kent, P, Kubica, GP. Public Health Mycobacteriology: A Guide for the Level III Laboratory. Centers for Disease Control and Prevention (CDC). 1985.
2. Kubica, GP, et al. Sputum digestion and decontamination with N-acetyl-L-cysteine-sodium hydroxide for culture of mycobacteria. Am Rev Respir Dis. 1963. 87:775–779.
3. Kubica, GP, et al. 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. 1964. 89:284–286.
4. Lennette, EH, et al. Manual of Clinical Microbiology. American Society for Microbiology. Third Edition. 1980.
5. Murray, P. Manual of Clinical Microbiology. American Society for Microbiology. 1985.
6. Mycobacterium Tuberculosis: Assessing Your Laboratory. Centers for Disease Control and Prevention (CDC). March, 1995.
7. Vestal, AL. Procedures for the Isolation and Identification of Mycobacteria. CDC. Atlanta, GA. 1975.
8. Yegian, D, Budd V. Toxic effect of sodium hydroxide on tubercle bacilli. Am J Clin Path. 1952. 22:456–460.

CONTACT

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.

WARRANTY

Alpha-Tec Systems, Inc. warrants this product to perform as described in the labeling and literature supplied. Alpha-Tec Systems, Inc. disclaims any implied warranty or merchantability or fitness for any other purpose, and in no event shall Alpha-Tec Systems, Inc. be liable for any consequential damages arising out of aforesaid express warranty.

TRADEMARKS

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PRODUCT CODES

0004813 NAC-PAC EA3 (3.0%) with NALC Powder / 60 patient tests
0004815 NAC-PAC EA3 (3.0%) with NALC Tablets / 60 patient tests
0004817 NAC-PAC EA3 (2.5%) with NALC Tablets / 60 patient tests
0004819 NAC-PAC EA3 (2.5%) with NALC Powder / 60 patient tests

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GLOSSARY OF SYMBOLS

LOT	Lot number / Númerode porción / Númerode lote / Numerodi lotto / Partienummer / Numéro de sort / Het Aantal van de partij
REF	Catalog number / Número de catálogo / Número de catálogo / Numero di catalogo / Katalogzahl / Numéro de catalogue / Het aantal van de catalogus
IVD	For in vitro diagnostic use only / Para el uso diagnóstico in vitro solamente / Para in vitro o uso diagnóstico somente / Solo per uso diagnostico in vitro / Für nur in vitrodiagnosegebrauch / Pour l'usage diagnostique in vitro seulement / Voor kenmerkend slechts gebruik in vitro
EC REP	Authorized European representative / Representante europeo autorizado / Representante europeu autorizado / Rappresentante europeo autorizzato / Autorisierter europäischer Repräsentant / Represent ant européen autorisé / Gemachtigde Europese vertegenwoordiger
⚠	Caution, consult accompanying documents / Precaución, consulte los documentos de acompañamiento / Cuidado, consulte originais acompanhando / Attenzione. Consulti i documenti di accompagnamento / Vorsicht, beraten Begleitdokumente / Attention, consultez les documents d'accompagnement / Voorzichtigheid, raadpleeg begeleidende documenten
	Store between temperatures indicated / Almacén entre las temperaturas indicadas / Loja entre as temperaturas indicadas / Deposito fra le temperature indicate / Speicher zwischen den Temperaturen angezeigt / Magasin entre les températures indiquées / Opslag tussen vermelde temperature
	Consult instructions for use / Consulte las instrucciones para el uso / Consulte instruções para o uso / Consulti le istruzioni per uso / Beraten Sie Anwendungsvorschriften / Consultez les instructions pour l'usage / Raadpleeg instructies voor gebruik
	Use by date indicated / Uso por la fecha indicada / Uso pela data indicada / Uso entro la data indicate / Gebrauch bis zum der Dattel angezeigt / Utilisation à la datte indiquée / Gebruik door vermelde datum
	Manufacturer / Fabricante / Fabricante / Fornitore / Hersteller / Fabricant / Fabrikant
Σ	Contains sufficient for <n> tests / Contiene suficiente para <n> las pruebas / Contem suficiente para <n> testes / Contiene sufficiente per <n> le prove / Enthält genügendes für <n> tests / Contient suffisamment pour <n> des essays / Bevat voldoende voor <n> tests