

- 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.

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.

#### CAS Numbers

10% Formalin	
Formaldehyde	50-00-0
DI Water	7732-18-5
Na <sub>2</sub> HPO <sub>4</sub>	7558-79-4
NaH <sub>2</sub> PO <sub>4</sub>	7558-80-7
Modified (Cu) PVA	
Acetic acid	64-19-7
Copper Sulfate	7758-99-8
DI Water	7732-18-5
Ethanol	64-17-5
Glycerol	56-81-5
PVA	9002-89-5
Methanol	67-56-1

#### BIBLIOGRAPHY

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- Brooke MM, Norman C. The effectiveness of the PVA fixative technique in revealing intestinal amebae in diagnostic cultures. Am J Trop Med Hyg 1955;4:479-482.
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- Erdman, Dean. Clinical comparison of ethyl acetate and diethyl ether in the formalin ether sedimentation technique. J Clin Microbiol 1981;14:483-485.
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- Scholten TH, Yang J. Evaluation of unpreserved and preserved stools for the detection and identification of intestinal parasites. Am J Clin Path 1974;62:563-567.
- Young, Kirk H, et al. Ethyl acetate as a substitute for diethyl ether in the formalin ether sedimentation technique. J Clin Microbiol 1979;10:852-853.

#### Protocol Modified PVA /Formalin Vials

	Item #
Protocol Modified (Cu) PVA Vials, 20/pk	23 005 33
Protocol Modified (Cu) PVA/Formalin, 2x10 vL/pk	23 005 27
Protocol Mod. (Cu) PVA/Formalin/Clean 3x10 vL/pk	23 005 30

#### Other Protocol Parasitology Products

	Item #
Protocol LV-PVA Vials, 20/pk	23 005 29
Protocol SAF Vials, 20/pk	23 005 41
Protocol Clean Vials, 20/pk	23 005 31
Protocol 10% Buff. Formalin Vials, 20/pk	23 005 46
Protocol C & S Vials, 20/pk	23 005 43
Protocol MIF Vials, 20/pk	23 005 35
Protocol Cary Blair Vials, 20/pk	23 005 47
Protocol Zn-PVA Vials, 20/pk	23 005 37
Protocol LV-PVA Formalin Kit, 2x10 vL/pk	23 005 23
Protocol SAF / Clean Kit, 2x10 vL/pk	23 005 44
Protocol LV-PVA/ Clean kit, 2x1 0 vL/pk	23 005 25
Protocol SAF / C&S Kit, 2x1 0 vL/pk	23 005 39
Protocol Zn-PVA / 10%Formalin Kit, 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 SAF/Clean/C&S, 3x10 vL/pk	23 005 26
Protocol Zn-PVA/ Formalin/ Clean, 3x10 vL/pk	23 005 28
Protocol Zn-PVA/ Formalin/ C&S, 3x1 0 vL/pk	23 005 32
Protocol MIF Kit	23 005 34
Protocol MIF Bulk Kit	23 005 36
Protocol Trichrome, 500 mL	23 005 38
Protocol Zn-PVA Bulk, 500 mL	23 005 40
Protocol LV-PVA Bulk, 500 mL	23 005 49
Protocol Bulk Tubes and caps, 15 mL	23 005 42
Protocol Iodine, 50 mL	23 005 48
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
Protocol Ethyl Acetate	23 005 68

840558 (R0)

# Protocol™

## Parasitology System

### Application for Modified PVA and Formalin Vial Systems

#### 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.

#### EXPLANATION

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. The two vial method, PVA & 10% formalin, has been demonstrated as an effective transport and fixative technique (2,3,4,6). Proper use of the Protocol Parasitology System assures the parasitologist that diagnostic stages of intestinal parasites, if present, will be preserved. Modified PVA is a copper sulfate solution that acts as a preservative/fixative for protozoan trophozoites. Slides may be stained using trichrome or hematoxylin. Formalin is an all purpose fixative that is appropriate for helminth eggs and larvae and for protozoan cysts. The buffering system of Protocol 10% formalin helps maintain organism morphology. Formalin preserved specimens may be examined directly or concentrated for recovery of eggs, larvae and protozoan cysts.

#### CONTENTS:

Protocol Modified (Cu) PVA Vials (23-005-33) contain twenty 15 mL vials of Modified (Cu) PVA fixative (cupric sulfate). Protocol Modified (Cu) PVA/Formalin Kits (23-005-27) contain ten kits each consisting of one 15 mL vial of Modified (Cu) PVA fixative and one 15 mL vial of 10% formalin. Protocol Modified (Cu) PVA/Formalin/Clean Kits (23-005-30) contain ten kits each consisting of one 15 mL vial of Modified (Cu) PVA fixative, one 15 mL vial of 10% formalin and one clean vial. Each vial has a built in collection spoon. 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 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 material 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).

8. 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 resulting slides are too dense (newsprint should be legible through them) more saline or 10% 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 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.