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[+1] (800) 221-6058 Nationwide
[+1] (360) 260-2779 Washington

Directions For Use for the following:

NAC-PAC® *N30* NALC Diluent

INTENDED USE

NAC-PAC® *N30* when used with NALC Diluent 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 (15–30ºC). After mixing NALC and NALC Diluent, store any unused portion of this reagent between 2–8ºC. 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: NALC Diluent, NALC powder. **NAC-PAC *N30***: 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. If the patient sample has already been liquefied with Dithiothreitol (DDT) or similar digestant, centrifuge the specimen so that the final volume is 1 ml and move to step 11. If the patient specimen has not yet been liquefied, use NALC Diluent, and start with step 4.
4. Open the bottle labeled "NALC Diluent". Add the NALC powder to the NALC Diluent. **NOTE:** Some residual NALC powder may remain in the vial. It is not necessary to liquefy the portion remaining in the vial. 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.
5. To a sterile centrifuge tube containing the specimen to be digested, add an equal volume of the NALC Diluent solution (up to 10 ml).
6. Tighten the caps on the centrifuge tubes. Mix each specimen on a vortex until liquefied (60 seconds per specimen).
7. Allow each specimen to stand for 5 minutes at room temperature.
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 1.0 ml of PRB. Do not resuspend the pellet with NPC-67, water, or saline.
11. Mix the sediment 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 QC1™ AFB Slide).
12. To each specimen sediment, add the contents of one NAC-PAC *RED* bottle.
13. Tighten the caps on the centrifuge tubes. Mix each specimen on a vortex mixer for 30 seconds per specimen.
14. Allow each specimen to stand for 15 minutes at room temperature.
15. Add the contents of one bottle of NPC-67 to each specimen. Tighten cap and swirl by hand to mix.
16. Centrifuge the specimen tubes at 3000 xg for 15 minutes. It is recommended but not required to use a refrigerated centrifuge.
17. 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.
18. 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.
19. 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.
20. 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

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.

WARRANTY

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PRODUCT CODES

0004820 NALC Diluent, 4 x 50 ml
0004823 NAC-PAC *N30*, 20 Sets

Manufactured by CalibreScientific US, Inc.
1311 SE Cardinal Court, Suite 170
Vancouver, WA 98683 USA

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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