

KIT CONTENTS

100 vials (15 ml) Total-Fix™

1 10-language instruction sheet

MATERIALS NOT PROVIDED

Ethyl acetate

Applicator sticks and cotton tipped applicators

Transfer pipets

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

Centrifuge

Microscope, slides and cover slips

Trichrome reagents

Physiological saline

Xylene or xylene substitute

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) Stool should be in Total-Fix for at least 30 minutes for complete fixation.

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. Total-Fix preserved stool samples stored at room temperature are preferred but refrigerated samples are acceptable.

EXAMINATION

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

Macroscopic examination

Examine the contents of the clean vial (unpreserved specimen) and if present, record the consistency of the

specimen, the presence of worms or proglottids, and blood if any.

Microscopic examination

Direct smears from Total-Fix 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 Total-Fix vial thoroughly.

Remove the cap from the Total-Fix vial and add 8-10 drops of surfactant if performing a concentration wet mount. Recap the vial making sure the lid is securely fastened.

NOTE: Do not use surfactant if the sediment will be used for permanent stains or fluorescent fecal immunoassay. For optional smear preparation directly from vial allow stool to settle to the bottom of the vial. Remove fecal material directly from the bottom of the vial using a pipet and without creating bubbles. Smear this fecal material onto a glass slide for staining. Dry for 30-45 min at 37°C or 60 min. at room temperature. Proceed to the staining procedure.

Permanent stain

1. Mix the contents of the vial by shaking vigorously, or vortexing for 30 seconds.
2. 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.
3. 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.
4. 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 1-3 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.
5. 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).
6. Decant. Mix the remaining Total-Fix preserved sediment with an applicator stick.
7. 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 stains for coccidia or modified trichrome for the microsporidia).

8. 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.

NOTE: Since Total-Fix does not contain PVA (which helps the smear adhere to the slide), the user can add albumin to the slide prior to preparing the stool smear. However, if the smear is thoroughly dry before staining the albumin should not be needed.

9. 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 than 10 minutes.
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.
5. Mount with a cover slip and mounting media.

Concentration Procedure using MCC Para-Sed #695A

1. To the remaining sediment from step 8 above add saline 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 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.

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

Thoroughly mix the contents of the Total-Fix 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 Total-Fix 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 (about 1/4 inch). Decant the supernatant fluid.
5. Mix the sediment and prepare a permanent slide (trichrome, modified AFB, iron hematoxylin, modified trichrome) as described above.
6. Use the remaining sediment for your method of concentration.

Fecal Immunoassays

1. If using the enzyme immunoassay procedure (EIA), shake the stool/Total-Fix vial and allow it to stand on the counter for at least 5 min prior to taking the specimen from the top of the vial (without stool particulate material). Continue with the fecal immunoassay procedure.
2. If using the fluorescence procedure (FA), see note under "Permanent stain and concentration procedure" regarding the use of centrifuged stool (recommended). Continue with the fecal immunoassay FA procedure.
3. If using the rapid/cartridge/lateral flow procedure (rapid cartridge) shake the stool/Total-Fix vial and allow it to stand on the counter for at least 5 min prior to taking the specimen from the top of the vial (without stool particulate material). Continue with the fecal immunoassay rapid cartridge procedure.

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 Total-Fix solution with skin or eyes. If contact occurs, flush effected area with water. If irritation develops contact a physician immediately.
3. Total-Fix 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 Total-Fix 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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3. Garcia, L.S. 2016. Diagnostic Medical Parasitology, 6th ed., ASM Press, Washington, D.C.
4. Garcia, L.S. (Coordinating Editor), 2003. Selection and Use of Laboratory Procedures for Diagnosis of Parasitic Infections of the Gastrointestinal Tract, Cumitech 30A, ASM Press, Washington, D. C.
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7. Scholten, Th., 1972. An Improved Technique for the Recovery of Intestinal Protozoa. J. Parasitol. 58:603-634
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.

OTHER MEDICAL CHEMICAL PRODUCTS

C&S Medium Vials	2805-05
Clean Vials	310
Para-Sed™ (50 ml concentration system)	695A
Micro-Sed™ (15 ml concentration system)	694A
Sed-Connect™ (15 ml, 50 ml closed system)	693A
(with ethyl acetate)	693A-E
Wheatley's Gomori Trichrome	602A
Modified Trichrome Blue for Microsporidia	601A
Iron Hematoxylin 1	6185A
Iron Hematoxylin 2	6188A
D'Antoni Iodine	628A
Giemsa Stain	591A
Giemsa Buffer	592A

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Total-Fix™

Stool Collection System

Catalog # 2807-05

Patent # 8,338,130

INTENDED USE

The Total-Fix 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 stains, concentrations and most fecal immunoassays can be performed from a Total-Fix preserved specimen. Total-Fix is also an excellent DNA and RNA preservative for stool pathogen molecular assays. 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 specimens 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.

Total-Fix is a mercury, formalin and PVA 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.

