

- Swabbing centrifuge tube: Failure to swab the sides of the centrifuge tube after decanting the ethyl acetate, fecal debris plug and aqueous layer can result in a poor wet mount preparation. Sediment should appear dry and gritty. Sediment When the Concentrate procedure is followed correctly, the sediment will appear dry and gritty.

#### PRECAUTIONS

CAUTION: Ethyl acetate is flammable. Perform all procedures in a well ventilated area. Avoid open flames and ignition devices. Avoid contact with the skin or eyes. Should contact occur, call a physician immediately. Flush with plenty of water. Avoid breathing fumes. DO NOT DRINK. If ingested, call a physician immediately. This product is for in vitro diagnostic use only by trained, qualified personnel. Occupational Safety and Health Act regulations (including Universal Precautions) should be used for handling all specimens.

Concentration of fecal specimens for parasitic examination is only one part of a complete examination. See appropriate literature for additional tests and procedures.

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

<b>Surfactant</b>	9002-93-1
DI Water	7732-18-5

#### BIBLIOGRAPHY

- Ash US, Orihel TC, Parasites: a guide to laboratory procedures and identification. Chicago:ASCP Press, 1991:23-28.
- American Society of Parasitologist. Procedures Suggested for Use in Examination of Clinical Specimens to Parasitic Infection. J Parasitol 1977;63:959-960.
- ASMT. Recommended Procedures for the Examination of Clinical Specimens Submitted for the Diagnosis of Parasitic Infections. Am J Clin Pathol 1978;44:1101-1106.
- Brook MM, Goldman M. Polyvinyl alcohol fixative as a preservative and adhesive for protozoa in dysenteric stools and other liquid materials. J Lab and Clin Med 1944;34:1554-1560.
- Burrows RB. Microscopic Diagnosis of the Parasites of Man, New Haven: Yale University Press, 1965.
- Carroll MJ, Cook J, Turner JA. Comparison of polyvinyl alcohol and formalin-preserved fecal specimens in the formalin-ether sedimentation technique for parasitological examination. J Clin Microbiol 1983;18:1337-1341.
- Erdman, Dean. Clinical comparison of ethyl acetate and diethyl ether in the formalin-ether sedimentation technique. J Clin Microbiol 1981;14:483-485.
- Garcia LS, Brewer TS, Bruckner DA, Shimizu RY. Clinical Laboratory Diagnosis of Cryptosporidium from

Human Fecal Specimens. Clin Microbiol News 1982;4:136-137.

- Garcia LS, Shimizu R. Comparison of Clinical Results for the use of Ethyl Acetate and Diethyl Ether in the Formalin-Ether Sedimentation Technique Performed on Polyvinyl Alcohol Preserved Specimens. J Clin Microbiol 1981;13:709-713.
- Garcia MS, Bruckner D. Diagnostic Medical Parasitology, 3rd ed. Washington D.C.: ASM Press, 1997: 594-822.
- Horen, WP. Modification of Schaudin's fixative. J Clin Microbiol 1981;13:204-205.
- Melvin DM, Brooke MM. Laboratory Procedures for the Diagnosis of Intestinal Parasites. Atlanta: U.S.H.H.S.(CDC), 1982;82-8282.
- 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 50 mL Concentrate System

Protocol 50 mL Concentration System, 120 ea. 23-005-50

#### Other Protocol Parasitology Products

	Item #
Protocol LV-PVA Vials, 20/pk	23-005-29
Protocol Clean Vials, 20/pk	23-005-31
Protocol Modified (Cu) PVA Vials, 20/pk	23-005-33
Protocol MF Vials, 20/pk	23-005-35
Protocol Zn PVA Vials, 20/pk	23-005-37
Protocol SAF Vials, 20/pk	23-005-41
Protocol C&S Vials 20/pk	23-005-43
Protocol 10% Buff. Formalin Vials, 20/pk	23-005-46
Protocol Cary Blair Vials, 20/pk	23-005-47
Protocol LV-PVA/Formalin Kit, 2x10 vI/pk	23-005-23
Protocol LV-PVA / Clean Kit, 2x10 vI/pk	23-005-25
Protocol Modified (Cu) PVA/Formalin, 2x10 vI/pk	23-005-27
Protocol SAF / C&S Kit, 2x10 vI/pk	23-005-39
Protocol SAP / Clean Kit, 2x10 vI/pk	23-005-44
Protocol Zn-PVA/ 10%Formalin Kits, 2x10 vI/pk	23-005-45
Protocol PVA/ Formalin/Clean, 3x10 vI/pk	23-005-22
Protocol LV-PVA/Formalin/C&S, 3x10 vI/pk	23-005-24
Protocol SAF/Clean/C&S, 3x10 vI/pk	23-005-26
Protocol Zn-PVA/ Formalin/ Clean, 3x10 vI/pk	23-005-28
Protocol Mod. (Cu) PVA/ Formalin/Clean, 3x10	23-005-30
Protocol Zn-PVA/ Formalin/ C&S, 3x10 vI/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 LV-PVA Bulk, 500 mL	23-005-49
Protocol Zn-PVA Bulk, 500 mL	23-005-40
Protocol Bulk Tubes and caps, 15 mL	23-005-42
Protocol Iodine, 50 mL	23-005-48
Protocol 15 mL Concentration System, 50 ea.	23-005-51
Protocol 1 5 mL Concentration System, 450 ea.	23-005-52
Protocol Ethyl Acetate	23-005-68

840561 (R0)

# Protocol™

## Parasitology System

### 50 mL Concentrate System

#### PURPOSE:

The Protocol 50 mL Concentrate System is a complete system for concentrating and recovering helminth eggs, larvae and protozoan cysts from stool specimens. The Protocol 50 mL Concentrate System creates a closed system that minimizes exposure to infectious agents and fits with any of the Protocol system vials to insure complete usage of available specimen.

#### EXPLANATION

Diagnosis of enteric parasitic disease is confirmed by" isolation and identification of pathogenic organisms in stool specimens. When small numbers of organisms are present, large volumes of stool are required to insure recovery and identification of all intestinal parasites. The convenient 50 mL Concentrate system allows for complete usage of the specimen in the Protocol collection vial while the closed system minimizes exposure to potential infectious agents.

Concentration of stool specimens is an integral part of a complete examination. Concentration is essential to separate protozoan organisms and helminth eggs and larvae from fecal debris. Proper use of the Protocol Parasitology 50 mL Concentrate System optimizes isolation and identification while offering an easy to use processing system that minimizes specimen handling.

#### CONTENTS:

Each Kit contains:

- 120 conical centrifuge tubes
- 120 filtration units
- 120 screw caps
- 55 mL surfactant

Directions for use are also included.

## MATERIALS NOT PROVIDED

Ethyl acetate  
Cotton tipped applicator sticks  
Microscope slides and coverslips  
Centrifuge  
Transfer pipettes  
Physiological saline or 10% formalin  
10% buffered formalin  
Microscope

## SPECIMEN COLLECTION

Specimens collected and transported in 10% formalin, PVA\*, SAF, MIF or Clean vials may be used with the Protocol 50 mL Concentrate System. Patients should be properly instructed on collection and handling of stool specimens. Refer to Protocol Parasitology System applications of the above items for appropriate guidelines.

\*Although it is possible to perform concentration procedures on PVA fixed specimens, eggs and cysts have been demonstrated to be more abundant when concentrated from formalin fixed feces (6).

## SPECIMEN PROCESSING

### **Unpreserved Specimens:**

Unpreserved specimens must be processed immediately to insure recovery of organisms. Liquid specimens should be examined within 30 minutes of passage, soft or formed specimens should be examined within 1 hour of passage, if this time frame is not possible, preservatives should be used. For optimum results, it is recommended that specimens be preserved at the time of collection. Unpreserved specimens delayed in transport may have limited diagnostic value.

- a. Transfer 4-6 grams of unpreserved stool into a Protocol clean vial and add 10-15 mL of 10% formalin. Mix thoroughly until mixture is uniform. Allow specimen to fix for at least 30 minutes.
- b. Follow directions for processing formalin preserved specimens.

### **Formalin, MAP or PVA preserved specimens:**

Allow the specimen to fix for at least 30 minutes. Specimen mixture should be maintained at room temperature.

- a. Mix the fixed specimen thoroughly. (It is recommended that you use Protocol 10% formalin or MIF vials and follow the instructions on proper specimen collection.) Approximately 2-4 gm of stool in 15 mL of fixative is usually sufficient.
- b. PVA preserved specimens: If a permanent stain is desired, it is recommended to remove a portion of the fixed specimen prior to concentration procedure.
- c. Add 8-10 drops of surfactant to the fixed specimen. (Additional surfactant may be added if the specimen is highly mucoid.) Cap and mix the contents thoroughly by shaking vigorously for at least 1 minute.
- d. Remove the cap from the specimen vial and place concentrate filter unit with conical tube on top of the specimen vial. Screw filter unit clockwise until securely tightened to vial.

- e. Invert concentrate system to allow the fecal suspension to flow through the filtering device into the centrifuge tube. If specimen is thick tapping the unit may facilitate filtering.
- f. Once filtration is complete, discard specimen vial and filtering device. Add 10 mL physiological saline to the 50 mL centrifuge tube, cap with the lid provided and centrifuge for 10 minutes at 500 x g (1800-2200 rpm).
- g. Decant the supernatant fluid. Approx. 0.5 - 1 mL of sediment should be present. A portion of the sediment may be used for detection of Cryptosporidium. Consult available literature for proper preparation and examination.
- h. Add 10% formalin to the 25 mL mark as indicated on the label. Resuspend the sediment. Allow mixture to stand for at least 5 minutes before proceeding.
- i. Add approximately 5 mL of ethyl acetate, cap the tube and shake vigorously for 30 seconds. CAUTION: Pressure may build up within the tube during shaking. Carefully release the pressure by opening the cap slowly, while pointing the end away from you.
  - l. Centrifuge the tube for 10 minutes at 500 x g (1800-2200 rpm). Pour off the supernatant fluid from the tube and keep inverted.
- k. While the centrifuge tube is inverted, clean the sides of the tube with cotton-tipped applicator sticks to remove debris and any remaining fluid. IMPORTANT: Clean the sides of the tube thoroughly with cotton swabs, before turning the tube upright. Allowing excess fluid to run into sediment will cause poor wet mount.
- l. Transfer a portion of the sediment to a clean glass microscope slide and prepare the mount of choice. Examine and record results.

### **SAP preserved specimens**

Allow the specimen to fix for at least 30 minutes. Specimen mixture should be maintained at room temperature.

- a. Mix the fixed specimen thoroughly. (It is recommended that you use Protocol SAF vials and follow the instructions on proper specimen collection.) Approximately 2-4 gm of stool in 15 mL of fixative is usually sufficient.
- b. If a permanent stain is desired, it is recommended to remove a portion of the fixed specimen prior to concentration procedure.
- c. Add 8-10 drops of surfactant to the fixed specimen. (Additional surfactant may be added if the specimen is highly mucoid.) Cap and mix the contents thoroughly by shaking vigorously for at least 1 minute.
- d. Remove the cap from the specimen vial and place concentrate filter unit with conical tube on top of the specimen vial. Screw filter unit clockwise until securely tightened to vial.
- e. Invert concentrate system to allow the fecal suspension to flow through the filtering device into the centrifuge tube. If specimen is thick tapping the unit may facilitate filtering.
- f. Once filtration is complete, discard specimen vial and filtering device. Add 10 mL physiological saline to the 50 mL centrifuge tube, cap with the lid provided and centrifuge for 10 minutes at 500 x g (1800-2200 rpm). Decant the supernatant fluid.

- g. Prepare a smear for permanent staining as follows: Place 1 drop of Mayer's Albumin on the slide and wipe so that only a thin layer remains on the slide. Add 1 drop of the sediment to the slide and allow the smear to air dry at room temperature for 30 minutes prior to staining.
- h. 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.
- i. Resume processing: Add 10% formalin to the 25 mL mark as indicated on the label. Resuspend the sediment. Allow mixture to stand for at least 5 minutes before proceeding.
- l. Add approximately 5 mL of ethyl acetate, cap the tube and shake vigorously for 30 seconds. CAUTION: Pressure may build up within the tube during shaking. Carefully release the pressure by opening the cap slowly, while pointing the end away from you.
- k. Centrifuge the tube for 10 minutes at 500 x g (1800-2200 rpm). Pour off the supernatant fluid from the tube and keep inverted.
- l. While the centrifuge tube is inverted, clean the sides of the tube with cotton-tipped applicator sticks to remove debris and any remaining fluid. IMPORTANT: Clean the sides of the tube thoroughly with cotton swabs, before turning the tube upright. Allowing excess fluid to run into sediment will cause poor wet mount.
- m. Transfer a portion of the sediment to a clean glass microscope slide and prepare the mount of choice. Examine and record results.

## PERFORMANCE SUGGESTIONS:

Experience will dictate appropriate techniques and volumes to assure an adequate sediment for proper microscopic examination. The following is a list of recommendations to maximize your technique:

1. A sediment of 0.5 - 1 mL is optimum. When filtering the fixed specimen, the stool/fixative mixture density determines the necessary volume. If the suspension is dense, 3 mL should be sufficient. In less dense stool mixes, larger volumes of the filtrate are necessary. (Up to 10 - 12 mL of suspension). Attention to detail and experience will provide the anticipated results.
2. Do not force dense fecal matter through the filter device. If vegetative matter is present, remove by gently running an applicator stick across the screen. Allow the suspension to filter naturally. The problem is minimized by adding the recommended amount of surfactant and thoroughly mixing the specimen/fixative mixture.

Fisher Diagnostics

a division of Fisher Scientific Company, LLC

a subsidiary of Thermo Fisher Scientific Inc.

Middletown, VA 22645-1905 USA

Phone: 1-800-528-0494

1-540-869-3200

Fax: 1-540-869-8132

**Thermo**  
SCIENTIFIC