THIS INFORMATION IS TO BE USED IN CONJUNCTION WITH DFU L004302, NAC-PAC *RED*

PROCESSING BLOODY AFB SPECIMENS

If an AFB specimen submitted for processing contains a significant amount of blood it may be difficult to visualize the color change from pink to colorless when adding the AFB Neutralization Buffer (XPR-*PLUS*® or NPC-67®) to the specimen and NAC-PAC *RED* solution after digestion. This is a modification to ensure proper neutralization when color change cannot be visualized. When adding the NAC-PAC *RED* to the bloody specimen, the NaOH in the solution will lyse the red blood cells and the residual hemoglobin can mask the visualization of the pH indicator in the solution in the neutralization step.

1. To the sterile 50 ml centrifuge tube, add the NAC-PAC *RED*/NALC solution in the following amounts: for specimens 1-5 ml, add a volume equal to that of the specimen volume; to specimens 6-7 ml, add 5 ml of base solution; for specimens 8-10 ml, add an equal volume of base digestant and split the specimen after step 3 equally into two centrifuge tubes, proceed with steps 4-6 and then combine the sediments from both tubes into one centrifuge tube and proceed with step 8.
2. Mix each specimen on a vortex until liquefied.
3. Allow each specimen to stand for 15 minutes, vortexing every 5 minutes during this step. Each specimen should stand for 15 minutes, but no more than 20 minutes.
4. Add the Neutralization Buffer up to the 50mL mark on the centrifuge tube to bring the solution pH below 8.1. This will ensure optimal recovery of any Mycobacterium that may be present in the specimen.
5. Centrifuge the specimen tubes at 3000 xg for 15 minutes. It is recommended but not required to use a refrigerated centrifuge.
6. Working in a biosafety hood, pour off all of the supernatant into a splash-proof container holding an appropriate disinfectant.
7. Resuspend the pellet with 0.5-1.0 ml of PRB™ Pellet Resuspension Buffer.
8. Prepare appropriate smears and/or inoculate culture media according to laboratory protocols.

The lysed red blood cells will also result in a significant amount of cell debris in the solution. The debris may attach to the sides of the centrifuge tube due to the natural charge associated with the plastic.

PROCESSING SMALL VOLUME AFB SPECIMENS

Small volume specimens with corresponding low post-neutralization volumes can make centrifuge balancing difficult. This is a modification to accommodate small volumes (under 5 mL).

1. Add **sterile** saline to the sample in a sterile 50 ml centrifuge tube to reach a total volume of 5 mL.
2. Add 5 mL of NAC-PAC *RED* containing N-acetyl-L cysteine.
3. Mix each specimen on a vortex until liquefied.
4. Allow each specimen to stand for 15 minutes, vortexing every 5 minutes during this step. Each specimen should stand for 15 minutes, but no more than 20 minutes.
5. Centrifuge the specimen tubes at 3000 xg 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 holding an appropriate disinfectant.
7. Resuspend the pellet with 0.5-1.0 ml of PRB.
8. Prepare appropriate smears and/or inoculate culture media according to laboratory protocols.

PROCESSING SPECIMENS CONTAMINATED WITH *PSUEDOMONAS* SPP.

Specimens contaminated with *Pseudomonas* spp. will need additional treatment with 5% Oxalic Acid to prevent overgrowth of *Pseudomonas* spp. Refer to the 5% Oxalic Acid Directions For Use (DFU L003447) for complete instructions. For additional information on the pH effects of the oxalic acid procedure and the appropriate buffering requirements, call Alpha-Tec Technical Services or the Alpha-Tec Sales Department.

RE-PROCESSING CONTAMINATED AFB SPECIMENS:

If there is non-AFB overgrowth on the test media or rapid growth detection systems, the specimens must be re-processed to remove the contaminating bacteria.

1. Add **sterile** saline or Pellet Resuspension Buffer to the sample in a sterile 50 ml centrifuge tube to reach a total volume of 5 mL.
2. Add 5 mL of NAC-PAC *RED* containing N-acetyl-L cysteine.
3. Mix each specimen on a vortex until liquefied.
4. Allow each specimen to stand for 15 minutes, vortexing every 5 minutes during this step. Each specimen should stand for 15 minutes and can be increased up to 20 minutes to ensure adequate decontamination.
5. Centrifuge the specimen tubes at 3000 xg for 15 minutes. It is recommended but not required to use a refrigerated centrifuge.
6. Working in a biosafety hood, pour off all of the supernatant into a splash-proof container holding an appropriate disinfectant.
7. Resuspend the pellet with 0.5-1.0 ml of PRB.
8. Prepare appropriate smears and/or inoculate culture media according to laboratory protocols.

PROCESSING STOOL AFB SPECIMENS:

If a stool specimen is submitted to the laboratory for AFB processing it may be difficult to visualize the color change from pink to colorless when adding the AFB Neutralization Buffer (XPR-*PLUS* or NPC-67) due to the opacity of fecal material. This is a modification to the digestion and decontamination procedure (NAC-PAC *RED* DFU L004302) to accommodate stool specimens.

1. In a sterile 50 ml centrifuge tube, suspend approximately 1 gram of fecal material in 5 ml of saline. Vortex to mix well.
2. Add 5 ml of NAC-PAC *RED* containing N-acetyl-L-cysteine and allow the specimen to liquefy and decontaminate for 15 minutes, vortexing every 5 minutes.
3. Add enough Neutralization Buffer to neutralize the NAC-PAC *RED* (liquid goes from red to colorless). If you are unable to see the color change due to the fecal material, add the Neutralization Buffer up to the 50 ml mark on the centrifuge tube to bring the pH below 8.1.
4. Centrifuge the specimen tube at 3000 xg for 15 minutes. It is recommended, but not required to use a refrigerated centrifuge.
5. Working in a biosafety hood, pour off all of the supernatant into a splash-proof container holding an appropriate disinfectant.
6. Resuspend the pellet with 0.5 – 1.0 ml of Pellet Resuspension Buffer.
7. Prepare appropriate smears and/or incoculate culture media according to laboratory protocols.
8. Save the remaining resuspended pellet in case of excessive breakthrough contamination. If the breakthrough contamination is unacceptable, re-process the pellet following the directions in the section “Re-processing Contaminated Specimens” in this document, increasing the decontamination time to 20 minutes.
9. Due to the inherent high bioburden of stool specimens, you may need to use a higher (up to 4.0%) concentration of NAC-PAC *RED* solution to help control breakthrough contamination.

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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