KIT CONTENTS

50 filter Funnels

50 15 ml centrifuge vials

50 vial caps

1 bottle surfactant

1 instruction sheet

ethyl acetate (optional)

MATERIALS NOT PROVIDED

Cotton tip applicators

Centrifuge

Transfer pipettes

Microscope slides and coverslips

Microscope

Physiological saline

Applicator sticks

Lugol's iodine

SPECIMEN COLLECTION

1. A well preserved specimen is critical to the detection process. The MICRO-SED is designed to be used with the Para-Fix collection kits. For collection procedures see insert provided with each kit, or the procedure sheet provided with each case.

2. For the concentration of fresh material collected in the Para-Fix clean vial, add 15 ml of 5% formalin, 10% formalin, SAF or Total-Fix to 3-5 g of sample. Mix well, and allow to stand for at least thirty minutes before processing.

3. If a permanent stained slide is going to be prepared from the SAF or Total Fix sample, remove some of the preserved material prior to concentration or the concentrated stool can be used to prepare this smear.

SPECIMEN PROCESSING

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

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

3. Place a filter funnel in one of the 15 ml centrifuge vials.

4. The approximate specimen volumes will be 3 ml for thick specimens to 10 ml for thin specimens. Filter a sufficient volume of specimen through the funnel so that 1 ml of sediment remains after initial centrifugation.

5. Do not force fecal material through the filter funnel. After filtration is complete, discard filter funnel using established laboratory procedures for fecal specimens. **If using only a single rinse, omit steps 6 and 7.**

6. Add physiological saline* to the 13 ml mark, on the centrifuge vial. Centrifuge at 500xg (1800-2500 rpm) for 10 min.

7. Decant the supernatant, retaining the fecal sediment at the bottom of the vial.

8. Add physiological saline* to bring the tube contents to ~9 ml.

9. Add 3-4 ml of ethyl acetate, Hemo De, Med-Chem Clearant or other ether substitute to the 15 ml centrifuge tube. Recap the tube with the cap provided with the kit.

10. Shake vigorously for 30 seconds. If diethyl ether is used (not recommended) pressure may build up in the vial during shaking, and the cap should be carefully loosened after shaking to release the pressure, then retightened.

11. Centrifuge at 500xg (1800-2500 rpm) for 10 min.

12. Carefully remove the cap. The resulting solution should have four layers:

Top: ethyl acetate or ethyl ether.
Second: debris plug.
Third: saline*
Fourth: sediment

13. Ring the debris layer with an applicator stick to loosen the debris.

14. Invert the tube to pour off the supernatant and debris layer. While the tube is still inverted, wipe the sides of the tube with one or two cotton tip applicators to remove 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 vial will contain the parasites.

15. Resuspend the pellet at the bottom of the tube with saline*. If using SAF, do not resuspend the sediment. Remove some sediment and prepare smears for permanent staining prior to resuspension of the remaining sediment for wet preparation examination.

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

17. 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.

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

STABILITY

The product is stable for two years from the date of manufacture, when stored at room temperature. The user should verify this by examining the concentrator unit for cracks and the surfactant for bacterial or fungal contamination.

* 5% formalin, 10% formalin, Total-Fix or SAF may be

used in place of physiological saline.

BIBLIOGRAPHY

1. ASMT, 1978. Recommended Procedures for the Examination of Clinical Specimens Submitted for the Diagnosis of Parasitic Infections. *Am. J. Med. Technol.*, 44:1101-1106.

2. Brooke, M.M., 1974 "Intestinal and Urogenital Protozoa", Manual of Clinical Microbiology, ASM Washington D.C., 2nd Edition, 582-601

3. Garcia, L.S. and Bruchner, D.A., 1997 Diagnostic Medical Parasitology, 3rd Ed.; ASM Press: Washington D.C.

4. Garcia, L.S. and R. Shimizu, 1981. 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.*, 13:709-713

5. Melvin, D.M. and M.M. Brooke, 1980. "Laboratory Procedures for the Diagnosis of Intestinal Parasites", U.S. D.S.E.W., 79:8282, CDC Atlanta, GA, 23-65

6. 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

7. Young, Kirk H., et al, 1979. Ethyl Acetate as a Substitute for Diethyl Ether in the Formalin-Ether Sedimentation Technique. *J. Clin. Microbiol.*, 10:852-853.

OTHER MEDICAL CHEMICAL PRODUCTS

	Catalog#
Total-Fix Vials	2807-05
Bulk 15 ml tubes and caps	895A-TC
Bulk 50 ml tubes and caps	896A-TC
C&S Medium Vials	2805-05
Clean Vials	310
D'Antoni Iodine	628A
Ethyl Acetate	4992
Formalin 10% Vials	575-05
Giemsa Stain	591A
Giemsa Buffer	592A
Hemo De (xylene substitute)	930E
Iron Hematoxylin 1	6185A
Iron Hematoxylin 2	6188A
Med-Chem Clearant (xylene substitute)	9350A
LV-PVA Vials	2802-05
Modified Trichrome Blue for Microsporidia	601A
SAF Vials	574-05
Wheatley's Gomori Trichrome	602A
Z-PVA Vials	2803-05

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MICRO-SED™
15 ml Fecal Concentration Kits
Catalog # 694A, 694A-E

INTENDED USE

MICRO-SED™ is a concentration system for the recovery of eggs, larvae, and protozoa from preserved fecal specimens. MICRO-SED is designed to be used with 5% or 10% formalin, SAF, PVA, Z-PVA and Total-Fix preserved material. When used with Para-Fix collection vials, MICRO-SED provides a convenient and reproducible method for detecting parasites even when present in very low numbers.

SUMMARY AND EXPLANATION

The diagnosis of parasitic infection is confirmed by the recovery of helminth larvae and eggs, protozoan trophozites and cysts, coccidian oocysts, and microsporidian spores. A concentration procedure should be performed as a routine part of a complete examination for parasites. Concentration procedures permit the detection of organisms present in small numbers, that may be missed using only the direct mount. Organisms that can generally be identified using a concentration procedure include: helminth eggs and larvae; cysts of *Giardia lamblia, Entamoeba histolytica, Entamoeba coli,* and *Iodamoeba bütschlii*; and oocysts of *Isospora belli.* The identification of other protozoa should be considered tentative, and confirmed with a permanent stained smear.