

INTENDED USE

NAC-PAC® N30 contains the necessary reagents—NAC-PAC RED, N-acetyl-L-cysteine (NALC), NALC Diluent, 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.

SUMMARY

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 decontaminant 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 in the 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 Neutralizing Buffer is used to neutralize the NAC-PAC RED following the appropriate 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 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**

NAC-PAC RED 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.

STABILITY AND STORAGE

NAC-PAC RED, NALC, NALC Diluent, NPC-67, and PRB are stable to the stated expiration dates when stored at the required temperature. Store at room temperature (15–30°C). After mixing NALC and NALC Diluent, store any unused portion of this reagent between 2–8°C. Do not freeze or heat above 30°C. Allow the product to come to room temperature prior to use.

USER 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.

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 Diluent Solution, NALC powder, NAC-PAC RED, NPC-67 Neutralizing Buffer, PRB Pellet Resuspension Buffer.

Materials Not Provided: Centrifuge, vortex mixer, sterile pipettes, microscope slides, TB media, centrifuge tubes, 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 bottle labeled "NALC Diluent". With the plastic sleeve still attached, carefully break off the top of the ampule containing NALC powder. Add the NALC powder to the NALC Diluent. **NOTE:** Some residual NALC powder may remain in the ampule. It is not necessary to liquefy the portion remaining in the ampule. Store any unused portion at 2–8°C for up to 48 hours. Allow the refrigerated portion to come to room temperature prior to use. Discard the mixed solution after 48 hours and mix a fresh solution.
4. To a sterile centrifuge tube containing the specimen to be digested, add an equal volume of the NALC Diluent solution (up to 10 ml).
5. Tighten the caps on the centrifuge tubes. Mix each specimen on a vortex until liquefied (60 seconds per specimen).
6. Allow each specimen to stand for 5 minutes at room temperature.
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 1.0 ml of PRB. Do not resuspend the pellet with NPC-67, water, or saline.
10. Mix the sediment and buffer well and make smears for acid-fast staining. Use 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. Call Alpha-Tec for a complete list of acid-fast stains. Stain the prepared specimen slides using appropriate stains. By using a concentrated sample prior to decontamination for microscopic diagnostics, the sensitivity of microscopy can be improved. **NOTE:** An acid-fast stain control slide should be stained in conjunction with the patient smears to verify the staining technique and components (#0003240 AFB Quality Control Slide).
11. To each specimen sediment, add the contents of one NAC-PAC RED bottle.
12. Tighten the caps on the centrifuge tubes. Mix each specimen on a vortex mixer for 30 seconds per specimen.
13. Allow each specimen to stand for 15 minutes at room temperature. Vortex every five minutes during this step.
14. Add the contents of one bottle of NPC-67 to each specimen. Tighten cap and swirl by hand to mix.
15. Centrifuge the specimen tubes at 3000 xg for 15 minutes. It is recommended but not required to use a refrigerated centrifuge.
16. 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.
17. Resuspend the pellet in 1–2 ml of PRB (according to the laboratory's set-up procedure volume requirements). Do not resuspend the pellet with NPC-67, water, or saline.
18. Place two drops of the sediment onto the surface of each of the TB media used and inoculate with your manual or instrumented mycobacterial growth system, per the manufacturer's recommendations. **NOTE:** A contamination control plate (BAP or TSA) can be inoculated at this point and incubated at 35–37°C for 48 hours.
19. Add the remaining PRB to the pellet specimen and refrigerate at 2–8°C to save for further diagnostic procedures or reprocessing, if necessary.

PROCEDURE NOTES

1. NAC-PAC N30 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.
2. Specimens contaminated with *Pseudomonas* spp. will need additional treatment with 5% Oxalic Acid (#0003447) or the OxA® Oxalic Acid Reagent Kit (#0004805). Refer to the Oxalic Acid Directions For Use for complete instructions, or contact Alpha-Tec Technical Services or the Alpha-Tec Sales Department for information on the pH effects of the Oxalic Acid procedure and the appropriate buffering requirements.
3. Following the decontamination of the specimen with NAC-PAC RED, bloody specimens may remain pink after the addition of the NPC-67 Neutralizing Buffer due to the residual hemoglobin in the specimen. 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 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 the addition of PRB to the pellet are vital to the recovery of *Mycobacterium* spp. Failure to follow the listed procedures may result in decreased numbers or total loss of *Mycobacterium* spp. resulting in an inaccurate culture report.

BIBLIOGRAPHY

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CONTACT

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WARRANTY

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GLOSSARY OF SYMBOLS



Lot number / Número de porción / Número de lote / Numerodi lotto / Partienummer / Numéro de sort / Het Aantal van de partij



Catalog number / Número de catálogo / Número de catálogo / Numero di catalogo / Katalogzahl / Numéro de catalogue / Het aantal van de catalogus



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



Authorized European representative / Representante europeo autorizado / Representante europeu autorizado / Rappresentante europeo autorizzato / Autorisierter europäischer Repräsentant / Representant européen autorisé / Gemachtigde Europese vertegenwoordiger



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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 date indiquée / Gebruik door vermelde datum



Manufacturer / Fabricante / Fabricante / Fornitore / Hersteller / Fabricant / Fabrikant



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