KIT CONTENTS

100 vial (15 ml) UNIFIX™

1 10-language instruction sheet

MATERIALS NOT PROVIDED

ethyl acetate, saline

applicator sticks and cotton tipped applicator

transfer pipets

concentration system (Sed-Connect™, Para-Sed™, Micro-Sed™)

centrifuge

microscope, slides and cover slips

trichrome reagents

5% formalin, 10% formalin or physiological saline

COLLECTION

1. Collection of fecal specimens for intestinal parasites should always be performed prior to the use of any antacids, barium, bismuth, antidiarrheal medication, or oily laxatives.

2. Routine examination for parasites prior to treatment, a minimum of three specimens, collected on alternate days, is recommended. Two of the specimens should be collected after normal movements, and one after a cathartic, such as magnesium sulfate or fleet Phospho-Soda. If the patient has diarrhea, do not use a laxative.

3. Fecal specimens should be collected in a clean, dry wide mouthed container; a bedpan is ideal. However a waxed, cardboard half-pint container with a tight-fitting lid, a clean, dry milk carton with the top two thirds removed or a plastic bag or plastic wrap placed over the toilet seat opening is acceptable. Contamination with urine should be avoided.

4. Small samples of the specimen should be placed into the vial using the spork built into the lid of the vial. Pay particular attention to the areas that appear bloody or contain a lot of mucus. Add samples until the fluid level reaches the red “fill line”. This will insure the appropriate three to one ratio of fixative to sample.

5. Use the spork to thoroughly stir and mix the stool with fixative. Recap the vial, making sure the lid is securely fastened. Firmly shake the vial until contents are thoroughly mixed (the solution should appear homogeneous).

6. Fill out the patient information on the side of each vial. Reseal the vials in the plastic bag. **Caution**: Every sample should be treated as a potential source of contamination.

EXAMINATION

The use of UNIFIX allows for a wide variety of examination procedures including gross examination (only if the kit contains a clean vial), direct microscopic examination, permanent staining, and concentration procedures.

Macroscopic examination

Examine the contents of the clean vial (unpreserved specimen) and record the consistency of the specimen, the presence of worms or proglottids, and blood if any.

Microscopic examination

Direct smears from UNIFIX preserved specimen. Prepare the smear by mixing a small amount of preserved fecal material (approximately 2 mg) with a drop of physiologic saline on a glass slide. Cover with a 22 by 22 mm coverslip. Examine the entire coverslip immediately using the low powered objective. Suspect objects may be examined under high-dry power. Since the fecal material has been preserved, there will be no organism mobility visible (the purpose of the direct wet mount). With preserved material, the routine Ova and Parasite examination can begin with the fecal concentration rather than the direct wet mount.

Permanent stain and concentration procedure using MCC’s Para-Sed (50 ml) and Sed-Connect (15 ml)

Mix contents of the UNIFIX vial thoroughly.

1. Remove the cap from the UNIFIX vial and add 8-10 drops of surfactant. Recap the vial making sure the lid is securely fastened.

2. Mix the contents of the vial by shaking vigorously, or vortexing for 30 seconds.

3. With the 50 ml centrifuge vial or 15 ml vial still loosely attached to the filter unit (loose attachment will facilitate the release of air pressure during use), insert the open end of the filter unit into the specimen vial until the sealing ring is firmly seated. Tighten the 15 ml or 50 ml centrifuge vial onto the filter unit.

4. Invert the tube and filter the specimen through the mesh into the 15 ml or 50 ml centrifuge tube. If the flow does not start immediately, or the specimen is thick, the flow may be initiated by sharply tapping the centrifuge tube on a counter top.

5. After filtration is complete, tap the centrifuge tube on the counter top 2 or 3 times to insure that all the fluid (Par-Sed) or 3-5 ml of material (Sed-Connect) has drained into the tube. Tilt the filter unit at a slight angle. Unscrew the concentrator unit and specimen vial and discard using established laboratory procedures for fecal specimens.

6. Place the screw cap on the 50 ml centrifuge tube or push cap on the 15 ml centrifuge tube and centrifuge for 10 min at 500xg (1800-2000 rpm for most table top centrifuges).

7. Decant. Mix the remaining UNIFIX preserved sediment with a applicator stick.

8. Prepare a slide for permanent staining by adding a small sample of the suspended sediment to the slide. The sediment can also be used to prepare smears for special staining (modified acid-fast for coccidia or modified trichrome for the microsporidia).

9. Spread the sample over the slide to prepare a thin smear which varies in thickness. Allow to dry overnight at room temperature or for several hours (minimum of 30min; 60 min if slide is thicker) in a 37°C incubator or slide warmer (smear will appear opaque when dry). Do not use a heating block ; the temperature will be detrimental to any organisms present.

10. Proceed with staining regimen of choice. We recommend Wheatley’s Gomori Trichrome stain although iron hematoxylin may also be used.

Staining Procedure

1. Place in Trichrome stain 6-10 minutes.

2. Dip twice in 90% alcohol with 0.5% acetic acid. If the slide appears pale, substitute 90% alcohol without acid or stain longer.

3. Place in two changes of 100% alcohol for 2 to 5 minutes.

4. Place in two changes of xylene or xylene substitute for 5 to 10 minutes.

Concentration Procedure using MCC Para-Sed #695A

1. To the remaining sediment from step 8 above add saline (5% or 10% formalin may be used instead) to bring the level of the filtered sediment to the fill line on the Para-Sed centrifuge tube.

2. Add approximately 3 ml-5 ml of ethyl acetate (or other ether substitute) and recap the tube with cap provided with the kit.

3. Hold the tube so that is directed away from your face and shake vigorously for 30 seconds. If diethyl ether is used (not recommended) pressure may build up during shaking, and the cap should be carefully loosened after shaking to release the pressure and then retightened.

4. Centrifuge at 500Xg for 10 min.

5. Carefully remove the stopper. The resulting solution should have four layers:

Top: ethyl acetate or ethyl ether

Second: debris plug

Third: saline (or formalin)

Fourth: sediment

6. Ring the debris layer with an applicator stick to loosen the debris. Invert the tube to pour off the supernatant fluid and debris layer. While tube is still inverted use a cotton tipped applicator to clean the sides of the tube, making certain to remove any ethyl acetate or debris left behind. Failure to remove the excess ethyl acetate may result in the formation of solvent bubbles in the wet mount. The sediment at the bottom of the tube will contain the parasites.

7. Resuspend the remaining sediment with a few drops of 5% or 10% formalin or saline.

8. To prepare a wet mount, draw a sample from the resuspended material with a capillary or transfer pipette. Place one or two drops on a microscope slide and cover with a coverslip. Examine immediately.

9. If an iodine mount is preferred, place one drop of Lugol’s iodine on a slide, and one drop of the

resuspended material. Place a coverslip on the slide and examine immediately.

10. If smears will be prepared for special staining (*Cryptosporidium* spp, *Isospora belli*, *Cyclospora cayetanensis*, or the microsporidia), the remaining sediment can be used for making the smears.

If using a Sed-Connect or Micro-Sed for concentration use the following procedure.

Thoroughly mix the contents of the UNIFIX vial. Proceed with the specimen processing instructions in the Micro-Sed or Sed-Connect instruction sheet. NOTE: You may skip steps 6 & 7 if you prefer a single wash.

If neither MCC Para-Sed, Micro-Sed or Sed-Connect are available the following procedure for permanent slides and concentration may be used:

1. Mix the material in the UNIFIX vial thoroughly.

2. Strain approximately 2-3 ml of the fixed material through the gauze into a 15 ml centrifuge tube.

3. Centrifuge for ten min. at 500xg.

4. The sediment should be approximately 1 ml in volume. Decant the supernatant fluid.

5. Mix the sediment and prepare a permanent slide as described above.

6. Use the remaining sediment for your method of concentration.

PRECAUTIONS

1. Ethyl acetate and diethyl ether are flammable. Use in a well ventilated area. Keep away from direct flame. Avoid contact of the solution with skin and eyes. Should contact occur flush with running water. Avoid breathing fumes.

2. Avoid contact of UNIFIX solution with skin or eyes. If contact occurs, flush effected area with water. If irritation develops contact a physician immediately.

3. UNIFIX solution is poisonous. If ingestion occurs drink milk or water. Contact a physician immediately.

4. Every sample should be treated as a potential source of infection. Good laboratory practice should be followed at all times. The use of gloves and hand washing is recommended.

STABILITY

The expiration date of each kit is printed on the outer label. The expiration dates of each vial are printed on the individual vial label. The kits should be stored at room temperature. If the UNIFIX vials are exposed to freezing temperatures for an extended period of time they will freeze. If the vials are restored to room temperature, there will be no change in performance.

BIBLIOGRAPHY

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8. Yang, J., and Th. Scholten, 1977. A Fixative for Intestinal Parasites Permitting the Use of Concentration and Permanent Staining Procedures. Am. J. Clin. Pathol., 67:300-304.

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Micro-Sed™ (15 ml concentration system)	694A
Sed-Connect™ (15 ml, 50 ml closed system)	693A
(with ethyl acetate)	693A-E
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UNIFIX™

Stool Collection System
Catalog # 2804-05

INTENDED USE

The UNIFIX stool collection kit is a single vial system that provides a standardized method for untrained personnel to properly collect and preserve stool specimens for the detection of helminth larvae and eggs, protozoan trophozoites and cysts, coccidian oocysts, and microsporidian spores. Permanent stain and concentration may be performed from a UNIFIX preserved specimen. A ten language instruction sheet is provided to assist patients or healthcare professionals with the proper use of the kits at home or in the hospital.

SUMMARY AND EXPLANATION

The diagnosis of intestinal parasitic infection is confirmed by the recovery of helminth larvae and eggs, protozoan trophozoites and cysts, coccidian oocysts, and microsporidian spores. The ability to detect and identify intestinal parasites in fresh stool specimen depends on immediate collection, transportation, and examination by the laboratory, all of which are difficult to guarantee. The use of a stool preservative is highly recommended to preserve parasite morphology during situations where time constraints for collection, delivery and examination cannot be reasonably met.

UNIFIX is a mercury and formalin free fixative that preserves parasite morphology and helps with disposal and monitoring problems encountered by laboratories. A clean vial can be incorporated for collection of unpreserved specimens, for examination for stool fat, occult blood, enteric or amebic culture.