K745125 Oxalic Acid Solution (125 ml.)
K7405030 Brom Thymol Blue Indicator (K74505030)

**INTENDED USE:**
The oxalic acid method is preferable to alkali methods for processing specimens that are expected to be contaminated with *Pseudomonas*. This method may be used by itself or in conjunction with NAOH-NALC method (also available from Key) The specimens processed with oxalic acid are compatible with BACTEC 460TB (BD) automated system.

**APPROXIMATE FORMULATIONS:**
K745125 contains 5% Oxalic Acid
K7405030 contains 0.05% Bromthymol Blue

**STORAGE AND SHELF LIFE:**
Do not use any of the material if there are any signs of discoloration, contamination, deterioration or if the expiration has passed. Store at room temperature. Bromthymol blue should be stored out of direct light.

**CAUTIONS:**
This product is for in vitro diagnostic use only and is to be used only by adequately trained and qualified laboratory personnel. All laboratory specimens should be considered infectious and handled according to approved laboratory guidelines for infectious material.
Sterilize all biohazard waste before disposal.

**SPECIMEN COLLECTION:**
Recommended collection and handling of specimens are available from sources including the World Health Organization (1) and the CDC Public Health Lab. Work within a biological safety cabinet and wear gloves

**PROCEDURE:**
**Materials provided: 5% Oxalic Acid.**
1.Add an equal volume of 5% oxalic acid to the sputum specimen in a 50 ml centrifuge tube (1:1 ratio). Sputum volume may not exceed 10 ml.
2. Vortex the above mixture and let it stand at room temperature for 30 minutes with occasional mixing.
3. Add sterile saline to the 50 ml mark on the centrifuge tube. Cap the tube and mix well by inversion.
4. Centrifuge for 15 minutes $\geq$ 3000 x *g*, decant the supernatant.
5. Add two drops of bromthymol indicator to each ml of sediment.
6. Neutralize carefully with 4% Sodium hydroxide to achieve a light green/apple green color indicating a neutral pH (it will take approximately 130 µl of 4% NaOH for each ml of the sediment)**.** Any blue coloration indicates excessive alkalinity, and a yellow color signifies too much acidity.
7.Completely resuspend the sediment, inoculate the appropriate medium, and make smears for AFB

**OPTIONAL PROCEDURE:**
If the KEY SCIENTIFIC NAOH-NALC digestion and decontamination procedure has been done and the culture has grown on the subculture purity plate perform a gram stain and oxidase test. If the culture is gram-negative and oxidase positive, perform the Oxalic acid decontamination as follows:
1. In a 50 ml centrifuge tube, add 1 ml of processed specimen, resuspended in Bovine Albumin. (resuspend up to 1 ml if needed).
2. Add 5 ml of 5% Oxalic Acid, vortexing for 30-60 seconds.
3. Using a sterile pipette, add the appropriate volume of NAOH-NALC as follows:

| KEY PREFIX * | NAOH-NALC | ADD AMT |
|---|---|---|
| K7620### | 2% | 13 ml |
| K7625### | 2.5% | 11 ml |
| K7640### | 4.0 % | 7 ml |
| * LAST 3 DIGITS VARY WITH SIZE OF BOTTLE | | |

4. Add 0.5 - 1.0 ml (12-24) drops of K7405030 BTB indicator and mix gently. Solution should turn blue. If not, then add more NAOH-NALC, a drop at a time, until the blue color is apparent,
5. Add K7670* PHOSPHATE BUFFER to the 50 ml mark on the centrifuge tube. Mix well.
6. The solution should turn clear with a slight blue/green tint.
7. Centrifuge the specimen tubes in a refrigerated centrifuge at 3000 xg for 15 minutes.
8. Working is 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 fo the specimen tube. Do not allow the disinfectant to run down the inside of the specimen tube.
9. Resuspend the pellet with 0.5 ml of pellet resuspension buffer. (do not use neutralizing buffer or phosphate buffer)
10. Mix the sediment and buffer well and make smears for AFB staining using appropriate sterile albumin adhesive solutions to attach the specimen to the slide. Dry the smears and process with AFB staining following the manufacturers directions. NOTE: preparation of the smear with only 0.5 ml of pellet resuspension buffer allows for greater sensitivity in the visualization of AFB in the stained ???Procedure.

**LIMITATIONS:**
Timing is important during the digestion process. A digestion time of longer than 15 minutes should not be used. Many Mycobacterium spp. are killed by over decontamination. Occasional specimens are so contaminated with resistant bacteria that the decontamination process is not effective and the contaminating bacteria will overgrow the slower growing mycobacteria. Sediment material may be redigested and a selective medium, with antibiotics such as Lowenstein Jensen, Selective or Middlebrook 7H11, can be used to decrease the growth of contaminating organisms.

**MATERIALS REQUIRED BUT NOT PROVIDED**
Microbiological supplies and equipment such as vortex mixers, biological safety cabinets, centrifuge tubes, slides, media, loops, incinerators, incubators, pasteur pipets, etc., as well as serological and biochemical reagents, are not provided. Optional items:

**QUALITY CONTROL:**
REFERENCES:
1. Kevin Weyer, et al , Laboratory Services in Tuberculosis Control, 1998
2. Patrick RM; Barron EJ; Jorgansen JH; Landry ML; Pfaller MA; Manual of Clinical Microbiology,page 564, 9th edition, ASM press
3. BANGE FC, KIRSCHNER PAND. BOTTGER EC; Recovery of Mycobacteria from Patients with Cystic Fibrosis; JOURNAL OF CLINICAL MICROBIOLOGY,Nov. 1999, p. 3761–3763

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