

PRINCIPLE

NPC-67® Neutralizing Buffer is used to neutralize the base pH reagents in the N-acetyl-L-cysteine (NALC) digestion and decontamination procedure for the increased recovery of *Mycobacterium* spp. from sputum and other clinical specimens.

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 decontaminant 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. A pH indicator is incorporated into the NAC-PAC® RED decontamination reagent to monitor the pH throughout the decontamination and buffering procedure, allowing the laboratory technologist to visually see when neutralization has been achieved. Bringing the pH to a neutral range can stop the decontamination process. The NPC-67 is used to neutralize the NaOH following the appropriate digestion and decontamination time, resulting in a pH of below 8.10. Adding conventional M/15 phosphate buffer or phosphate buffered saline will result in a pH range of 9.40-12.20, 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. NPC-67 Neutralizing Buffer
2. **Not Provided**
 - a. NAC-PAC RED or NAC-PAC decontamination reagent with NALC
 - b. Centrifuge
 - c. Vortex mixer
 - d. Sterile pipettes
 - e. Microscope slides
 - f. TB media
 - g. Centrifuge tubes
 - h. PRB Pellet Resuspension Buffer
 - i. CELL-BOND® Slides.
3. **Storage:** Prior to opening, store at room temperature (15-30° C). After opening, store between 2-8° C. Allow the product to come to room temperature prior to use. Do not freeze or heat above 30° C.
4. **Stability:** NPC-67 Neutralizing Buffer 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 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

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 bottle labeled "NAC-PAC" or "NAC-PAC RED". Add the NALC powder to the NAC-PAC or NAC-PAC RED 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. To the sterile 50 ml centrifuge tube containing the specimen to be digested, add the NAC-PAC RED / NALC solution as follows:
 - a. For specimens **1-5 ml add a volume of NAC-PAC RED / NALC equal to that of the specimen volume.**
 - b. For specimens **6-7 ml add 5 ml of NAC-PAC RED / NALC.**
 - c. For specimens **8-10 ml add an equal volume of NAC-PAC RED / NALC and 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. If you routinely encounter specimens greater than 10 ml in volume, please contact Alpha-Tec Systems Technical Services for special instructions.
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. When using NAC-PAC RED, 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:** NPC-67 will achieve a colorless solution [basic pH neutralization] when added to a decontamination reagent [NAC-PAC or NAC-PAC RED] with a NaOH concentration of 3% or lower. When using NAC-PAC (without the red color indicator), add NPC-67 until the pH is less than 8.10.
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 ml-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 detection systems, resuspend the pellet with 1.0 ml of PRB. Depending on the needs of your laboratory, the pellet may be resuspended in 0.5 ml of PRB to create a more concentrated sample for increased acid-fast smear 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 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. **NOTE:** An acid-fast stain control slide should be stained in conjunction with the patient smears to verify the staining technique and components. Call Alpha-Tec Systems, Inc. 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 2-8° C to save for future 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. NPC-67 was 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 Pellet Resuspension Buffer 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.

NOTES**1. Procedure Notes**

- a. Molecular Diagnostics
Alpha-Tec NPC-67 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 NAC-PAC RED / NALC solution. In this case, the sample should be decontaminated with 5 ml of NAC-PAC RED / NALC solution. 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® 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.
- 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 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* spp. 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 \leq 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 incorporated in the digestion decontamination reagents to monitor the pH throughout the decontamination and buffering procedure allows the laboratory technologist to visually see when neutralization has been achieved.

BIBLIOGRAPHY

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CONTACT

For Technical Assistance email Technical@AlphaTecSystems.com and for Customer Service, email Sales@AlphaTecSystems.com or call (800) 221-6058 or (360) 260-2779 between 8am and 4pm Monday through Friday, Pacific Time.



[+1] 800.221.6058 United States
[+1] 360.260.2779 International

Directions For Use for the following:

NPC-67[®]

Neutralizing
Buffer

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

0003939 NPC-67 Neutralizing Buffer, 50 x 40 ml
0003940 NPC-67 Neutralizing Buffer, 50 x 50 ml
0003941 NPC-67 Neutralizing Buffer, 8 x 250 ml
0003942 NPC-67 Neutralizing Buffer, 10 x 500 ml



Manufactured by Alpha-Tec Systems, Inc.
 1311 SE Cardinal Court, Suite 170
 Vancouver, WA 98683 USA



MOSE GmbH
 Schiffgraben 41
 30173 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 number / 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 / Gemachtigde Europese vertegenwoordiger / Auktoriserad europeisk representant / Pooblaščen evropski predstavnik / Representante Europeu Autorizado
-  Use-by date / Utiliser avant la date de péremption indiquée / Use antes de la fecha indicada / Utilizzare entro la data indicata / Bis zum angegebenen datum verbrauchen / Gebruik door vermelde datum / Använd innan angivet datum / Porabiti do navadenega datuma / Usar até à data indicada
-  Manufacturer / Fabricant / Fabricante / Produttore / Hersteller / Fabrikant / Fabrikant / Proizvajalec / Fabricante
-  Caution / Attention / Cuidado / Attenzione / Achtung / Voorzichtig / iaktag försiktighet / Previdno / Atenção
-  Temperature limit / Conserver aux températures indiquées / Almacene entre las temperaturas indicadas / Conservare a temperatura compresa fra quelle indicate / Im angegebenen Temperaturbereich aufbewahren / Opslaan bij een temperatuur tussen / Förvara mellan angivna temperaturer / Šranjevali med navedenimi temperaturami / Armazene entre as temperaturas indicadas
-  Contains sufficient for <n> tests / Contenu suffisant pour <n> tests / Contiene suficiente para <n> pruebas / Contenido suficiente per <n> tests / Enthält ausreichend für <n> untersuchungen / Inhoud voldoende voor <n> testen / Innehåller tillräckligt för <n> tester / Vsebinsa zadostuje za <n> testov / Contém quantidade suficiente para <n> testes
-  Consult instructions for use / Consulter la notice d'utilisation / Consulte las instrucciones para el uso / Consultare le istruzioni per l'uso / Bitte beachten Sie die Anwendungsvorschriften / Raadpleeg instructies voor gebruik / Konsultera bruksanvisningen innan användning / Glej navodila za uporabo / Consulte instruções para o uso
-  Do not reuse / Ne pas réutiliser / No reutilizar / Non riutilizzare / Nicht wiederverwenden / Niet hergebruiken / Återanvänd inte / Ne uporabljajte znova / Não reutilize