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Directions For Use for the following:

Sodium Hydroxide, 4%
Sodium Citrate Solution
(Trisodium Citrate), 2.94%

INTENDED USE
Sodium hydroxide, sodium citrate (trisodium citrate), and NALC are used in the qualitative procedure in the N-acetyl-L-cysteine (NALC) digestion and decontamination procedure of clinical specimens for the increased recovery of *Mycobacterium* species.

SUMMARY
The decontamination and digestion procedure, utilizing the compound N-acetyl-L-cysteine (NALC) [per Kubica and Associates], and 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). Timing of the decontamination is critical, so as not to kill *Mycobacterium* spp. present in the patient specimen (or to limit the amount of *Mycobacterium* spp. killed by the basic pH). Bringing the pH to ≤ 8.10 can stop the decontamination procedure. The XPR-*PLUS*® Buffer or NPC-67® Neutralizing Buffer can be used to neutralize the NALC reagents following the appropriate digestion and decontamination time, resulting in the desired pH range.
Adding M/15 Phosphate Buffer will result in a pH range of 9.40 to 12.20, requiring a titration to a neutral pH with 1N HCL, or it will result in continued decontamination of *Mycobacterium* spp. Studies have documented that pH values above 8.10 are toxic to mycobacteria, including *Mycobacterium tuberculosis*.

FOR IN VITRO DIAGNOSTIC USE ONLY

PRECAUTIONS
The decontamination reagent contains a caustic chemical (sodium hydroxide). Use appropriate care in the handling of this reagent. All clinical specimens submitted for AFB testing must be treated with appropriate care so as not to contaminate other specimens or laboratory personnel. Use all approved and regulated equipment for AFB processing and detection procedures.

STABILITY AND STORAGE
AFB Reagents are stable to the stated expiration date when stored at the required temperature. Upon receipt, store unopened bottles at room temperature (15-30ºC). Do not freeze or heat above 30ºC. Refrigerate open bottles at 2-8ºC. Do not freeze or overheat. Allow the product to come to room temperature prior to use. Once NALC is mixed with the sodium citrate and sodium hydroxide, the product should not be used beyond 72 hours.

USER 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.

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: Sodium Hydroxide 4% and /or Sodium Citrate Solution 2.94%.

Materials Not Provided: 250 mg NALC ampoule, Vortex mixer, pipettes, centrifuge, centrifuge tubes, neutralization buffer, resuspension buffer, 1N HCL.

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. Mix 25 ml of Sodium Hydroxide 4% and 25 ml of Sodium Citrate Solution 2.94%. This will create a working digestion solution of 2% sodium hydroxide and 1.47% sodium citrate. With the plastic sleeve still attached, carefully break off the top of the ampoule (*N*-Acetyl-L-cysteine (NALC) powder). Add the NALC powder to the digestion solution. Mix well to dissolve the NALC powder.
   NOTE: Some residual NALC powder may remain in the ampule. It is not necessary to liquefy the portion remaining in the ampule. *THIS SOLUTION WILL BE GOOD FOR ONLY 72 HOURS AFTER MIXING*. Discard the mixed solution after 72 hours.
4. To a sterile 50 ml centrifuge tube containing the specimen to be digested, add an equal volume of NALC / digestion solution. Do not use more than 8 ml of specimen.
   NOTE: The amount of the specimen to be processed in the centrifuge tube must never exceed more than 1/6 the volume of the tube (i.e., for a 50 ml centrifuge tube, maximum of 8 ml of specimen). If it is necessary to process the entire specimen and the specimen exceeds 8 ml, add a volume of NALC / digestant solution equal to the volume of the patient sample, but split it into two centrifuge tubes immediately 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 minutes. (Each specimen should stand for 15 minutes but no longer than 20 minutes before the buffer is added.)
7. To complete the AFB diagnostic process, follow the neutralization and diagnostic procedures of your choice. CalibreScientific US, Inc. strongly recommends the use of either XPR-*PLUS* Neutralizing Buffer or NPC-67 Neutralizing Buffer along with PRB™.
   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.

SPECIAL PROCEDURES
Specimens contaminated with *Pseudomonas* spp. will need additional treatment with 5% Oxalic Acid (#0003447). Refer to the Oxalic Acid Directions For Use for complete instructions, or call CalibreScientific US, Inc. Technical Services or the Sales Department for information on the pH effects of the oxalic acid procedure and the appropriate buffering requirements.

OPTIONAL: The use of 0.2% Bovine Albumin Fraction V, (BA) is optional. However, if BA is not used, some type of material or CELL-BOND® Slides must be used to adhere the specimen pellet material to a microscope slide. Using a sterile pipette, add 1 or 2 drops of the 0.2% Bovine Albumin Fraction V Solution to the pellet. Shake gently by hand to mix.

EXPECTED RESULTS
To avoid the loss of any mycobacteria due to extended exposure to an elevated pH, specimens must be neutralized immediately following the addition of M/15 Phosphate Buffer. This neutralization can best be accomplished by titration with 1N HCL until the pH of the specimen falls below 8.10. A pH indicator can be added to the solution or NAC-PAC® *RED* can be used in place of sodium hydroxide / sodium citrate as it contains an integral pH indicator which visually confirms neutralization. Other buffers can be used to avoid the use of 1N HCL acid. Contact Sales for additional information.

LIMITATIONS OF PROCEDURES
This procedure is designed to be most effective with XPR-*PLUS* Neutralizing Buffer or NPC-67 Neutralizing Buffer. If M/15 Phosphate Buffer is used without proper subsequent neutralization, additional mycobacteria can be lost due to extensive 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.

SPECIFIC PERFORMANCE CHARACTERISTICS
Sodium hydroxide / sodium citrate was tested on clinical samples and recovered all culture appropriate *Mycobacterium* spp. when the designated procedures were followed.

BIBLIOGRAPHY
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9. Data on file.

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.

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PRODUCT CODES:
0003436 4% Sodium Hydroxide, 5 x 250 ml
0003458 Sodium Citrate Solution, 5 x 250 ml
0003471 4% Sodium Hydroxide, 250 ml

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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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