I. Transfer a portion of the sediment to a dean glass microscope slide and prepare the mount of choice. Examine and record results.

1. 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 - I mL is optimum. When filtering thefixed 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, a larger volume of the filtrate is 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 the applicator stick across the screen. Allow the suspension to filter naturally. Filtering problems may be minimized by adding the recommended amount of Reagent A and thoroughly mixing the specimen/fixative mixture.
3. Swabbing centrifuge tube: Failure to swab the sides of the centrifuge tube after decanting the Reagent B, fecal debris plug and aqueous layer can result in a poor wet mount preparation. Sediment: When the Concentrate procedure is followed correctly, the sediment will appear dry and gritty.

PRECAUTIONS

CAUTION: Protocol Concentrate System Reagent B 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 AM. and 5 P.M. Eastern Standard Time.

CAS Numbers

Reagent A		Reagent B	
Surfactant	9002-93-1	Ethyl acetate	141-78-6
Dl Water	7732-18-5		

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Protocol 15 mL Concentrate System	Item #
Protocol 15 mL Concentration System, 50 ea.	23-005-51
Protocol 15 mL Concentration System, 450 ea.	23-005-52

Other Protocol Parasitology Products	Item #
Protocol Trichrome, 500 mL	23-005-38
Protocol LV-PVA Bulk, 500 mL	23-005-49
Protocol Iodine, 50 mL	23-005-48
Protocol Ethyl Acetate	23-005-68

840562 (RO)

Protocol™

Parasitology System

15 mL Concentrate System

PURPOSE:

The Protocol 15 mL Concentrate System is a complete system for concentrating and recovering helminth eggs, larvae and protozoan cysts from stool specimens.

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.

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 15 mL Concentrate System optimizes isolation and identification while offering an easy to use processing system that minimizes specimen handling.

CONTENTS:

Each Kit contains:

- 50 filtering devices
- 50 disposable centrifuge tubes with caps
- Reagent A : 15 mL dropper bottle
- Reagent B : 225 mL

Directions for use are also included.

MATERIALS NOT PROVIDED

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

SPECIMEN COLLECTION

Specimens collected and transported in 10% formalin, SAF, MIF. PVAt or Clean vials may be used with theProtocol 15 rnL 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

1. ***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 I hour of passage, if this time frame is not possible, preservatives should be used. For optimal 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 46 grams of unpreserved stool into 10-15 mL of physiological saline. Mix thoroughly until mixture is uniform.
b. Add 4 drops of Concentrate Reagent A to the mixture. (Additional Reagent A may be added if the specimen is highly mucoid.) Cap and mix the contents thoroughly by shaking.
c. Insert one of the Concentrate filtering devices into the top of one of the disposable centrifuge tubes provided. Pour fecal suspension through the filtering device into the centrifuge tube. Usually 3-5 mL in the centrifuge tube is sufficient.
d. Discard filtering device, add 10 mL physiological saline and centrifuge for 10 minutes at 500 x g (1600-2200 rpm). 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.
e. Resuspend the sediment in 10 mL 10% formalin. Allow mixture to stand for at least 5 minutes before proceeding.
f. Add approximately 3 mL of Concentrate Reagent B, 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.
g. Centrifuge the tube for 10 minutes at 500 x g (1800-2200 rpm).
h. The resulting solution will separate into four layers:
   - Top layer. Reagent B
   - Second layer: plug of debris
   - Third layer: aqueous solution
   - Bottom layer: sediment
i. 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. IMPORTANT: Clean the sides of the tube thoroughly with cotton swabs, before turning the tube upright.
j. Transfer a portion of the sediment to a clean glass microscope slide and prepare the mount of choice. Examine and record results.

2. ***Formalin, MIF 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 4 drops of Concentrate Reagent A to the fixed specimen. (Additional Reagent A may be added if the specimen is highly mucoid.) Cap and mix the contents thoroughly by shaking.
d. Insert one of the Concentrate filtering devices into the top of one of the disposable centrifuge tubes provided. Pour fecal suspension through the filtering device into the centrifuge tube. Usually 3-5 mL in the centrifuge tube is sufficient.
e. Discard filtering device, add 10 mL physiological saline and centrifuge for 10 minutes at 500 x g (1800-2200 rpm).
f. Decant the supernatant fluid. Approx. 0.5 - I 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.
g. Resuspend the sediment in 10 mL 10% formalin. Allow mixture to stand for at least 5 minutes before proceeding.
h. Add approximately 3 mL of Concentrate Reagent B, 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.
i. Centrifuge the tube for ‘10 minutes at 500 x g (1800- 2200 rpm).
j. The resulting solution will separate into four layers:
   - Top layer. Reagent B
   - Second layer: plug of debris
   - Third layer: aqueous solution
   - Bottom layer sediment
k. 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. **IMPORTANT: Clean the sides of the tube thoroughly with cotton swabs, before turning the tube upright.**
l. Transfer a portion of the sediment to a clean glass microscope slide and prepare the mount of choice. Examine and record results.

3. ***SAF 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. Add 4 drops of Concentrate Reagent A to the fixed specimen. (Additional Reagent A may be added if the specimen is highly mucoid.) Cap and mix the contents thoroughly by shaking.
c. Insert one of the Concentrate filtering devices into the top of one of the disposable centrifuge tubes provided. Pour fecal suspension through the filtering device into the centrifuge tube. Usually 3-5 mL in the centrifuge tube is sufficient
d. Discard filtering device, add 10 mL physiological saline and centrifuge for ‘10 minutes at 500 x g (1800-2200 rpm).
e. Decant the supernatant fluid. Approx. 0.5 - I 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.
f. If permanent stained slide is desired: Place I drop of Mayers Albumin on the slide and wipe so that only a thin layer remains on the slide. Add I drop of the sediment to the slide and allow the smear to air dry at room temperature for 30 minutes. Proceed with staining (Refer to respective stain insert sheet).
g. Resuspend the sediment in 10 mL 10% farmalin. Allow mixture to stand for at least 5 minutes before proceeding.
h. Add approximately 3 mL of Concentrate Reagent B, 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.
I. Centrifuge the tube for 10 minutes at 500 x g (1800-2200 rpm).
j. The resulting solution will separate into four layers: Top layer: Reagent B . Second layer: plug of debris ‘Third layer: aqueous solution ‘Bottom layer, sediment
k. 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. IMPORTANT: Clean the sides of the tube thoroughly with cotton swabs, before turning the tube upright.

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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