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Directions For Use for the following:

# Bromothymol Blue Indicator

**INTENDED USE**
Bromothymol Blue Indicator is used as pH indicator during the oxalic acid decontamination of clinical respiratory source specimens contaminated with *Pseudomonas* spp. for the increased recovery of *Mycobacterium* species.

**FOR IN VITRO DIAGNOSTIC USE ONLY**

**PRECAUTIONS**
1. Bromothymol Blue is an IRRITANT.
2. Avoid contact with skin and eyes. Should contact occur, flush immediately with water. Contact a physician if irritation occurs.
3. Observe ALL laboratory standards for the handling of patient specimens. All clinical specimens submitted for the diagnosis of tuberculosis and other *Mycobacterium* spp. must be treated with the appropriate care to avoid contaminating other specimens or laboratory personnel. Use approved and regulated equipment for processing and detection procedures.

**STABILITY AND STORAGE**
Bromothymol Blue Indicator is stable to the stated expiration date when stored at 15- 30°C.

**USER QUALITY CONTROL**
Any reagent showing cloudiness, turbidity, precipitation, or discoloration should be discarded. Quality control organisms 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:** Bromothymol Blue Indicator

**Materials Not Provided:** Centrifuge, centrifuge tubes (50 ml), microscope slides, sterile pipettes, vortex mixer, AFB processing reagents, Oxalic Acid, NAC-PAC®, NPC-67®, AFB Phosphate neutralizing buffer, PRB™ (Pellet Resuspension Buffer), AFB stains, TB media.

**SPECIMEN PROCESSING**
1. Perform the NALC/NaOH digestion and decontamination procedure on the lower respiratory specimen. (Call Sales for a complete list of AFB digestion/decontamination and buffering products.)
2. If the specimen is identified as having *Pseudomonas* spp., proceed with the Oxalic Acid decontamination procedure.

**OXALIC ACID DECONTAMINATION PROCEDURE**
1. In a 50 ml centrifuge tube add up to 3 ml of NaOH processed specimen.
2. Add 3-5 ml of Oxalic Acid and vortex for 30 to 60 seconds.
3. Allow to stand for 30 minutes, vortexing every 10 minutes.
4. Add approximately 0.5 – 1.0 ml of Bromothymol Blue Indicator (12-24 drops) and mix gently.
5. Add sodium hydroxide solution or NAC-PAC® until the solution turns blue.
6. Using a sterile pipette, add an appropriate phosphate neutralizing buffer or NPC-67® Neutralizing Buffer until the color changes from blue to colorless or light yellow.
7. Centrifuge the specimen tubes in a centrifuge at 3000xg for 15 minutes. (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 3000xg.)
8. Working in a biosafety hood, pour off all supernatant into a splash-proof container holding an appropriate disinfectant. Use an appropriate disinfectant or flame 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 0.5 – 1.0 ml of an appropriate phosphate resuspension buffer or PRB™. Do not resuspend the pellet with water or saline. **NOTE:** To maximize time to detection for rapid growth automated detection systems, resuspend the pellet with 1.0 ml of buffer. Depending on the needs of your laboratory, the pellet may be resuspended with 0.5 ml of buffer to create a more concentrated sample for acid-fast smear staining. Once the smears have been made, add an additional 1.0 ml of buffer 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 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 appropriate sterile solutions to attach the specimens to slide. Dry the smears and proceed with AFB staining per the manufacturer's instructions. (Contact Sales for a complete list of AFB stains). **NOTE:** An AFB stain slide should be stained in conjunction with the patient smears to verify the staining technique and components (#0003240 QC1 AFB Slide).
13. Add an additional 1 - 2 ml of PRB (according to the laboratory's set-up procedure volume requirements) to the specimen and refrigerate at 2- 8°C for further diagnostic procedures.

**EXPECTED RESULTS**
If mycobacteria 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 can be expected.

**LIMITATIONS OF PROCEDURES**
Timing of the decontamination step, proper buffering, speed and timing of the centrifugation step, proper decanting and addition of the PRB to the pellet are vital to the recovery of mycobacteria. Failure to follow the listed procedures may result in decreased numbers of mycobacteria or total loss of mycobacteria resulting in an inaccurate culture report.

**BIBLIOGRAPHY**
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.
Lennette, E.H. et al., 1980. MANUAL OF CLINICAL MICROBIOLOGY American Society of Microbiology, Washington, D.C., pp. 150-179.
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.
Whittier, S., et al., 1993. "Improved Recovery of Mycobacteria from Respiratory Secretions of Patients with Cystic Fibrosis", J. Clin. Microbiol. 31:861-864.

**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:**
0003448 Bromothymol Blue Solution, 2 x 30 ml

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**GLOSSARY OF SYMBOLS**

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| 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 |
| | 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 / laktag försiktighet / Previdno / Atenção |
| | Temperature limit / Conserver aux températures indiquées / Almacene entre las temperaturas indicadas / Conservare a temperature comprese fra quelle indicate / Im angegebenen temperaturbereich aufbewahren / Opslaan bij een temperatuur tussen / Förvara mellan angivna temperaturer / Shranjevati med navedenimi temperaturami / Armazene entre as temperaturas indicadas |
| Σ | Contains sufficient for <n> tests / Contenu suffisant pour <n> tests / Contiene suficiente para <n> pruebas / Contenuto sufficiente per <n> tests / Enthält ausreichend für <n> untersuchungen / Inhoud voldoende voor <n> testen / Innehåller tillräckligt för <n> tester / Vsebina zadostuje za <n> testov / Contém quantidade suficiente para <n> testes |
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