KIT CONTENTS

50 Filter funnels
50 Funnel caps
50 15ml centrifuge vials
50 vial caps
1 bottle surfactant
1 instruction sheet
ethyl acetate (optional)

MATERIALS NOT PROVIDED

ethyl acetate
cotton tip applicators
centrifuge
transfer pipettes
microscope slides and coverslips
microscope
physiological saline

SPECIMEN COLLECTION

1. A well preserved specimen is critical to the detection process. The SED-CONNECT is a closed concentration system 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 15ml of 5% formalin, 10% formalin, SAF or Total-Fix to 3-5g 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.

4. NOTE: fecal smears prepared for permanent staining from all fixatives can be prepared from the sediment obtained from centrifugation of the original specimen (no rinse steps). Permanent stains can not be prepared from the sediment once the fecal specimen has been rinsed with saline, SAF (contains formalin), and/or formalin. the organism morphology will be very poor once the specimen has been rinsed. One exception would be SAF preserved specimens, which can be used to prepare permanent stained smears even if rinsed with SAF or formalin.

SPECIMEN PROCESSING

1. Remove the cap from the specimen vial and add 8-10 drops of surfactant (optional). 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. Remove cap from the specimen vial and insert a SED-CONNECT filter funnel unit into transport vial.

4. Tilt down the SED-CONNECT device at approximately a 30 degree angle (so specimen flows into centrifuge tube) and filter a sufficient volume of specimen so that 1ml of sediment remains after centrifugation. The approximate specimen volumes to filter into the centrifuge tube will be 5 ml for thick specimens or 5-9ml of specimen for thin specimens **(Tip for watery specimens: after insertion of the Sed-Connect device, remove the centrifuge tube from the Sed-Connect funnel and pour filtered specimen directly into the centrifuge tube, this will eliminate leakage and overflow)**. To facilitate the filtering of thick specimens into the 15ml centrifuge tube gently shake or gently tap the filtration device on counter top.

5. Tilt the device back to a horizontal angle (this will stop the flow of material and also prevent leaking or dripping of the specimen) and slide off the 15ml centrifuge tube from the filter funnel unit. Plug filtration device with the caps provided and discard filter funnel unit using established laboratory procedures for fecal specimens.

6*. Add physiological saline* to the ~ 14ml mark, on the centrifuge tube and cap. Centrifuge at 500xg (1800-2500 rpm) for 10 minutes. One wash step may be eliminated to limit the possibility of losing organisms.

7. Decant the supernatant fluid, retaining the fecal sediment at the bottom of the vial. Make the smear for permanent stain from the sediment.

8. Add 5% formalin, 10% formalin, SAF, Total-Fix or saline to the remaining sediment to bring the tube contents to 8ml.

9. Add 4ml of ethyl acetate (or other ether substitute) to the 15ml 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 stopper. The resulting solution should have four layers:
Top: ethyl acetate or ethyl ether
Second: debris plug
Third: formalin
Fourth: sediment

13. Invert the tube to pour off the supernatant fluid and debris layer. While the tube is still inverted, ring 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.

14. Resuspend the pellet at the bottom of the tube with 5%formalin, 10% formalin or saline.

15. 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.

16. 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.

17. 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.

STABILITY

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

* 5% formalin, 10% formalin or SAF may be used in place of physiological saline.

BIBLIOGRAPHY

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OTHER MEDICAL CHEMICAL PRODUCTS

	Catalog#
C&S Medium Vials	2805-05
Bulk 15 ml tubes and caps	895A-TC
Bulk 50 ml tubes and caps	896A-TC
Clean Vials	310
D'Antoni Iodine	628A
Ethyl Acetate	4992
Formalin 10% Vials	575-05
Giemsa Stain	591A
Giemsa Buffer	592A
Iron Hematoxylin #1	6185A
Iron Hematoxylin #2	6188A
LV-PVA Vials	2802-05
SAF Vials	574-05
Total-Fix Vials	2807-05
Trichrome Blue Modified for Microsporidia	601A
UNIFIX™ Vials	2804-05
Wheatley's Gomori Trichrome	602A
Z-PVA Vials	2803-05

We also carry

Gram Stains
AFB Stains
Fluorescent AFB Stains
Quality Control Slides
QC Organisms

Revision date: Apr. 25, 2014

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SED-CONNECT™
15 ml Closed Concentration Kits
CAT # 693A, 693A-E

INTENDED USE

SED-CONNECTTM is a closed concentration system for the recovery of eggs, larvae, and protozoa from preserved fecal specimens. SED-CONNECT is designed to be used with 5% or 10% formalin, SAF, PVA, Z-PVA, UNIFIX or TOTAL-FIX preserved material. When used with Para-Fix collection vials, SED-CONNECT provides a closed, 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/E. dispar*, *Entamoeba coli, Iodamoeba bütschlii,* and oocysts of *Isospora belli*. The identification of other protozoa should be considered tentative, and confirmed with a permanent stained smear.

Patent numbers: 5,556,544; 377,031; and 382,963