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Directions For Use for the following:

OxA™ OXALIC ACID REAGENT KIT

PRINCIPLE

Alpha-Tec Systems, Inc. OxA™ Oxalic Acid Reagent Kit is used for decontamination of respiratory specimens containing *Pseudomonas aeruginosa*.

CLINICAL SIGNIFICANCE

Clinical specimens submitted to the laboratory for the isolation of acid-fast mycobacteria are often contaminated with commensal microbial flora. While most of the contaminating bacteria can be eliminated with a sodium hydroxide and N-acetyl-L-cysteine (NALC) procedure, *Pseudomonas aeruginosa* can survive this decontamination method. In 1930, Corper and Uyei described an oxalic acid decontamination procedure of clinical specimens. In 1993, Whittier et al. demonstrated the effectiveness of the oxalic acid decontamination procedure on *Pseudomonas aeruginosa* in cystic fibrosis patients.

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. 15 bottles of 5% oxalic acid
    b. 15 bottles of NAC-PAC *RED*.
    c. 15 bottles of NPC-67 Neutralizing Buffer.
    d. 15 bottles of Pellet Resuspension Buffer.
2. **Not Provided**
    a. Centrifuge
    b. Vortex mixer
    c. Sterile pipettes
    d. Microscope slides
    e. TB growth media
    f. Centrifuge tubes
    g. CELL-BOND™ Slides.
3. **Storage:** Store at room temperature (15º-30º C).
4. **Stability:** All products OxA Reagent Kit 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.

The products within the OxA Reagent Kit were tested on clinical samples and recovered all culture appropriate *Mycobacterium* spp. when the designated procedures were followed.

PROCEDURE

PRECAUTIONS

Oxalic Acid is POISONOUS. Avoid contact with skin and eyes. Should contact occur, flush immediately with water. Contact a physician if irritation occurs. 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.

**ATTENTION: Handle all specimens with extreme caution. Possible Pathogens. Wear gloves at all times. Work in biosafety cabinet.

1. Perform the NALC/NaOH digestion and decontamination procedure on the respiratory specimen. (Call your Alpha-Tec Account Executive for a complete list of our digestion/decontamination and buffering products.)
2. If the specimen is identified as having *Pseudomonas aeruginosa* contamination through either a positive contamination control plate or rapid broth test, or patient history of previous *Pseudomonas* infection, proceed with the OxA decontamination procedure.

OxA OXALIC ACID DECONTAMINATION PROCEDURE

1. Use up to 3 ml from the original NALC/NaOH processed specimen and add it to a sterile 50 ml centrifuge tube.
2. Add the entire contents of Reagent 1 (Oxalic Acid) and vortex for 30 to 60 seconds.
3. Allow to stand for 30 minutes, vortexing every 10 minutes.
4. Add entire contents of Reagent 2 (NAC-PAC *RED*).
5. After adding the NAC-PAC *RED,* mix gently.
6. Add the entire contents of Reagent 3 (NPC-67 Neutralizing Buffer). Tighten cap, and swirl to mix.
7. Centrifuge the specimen tubes at 3000 xg for 15 minutes. It is recommended 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. 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 0.5 – 1.0 ml of Reagent 4 (PRB). (Do not resuspend the pellet with any other reagents, water or saline.)
   Option 1: To maximize time to detection for rapid growth automated detection systems, resuspend the pellet with 1.0 ml of Reagent 4 (PRB).
   Option 2: Depending on the needs of your laboratory, the pellet may be resuspended with 0.5 ml of Reagent 4 (PRB) to create a more concentrated sample for acid-fast smear staining. Once the smears have been made, add an additional 1.0 ml of Reagent 4 (PRB) for rapid broth detection methods and media inoculation.
10. Mix the sediment and buffer well and inoculate the liquid broth for your automated detection equipment per the manufacturer's instructions.
11. Place two drops of the sediment onto the surface of each of the TB media used. A contamination control plate (BAP or TSA) can be inoculated at this point and incubated at 35-37°C for 48 hours.
12. Make smears for AFB staining. Use adhesive CELL-BOND™ Slides or your appropriate sterile solutions to attach the specimen to the slide. Dry the smears and proceed with AFB staining per the manufacturer's directions. (Call your Alpha-Tec Systems Account Executive for a complete list of AFB Stains). **NOTE:** An AFB Stain Control Slide should be stained in conjunction with the patient smears to verify the staining technique and components (#0003240 AFB Stain Control Slide).
13. Add the balance of Reagent 4 (PRB) to the unused portion of the specimen and refrigerate at 2-8°C for further diagnostic procedures or re-decontamination if the cultures or rapid detection methods indicate that a contaminant is present.

CALCULATIONS
N/A

RESULTS
If *Mycobacteria* 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 *Mycobacteria* spp. can be expected.

NOTES
1. **Procedure Notes**
   a. Molecular Diagnostics
      Alpha-Tec's OxA Reagent Kit 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. **Summary of Technology**
   a. Decontamination and Digestion
      i. Oxalic Acid
         1. Bacterial decontaminate.
         2. Digests bacteria (including *Pseudomonas aeruginosa*) utilizing an acidic pH.
      ii. Sodium Hydroxide
         1. Stops the decontamination process by bringing the pH above 8.
      iii. Timing
         1. Critical so as to limit the killing rate of *Mycobacterium* spp. present in the patient specimen due to low pH
   b. Neutralization
      i. NPC-67 Neutralizing Buffer
         1. Used to neutralize the reagents following the appropriate decontamination time, resulting in a pH of 6.87-7.70.
         2. Following the decanting step, Pellet Resuspension Buffer is added to achieve a tight neutral pH value (6.8-7.1) in the specimen sediment, optimizing Mycobacteria recovery.
         3. 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.

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 *Mycobacteria* spp. Failure to follow the listed procedures may result in decreased numbers of *Mycobacteria* spp. or total loss of *Mycobacteria* spp. resulting in an inaccurate culture report.

BIBLIOGRAPHY
1. Corper, H.J., and N. Uyei. 1930. "Oxalic Acid as a Reagent for Isolating Tubercule Bacilli and a Study of the Growth of Acid-Fast Nonpathogens on Different Mediums with Their Reactions to Chemical Reagents." J. LAB. CLIN. MED. 15:348-369.
2. Lennette, E.H. et al., 1980. MANUAL OF CLINICAL MICROBIOLOGY American Society of Microbiology, Washington, D.C., pp. 150-179.
3. Vestal, A., 1975. PROCEDURES FOR THE ISOLATION AND IDENTIFICATION OF MYCOBACTERIA. U.S. Dept. of Health, CDC Publication No. 79-8230, Center for Disease Control, Atlanta, GA. pp.21-31.
4. Whittier, S., et al., 1993. "Improved Recovery of Mycobacteria from Respiratory Secretions of Patients with Cystic Fibrosis", J. Clin. Microbiol. 31:861-864.

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 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.

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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

