AlphaTec™
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Directions For Use for the following:

MYCO-PAC™

INTENDED USE
This product is used in qualitative procedures as a mucolytic agent in the digestion of the disulfide bonds in mucus procedures for mycological and mycobacteriological culture. Mycobacterial processing requires additional decontamination and buffering procedures.

SUMMARY
Reep and Kaplan evaluated Dithiothreitol and N-acetyl-L-cysteine as mucolytic agents for sputum specimens, and demonstrated that either agent was successful in liquefying and decontaminating sputa for acid-fast culture. They also determined sodium hydroxide was toxic to fungus. By using MYCO-PAC™ (sodium citrate solution) without sodium hydroxide as the diluent, fungal toxicity is eliminated. N-acetyl-L-cysteine acts as a mucolytic compound by disrupting chemical bonds in mucus. MYCO-PAC stabilizes the NALC by chelating (binding) any heavy metal ions present in the specimen.

FOR IN VITRO DIAGNOSTIC USE ONLY

PRECAUTIONS
This product should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimen, containers and media after use. Directions should be read and followed carefully.

STABILITY AND STORAGE
This product can be stored in its original container at room temperature. When mixed, refrigerate at 2-8ºC. Do not freeze or overheat. Allow product to come to room temperature before use. Do not incubate prior to use. These products should not be used beyond the expiration date. Once the NALC is mixed with MYCO-PAC, 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
The laboratory should provide suitable sterile collection containers and instructions for their proper use. Specimens should be transported to the laboratory without delay and protected from excessive heat or cold.

General rules apply to all clinical specimens:
1. The specimen should be collected properly and should be representative of the infected area.
2. Care should be taken to prevent contamination of specimen.
3. Specimens should be taken to the laboratory promptly.
4. Specimens should be obtained before any antibiotics are administered to the patient. If therapy was initiated prior to collection, this should be noted on the forms sent with the specimen.

PROCEDURE
Materials Provided: MYCO-PAC (5 bottles); NALC (5 vials).

Materials Not Provided: Centrifuge, centrifuge tubes, vortex mixer, sterile pipettes, M/15 Phosphate Buffer, microscope slides, fungal media, AFB processing reagents (NAC-PAC® *RED*, NPC-67® Neutralizing Buffer, PRB™ (Pellet Resuspension Buffer), TB media, CELL-BOND® slides, QC1™ Quality Control Slides.

Instructions for Mycological Culture
1. Open the bottle labeled MYCO-PAC. Add NALC powder to the MYCO-PAC bottle. **NOTE**: Some residual NALC powder may remain in the vial. It is not necessary to liquefy the portion remaining in the vial. Re-cap the MYCO-PAC bottle and mix well. **Once dissolved, the MYCO-PAC/NALC solution will be good for only 72 hours. Store any unused portion at 2-8°C for up to 72 hours.**
2. Line up specimens and centrifuge tubes in numerical order.
3. Loosen cap of sputum container (work in sets equivalent to 1 centrifuge load).
4. Transfer sputum to sterile centrifuge tubes. Do not put more than 10 ml of sputum in a 50 ml tube. The amount of the specimen processed in the centrifuge tube must never be more than one-fifth the volume of the tube. When it is necessary to process the entire specimen, use more than 1 tube and combine the sediments after centrifugation (Step 9).
5. To the sterile 50 ml centrifuge tube containing the specimens, add a volume of MYCO-PAC/NALC equal to that of the specimen volume.
6. Tighten caps of centrifuge tubes. Beginning with the first specimen in the series, mix each specimen on the vortex until liquefied. This may take from 5-20 seconds per specimen.
7. Let each specimen stand for 5 minutes.
8. Fill each tube with M/15 Phosphate Buffer (pH 6.8, #0003442). This should be at least 30 ml in a 50 ml centrifuge tube. Tighten cap and swirl by hand to mix.
9. Centrifuge at 3000xg for 15 minutes. (Each laboratory must check the centrifuge head radius, and then use an appropriate nomogram for proper speed selection (rpm) to achieve the desired relative centrifugal field of 3000xg.) It is recommended but not required to use a refrigerated centrifuge.
10. Pour off all but approximately 1-2 ml of the supernatant into a splash-proof container containing 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.
11. Using a sterile pipette, resuspend the sediment.
12. With the pipette, transfer 1 or 2 drops of sediment to appropriate media for fungal culture and to slides for staining. A mycology quality control slide should be stained in conjunction with the patient smears to verify the staining technique and components (#0003265)

Instructions for Acid-Fast Culture
Use the remaining sample processed above (through Step 12).
1. Add additional M/15 buffer or sterile saline to bring the volume of the specimen up to 5 ml.
2. Add 5 ml of TB Base digestant (NAC-PAC *RED* #0004305 or 0004303) to the sediment. Vortex well for 5-10 seconds.
3. Allow each specimen to stand for 15 minutes. Each specimen should stand for 15 minutes but no longer than 20 minutes before buffer is added. Vortex every 5 minutes during this step.
4. Fill each tube with a neutralizing buffer (NPC-67 Neutralizing Buffer #0003941) until effective neutralization is indicated by a color change from red/pink to colorless. Once the colorless point has been reached, do not continue to add NPC-67 to the sample. Tighten cap and swirl by hand to mix.
5. Centrifuge at the specimen tubes at 3000xg for 15 minutes. It is recommended but not required to use a refrigerated centrifuge.
6. Working in a biosafety hood, pour off all the supernatant into a splash-proof container containing an appropriate disinfectant. Use and 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.
7. Resuspend the pellet with 0.5 – 1.0 ml of PRB #0004510). Do not resuspend the pellet with NPC-67, M/15 Phosphate Buffer, water or saline. Mix the sediment and buffer well, and inoculate the liquid broth for your automated detection equipment per the manufacturer's instructions.
8. Place two drops of sediment onto the surface of each of the TB media used. **NOTE:** A contamination control plate can be inoculated at this point, BAP or TSA, and incubated at 35 -37°C for 48 hours.
9. Make smears for acid-fast staining. Use adhesive CELL-BOND slides (#0003257) or appropriate sterile bovine albumin adhesive

solutions to attach the specimen to the slide. Dry the smears and proceed with appropriate acid-fast staining per the manufacturer's instructions. **NOTE:** Call Alpha-Tec for a complete list of acid-fast stains. 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 Slides).

10. Retain the balance of the specimen and refrigerate at 2-8°C to save for further diagnostic procedures or reprocessing if necessary.

EXPECTED RESULTS

If fungi or *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 fungi and *Mycobacterium* spp. can be expected.

LIMITATIONS OF PROCEDURES

This product is only part of the overall scheme for identification. Procedures for biochemical and serological tests for identification may be found in appropriate references.

BIBLIOGRAPHY

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CONTACT

Alpha-Tec Systems, 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

This product is warranted by Alpha-Tec Systems, Inc. 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:

PRODUCT CODES:

0003453 MYCO-PAC, 5 x 50 ml

Manufactured by Alpha-Tec Systems, Inc.
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30175 Hannover, Germany CE

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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