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 flame 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.

If gelled, the fixative may be liquefied by placing in a 50°C water bath until clear and fluid.

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
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, anti diarrheal 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 and replace the vials in the plastic bag.

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 formalin 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 formalin 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 modified PVA. Mix thoroughly with two applicator sticks. Pour a small amount of the Modified PVA fixed specimen onto a paper towel and allow to stand for three minutes. (Allow for absorption of the excess PVA). Do not eliminate this step.
   b. Using an applicator stick, apply (Do Not Smear) some of the stool material from the paper towel onto one or more clean glass slides to the edge of the slides.
   c. Dry the slides overnight at room temperature or for several hours in a slide warmer or 37°C incubator. Accelerated drying is not recommended and may cause distortion of the specimen morphology. Do not proceed until the slides are completely dry.
   d. Once the slides have dried, proceed with staining (Refer to respective stain insert sheet).

   NOTE: Slides made from very watery specimens may require an additional time to dry completely.

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. Formal in ether/ethyl acetate sedimentation:
      1. Mix the Formalin or Modified PVA 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. Add saline (or 10% 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 10% 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 10% 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).

   B. Zinc sulfate flotation
      1. Thoroughly mix a representative portion of the formalin 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.
      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.

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