12. Place the coverslip on a slide. It desired, iodine may be added to the wet mount for better detail. The edges may be sealed with a petroleum jelly/patatin mixture to prevent drying of the specimen.

*Alternate Method - directly after step 5, 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 "tissue", simply touch the surface with the loop portion. Proceed with examination.

PRECAUTIONS

Avoid contact of tissue 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 tissue 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 1-800-520-5454 between the hours of 8 AM and 5 PM, Eastern Standard Time.

CAS Numbers

10% Formalin
Formaldehyde 50-00-0
DI Water 7732-18-5
Na2HP04 7558-79-4
NaH2PO4 7558-50-7
LV-PVA
Glyceral 56-81-5
Ethyl Alcohol 64-17-5
Isopropyl Alcohol 67-63-0
Methyl Alcohol 67-56-1
Mercuro Chloride 7487-94-7

BIBLIOGRAPHY


Protocol LV-PVA/Formalin Vials

Item #
Protocol LV-PVA Vials, 29pk 23-905-29
Protocol LV-PVA Clean, 2x10 v/f 23-905-25
Protocol LV-PVA/Formalin 2x10 v/f 23-905-23
Protocol LV-PVA/Formalin/CAS, 2x10 v/f 23-905-22
Protocol LV-PVA/Formalin/CAS, 3x10 v/f 23-905-24

Other Protocol Parasitology Products

Item #
Protocol SAF Vials, 2x10 23-905-41
Protocol Clean Vials, 2x10 23-905-31
Protocol 10% Buff. Formalin Vials, 20/pk 23-905-46
Protocol Modified (Cu) PVA Vials, 20/pk 23-905-33
Protocol CAS Vials, 20/pk 23-905-43
Protocol MF Vials, 20/pk 23-905-35
Protocol Cary Blair Vials, 20/pk 23-905-37
Protocol Zip PVA Vials, 20/pk 23-905-39
Protocol SAF/Clean, 2x10 v/f 23-905-44
Protocol SAF/CAS, 2x10 v/f 23-905-39
Protocol Modified (Cu) PVA/Formalin, 2x10 v/f 23-905-27
Protocol Zip-PVA 10%Formalin, 2x10 v/f 23-905-45
Protocol SAF/Clean/CAS, 2x10 v/f 23-905-26
Protocol Mod. Cu PVA/Formalin/Cas 3x10 23-905-30
Protocol Zip-PVA/CAS, 2x10 v/f 23-905-28
Protocol Zip-PVA/CAS, 3x10 v/f 23-905-32
Protocol MF Kit 23-905-34
Protocol MF Bulk Kit, 50 ea. 23-905-36
Protocol Thickene, 500 mL 23-905-46
Protocol LV-PVA Bulb, 500 mL 23-905-49
Protocol Zip-PVA Bulk, 500 mL 23-905-40
Protocol Bulb Tubes and caps, 15 mL 23-905-42
Protocol Iodine, 56 mL 23-905-48
Protocol 50 mL Concentration System, 120 ea. 23-905-50
Protocol 15 mL Concentration System, 50 ea. 23-905-61
Protocol 15 mL Concentration System, 4x20 ea. 23-905-52
Protocol Ethyl Alcohol 23-905-68

PURITY:
Protocol Parasitology Systems provide standardized procedures for the routine collection, transportation, preservation and examination of stool specimens for intestinal parasitism. 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. The two-vial method, PVA & 10% formalin, has been demonstrated as an effective transport and fixation technique (2,3,4,8). Proper use of the Protocol Parasitology System assures the parasitologist that diagnostic stages of intestinal parasites, if present, will be preserved. Low viscosity PVA 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 hernhing eggs and larvae and for protozoan cysts. The buffering system of Protein 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 LV-PVA Vials (23-005-29) contain twenty 15 mL vials of LV-PVA fixative. Protocol LV-PVA/Clean Kits (23-005-23) contain ten kits each containing one 15 mL vial of LV-PVA fixative and one Clean vial. Protocol LV-PVA/Formalin Kits (23-005-26) contain ten kits each consisting of one 15 mL vial of LV-PVA fixative and one 15 mL vial of 10% formalin. Protocol LV-PVA/Formalin/Clean Kits (23-005-22) contain ten kits each consisting of one 15 mL vial of LV-PVA fixative, one 15 mL vial of 10% formalin and one Clean vial. Protocol LV-PVA/Formalin/CAS Kits (23-005-24) contain ten kits each consisting of one 15 mL vial of LV-PVA fixative, one 15 mL vial of 10% formalin and one 16mL vial of CAS medium. Each vial has a built-in collection spoon. Directions for use are also included.
3. Permanent slides for staining trichrome or ironhematoxylin:
   a. Allow specimen to fix for at least 30 minutes in 1X-PVA.
   MiX thoroughly with two applicator sticks. Pour a small amount of the 1X-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 soil 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 at 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 waxy or waxy-sandy specimens may require 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 potasDoc1070829192can organisms and nanoflagellates from local larvae by centrifugation and differences in specific gravity. Each procedure may vary in respect to the efficiency with which it recovers specific organisms. No one concentration procedure works equally well for all parasites and laboratory personal should consult available literature for additional information. The following are two common procedures:
   A. Formalin-fixed ethanol acetone sedimentation:
      1. Mix the formalin for PVA fixed specimen thoroughly. The specimen is now ready for processing with the protocol described in the protocol. See the appropriate package insert for further details.
      2. If the protocol described in the protocol is not available, strain a sufficient quantity through a layer of wet mesh gauze to proceed step 3. This will vary with the size and density of the specimen.
   B. Add saline (or 10% formalin) almost to the top of the tube, mix completely and centrifuge at 500 g x 10 minutes (1800-2000 rpm). If the resulting precipitate is not 0.5-1 ml, reprocess, add or remove specimen and reorient the centrifuge.
   C. Decant the supernatant fluid. A second wash may be used if necessary.
   D. Add approx. 10 ml of 10% formalin (fill the tube half full only), reprocess 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 to step 6; merely add the 10% formalin, spin, decant, and examine the remaining sediment.
   E. Add 3-5 ml of ethyl acetate or ethanol, tighten the cap and shake vigorously for at least 30 seconds.
   F. Centrifuge at 500 x g for 10 minutes (1800-2000 rpm).

8. The resulting solution will separate into four layers:
   a. Top layer: ethyl acetate or ethanol
   b. Second layer: plug of debris
   c. Third layer: formalin
   d. Bottom layer: sediment.

9. After removing the plug of debris from the sides 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 solidified 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 (newspaper 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 fixed formalin stained suspension or fresh specimen in a 15 ml centrifuge tube and fill to top 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 (1800-2000 rpm).
   3. Sediment should be 0.5 - 1 ml. If needed, adjust the suspension by adding 10% 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 with half full with zinc sulfate solution and resuspend the sediment by rocking 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 (1800-2000 rpm).
   7. Carefully remove the tube and place upright in a fast 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 disturbing 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 fluid is contained on the coverslip. This drop will contain cysts or eggs if they are present in the specimen.