10. Do not disturb the tube or coverslip for fifteen minutes.
11. With a swift motion, lift the coverslip straight upward so that a drop of liquid is contained on the coverslip. This drop will contain cysts or eggs if they are present in the specimen.
12. Place the coverslip on a slide. If desired, iodine may be added to the wet mount for better detail. The edges may be sealed with a petroleum jelly/ paraffin mixture to prevent drying of the specimen.
    *Alternate Method - directly after step 6, without removing the tube from the centrifuge, remove 1 or 2 drops of the surface film with a Pasteur pipette or a freshly flamed wire loop. Do not use the loop as a "dipper", simply touch the surface with the loop portion. Proceed with examination.

PRECAUTIONS

Avoid contact of fixative solutions with the skin and eyes. Should contact occur, call a physician immediately. Flush with plenty of water. DO NOT DRINK. If ingested, call a physician immediately.

Occupational Safety and Health Act regulations (including Universal Precautions) should be used for handling all specimens.

For assistance please call our Technical Service Department toll-free at 1-800-528-0494 between the hours of 8 A.M. and 5 P.M. Eastern Standard Time.

BIBLIOGRAPHY

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Protocol SAF Vials	**Item #**
Protocol SAF Vials, 20/pk	23-005-41
Protocol SAF/Clean, 2x10 vl/pk	23-005-44
Protocol SAF/C&S, 2x10 vl/pk	23-005-39
Protocol SAF/Clean/C&S, 3x10 vl/pk	23-005-26

Other Protocol Parasitology Products	**Item #**
Protocol Clean Vials, 20/pk	23-005-31
Protocol 10% Buff. Formalin Vials, 20/pk	23-005-46
Protocol Modified (Cu) PVA Vials, 20/pk	23-005-33
Protocol C&S Vials, 20/pk	23-005-43
Protocol Cary Blair Vials, 20/pk	23-005-47
Protocol Zn-PVA Vials, 20/pk	23-005-37
Protocol Modified (Cu) PVA/Formalin, 2x10 vl/pk	23-005-27
Protocol Zn-PVA / 10%Formalin, 2x10 vl/pk	23-005-45
Protocol PVA/Formalin/Clean, 3x10 vl/pk	23-005-22
Protocol PVA/Formalin/C&S, 3x10 vl/pk	23-005-24
Protocol Zn PVA/ Formalin/ Clean, 3x10 vl/pk	23-005-28
Protocol Mod. (Cu) PVA Formalin/Clean, 3x10 vl/pk	23-005-30
Protocol Zn-PVA/ Formalin/ C&S, 3x10 vl/pk	23-005-32
Protocol Ethyl Acetate	23-005-68
Protocol Trichrome, 500 mL	23-005-38
Protocol Zn-PVA Bulk, 500 mL	23-005-40
Protocol Bulk Tubes and caps, 15 mL	23-005-42
Protocol 50 mL Concentration System, 120 ea.	23-005-50
Protocol 15 mL Concentration System, 4x50 ea.	23-005-52
Protocol 15 mL Concentration System, 50 ea.	23-005-51

840655 (RO)

Protocol™

Parasitology System Application for SAF System

PURPOSE:

Protocol Parasitology Systems provide standardized procedures for the routine collection, transportation, preservation and examination of stool specimens for intestinal parasites. Protocol Systems are for use by patient or healthcare worker and help minimize the need for immediate transport and processing of specimens.

PRINCIPLES:

Diagnosis of enteric parasitic disease is confirmed by isolation and identification of pathogenic organisms in fresh stool specimens. Procedures such as freezing, incubation and refrigeration do not insure recovery and identification of all intestinal parasites. Proper use of the Protocol Parasitology System assures the parasitologist that diagnostic stages of intestinal parasites, if present, will be preserved. SAF (sodium acetate acetic acid formalin) lends itself to both the concentration technique and the permanent stained smear and has the advantage of not containing mercuric chloride, as is found in Schaudin's fluid and LV/PVA. Helminth eggs and larvae, protozoan trophozoites and cysts, and coccidian oocysts and microsporidian spores are preserved by this method. Slides may be stained using trichrome or hematoxylin.

CONTENTS:

Each vial contains 15 mL SAF with a built-in collection spoon. One vial of 5 mL Mayer's Albumin and directions for use are also included.

MATERIALS NOT PROVIDED

Ethyl acetate
Zinc sulfate solution
Physiological saline
Cotton tipped applicator sticks
Microscope slides and coverslips
Centrifuge
Microscope
Transfer pipettes

SPECIMEN COLLECTION

1. Caution: Patient should not use antacids, barium, bismuth, antibiotics, anti malarial agents, antidiarrheal medication or oily laxatives prior to specimen collection. After administration of any of these compounds, specimen collection should be delayed for 5 to 10 days or at least two weeks after barium or antibiotics.
2. Several specimens, collected intermittently over several days, should be examined to insure recovery of organisms.
3. Specimens must be collected properly to avoid contamination with urine or water (see collection instructions). Specimens are best collected in a bedpan. A clean dry container such as a milk container may be used by removing the top and washing thoroughly. Another option is to place plastic wrap over the toilet seat opening.
4. A suitable area (i.e. bloody, slimy, watery) from the sides, ends and middle of the stool should be selected using the collection spoon provided. Fill with sufficient stool to bring the liquid level up to the "Fill" line. This will result in approximately 5 mL of sample.
5. Stir each specimen with the spoon provided, tighten the cap and shake firmly until the specimen is adequately mixed. When mixing is complete the specimen should appear uniform.
6. Complete the label on each vial.

SPECIMEN PROCESSING

The Protocol System allows for a variety of procedures to be utilized. A complete examination should include at least four steps: gross examination, direct microscopic examination, slide staining and one or more concentration procedures. While each laboratory should follow its own established technique, the following gives directions for commonly accepted procedures:

1. Gross examination: record the presence of blood, worms, mucus or proglottids. If the consistency of the stool can be determined, it may give an indication of the types of organisms present and should be recorded.
2. Direct microscopic examination of SAF preserved specimen:
   a. Place a clean glass slide on a sheet of newsprint.
   b. Add a drop of 0.85% saline (iodine may be substituted) to the slide.
   c. Add a representative sample of SAF fixed specimen to the drop of saline and mix thoroughly. The newsprint should be just legible through the slide.
   d. Place a wide coverslip on the suspension and examine immediately.
   e. For a temporary seal, a cotton tipped applicator stick dipped in equal parts of heated paraffin and petroleum jelly should be used.
3. Permanent slides for staining with trichrome stain or iron hematoxylin:
   a. Allow specimen to fix for at least 30 minutes in SAF. The Protocol SAF vials may be used with 15 mL or 50 mL conical centrifuge tubes (Protocol 15 mL Concentrate System or 50 mL Concentrate System work well for this application). If specimen appears mucoid, add 10 drops of surfactant and mix gently by swirling tube.
   b. Filter the SAF fixed specimen. Add at least 5 mL of filtered specimen to the centrifuge tube (the entire specimen may be used if desired).
   c. Centrifuge the specimen for 10 minutes at 500 x g (1800 2200 rpm). Decant the supernatant. The final sediment should be approximately 0.5 1 mL. If necessary, adjust by adding specimen or resuspending the sediment in physiological saline and remove part of the suspension.
   d. Place I drop of Mayers Albumin on the slide and wipe so that only a thin layer remains on the slide. Add 1 drop of the SAF fixed sediment to the slide and allow the smear to air dry at room temperature for 30 minutes prior to staining. (The SAF stool smear can also be post fixed in Schaudin's fixative prior to staining).
   e. After drying, the smear can be placed directly into 70% alcohol to coagulate the albumin prior to staining. When proceeding with staining, the iodine alcohol step may be eliminated.
4. Concentration procedures: One or more concentration procedures should be employed. There are two types of concentration procedures, sedimentation and flotation, both of which are designed to separate protozoan organisms and helminth eggs and larvae from fecal debris by centrifugation and/or differences in specific gravity. Each procedure may vary in respect to the efficacy with which it recovers specific organisms. No one concentration procedure works equally well for all parasites and laboratory personnel should consult available literature for additional information. The following are two common procedures:

A. Forma lin ether/ethyl acetate sedimentation:

1. Mix the SAF fixed specimen thoroughly. The specimen is now ready for processing with the Protocol Stool Concentration System. See the appropriate package insert for further directions.
2. If the Protocol Stool Concentration System is not available, strain a sufficient quantity through a layer of wet mesh gauze to proceed with step 3. This will vary with the size and density of the specimen (3 4 mL will be sufficient unless there is very little stool in the vial).
3. Add saline (or formalin) almost to the top of the tube, mix completely and centrifuge at 500 x g for 10 minutes (1800 2200 rpm). If the resulting precipitate is not 0.5 1 mL, resuspend, add or remove specimen and recentrifuge.
4. Decant the supernatant fluid. A second wash may be used if necessary.
5. Add approx. 10 mL of formalin (fill the tube half full only), resuspend sediment mix and allow to stand for five minutes. If the amount of sediment left in the bottom of the tube is very small or the original specimen contained a lot of mucus, do not add ethyl acetate in step 6; merely add the formalin, spin, decant, and examine the remaining sediment.
6. Add 3 5 mL of ethyl acetate or ether, tighten the cap and shake vigorously for at least 30 seconds.
7. Centrifuge at 500 x g for 10 minutes (1800-2200 rpm).
8. The Protocol SAF vials may be used with 15 mL or 50 mL conical centrifuge tubes (Protocol 15 mL Concentrate System or 50 mL Concentrate System work well for this application). The resulting solution will separate into four layers:
   a) Top layer: ethyl acetate or ether
   b) Second layer: plug of debris
   c) Third layer: formalin
   d) Bottom layer: sediment
9. After ringing the plug of debris from the side of the tube with an applicator stick, carefully decant the top three layers. While keeping the tube inverted, a cotton swab may be used to remove debris sticking to the sides of the tube. This is particularly important for obtaining suitable results with ethyl acetate and prevents solvent bubbles in the wet mount.
10. Add a few drops of physiological saline or 10% formalin to resuspend the remaining sediment. Add a small amount of material to the slide. If the resuiting slides are too dense (newsprint should be legible through them) more saline or formalin may be added. Add a coverslip.
11. Examine and record results. As with the direct wet mount, iodine can be added to enhance morphological detail.

B. Zinc sulfate flotation

1. Thoroughly mix a representative portion of the SAF fixed stool suspension or fresh specimen in a 15 mL centrifuge tube and fill with tap water to approximately 10 mL. The specimen amount will vary according to the size and density.
2. Centrifuge at 500 x g for 10 minutes (1800-2200 rpm).
3. Sediment should be 0.5 - 1 mL. If needed, adjust the suspension by adding formalin fixed material or diluting with water. Once sediment is at the desired 0.5 - 1 mL amount, decant the supernatant. Repeat the wash if necessary.
4. Fill the tube approx. half full with zinc sulfate solution and resuspend the sediment by mixing thoroughly with applicator sticks. (Zinc sulfate solution must have a specific gravity of 1.2. Adjust as needed.)
5. Add additional zinc sulfate solution to within one inch of the top.
6. Centrifuge at 500 x g for 10 minutes (1800-2200 rpm).
7. Carefully remove the tube and place upright in a test tube rack without disturbing the contents. There should be two resulting layers: a small amount of sediment in the bottom of the tube and a layer of zinc sulfate.*
8. Without overflowing the tube, fill with additional zinc sulfate to the top of the tube.
9. Place a clean coverslip on top of the tube. If the coverslip does not make contact with the solution in the tube, carefully add more liquid.

Fisher Diagnostics
a division of Fisher Scientific Company, LLC
a part of Thermo Fisher Scientific Inc.
Middletown, VA 22645-1905 USA
Phone: 1-800-528-0494 / Fax:1-540-869-8132
1-540-869-3200

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