EVALUATION OF TWO STOOL PRESERVATIVES AND TWO CONCENTRATORS FOR DETECTION OF INTESTINAL PARASITES. Rose H, Schneider S, Rosenblatt J. Division of Clinical Microbiology, Mayo Clinic, Rochester, MN.

ABSTRACT

ECOFIX (EFX), (Meridian Diagnostics) is a formalin-free and mercury-free, alcohol based stool preservative for use with microscopy of concentrated specimens. The Mini Parasep (MP) concentrator (Diasys) does not require addition of ethyl acetate (ETAC) as compared to the Fecal Parasite Concentrator (FPC), (Evergreen Scientific) which does require ETAC. Initially, the two concentrators were compared using 60 formalin preserved stools. 27 were negative with both methods and 33 were positive for the same parasites except that individual FPC samples only were positive for hookworm, *Trichuris trichiura*, and *Ascaris lumbricoides* eggs. The reverse was true for individual MP samples in which *Chilomastix mesnili* and *Entamoeba coli* were found. Parasites detected by both methods included *Ascaris, Giardia, Cryptosporidium, Entamoeba coli, Endolimax nana, Isospora belli, Iodamoeba butschlii,* and *Strongyloides stercoralis*. Protozoan trophozoites could be detected in the MP samples, presumably because ETAC was not used. In the next phase of the study, the MP concentrator using stools preserved in EFX was compared to the FPC (with ETAC) using formalin preserved stools. The results for 22 positive specimens were essentially the same and the parasites found were the same as those listed above. Finally, results of antigen detection EIAs (Prospect, Remel) for stools from the two preservatives were compared. For 6 *Giardia* positive assays, 4 were from both preservatives and 2 were only positive from EFX stool. For 4 *Cryptosporidium* positives, 3 were from both preservatives and one other from formalin only was considered a false positive (DFA negative). In summary, the MP concentrator using EFX provides essentially the same parasite detection results as the FPC (with ETAC) using formalin preserved stools; trophozoites can also be observed with the EFX/MP system. In addition, EFX preserved stools are suitable for antigen detection EIAs and, when used with ECOSTAIN, provide adequate permanently stained smears, thereby avoiding the use of mercury-containing PVA.

INTRODUCTION

Traditional stool preservatives (PVA and formalin) present difficulties in handling and disposing of samples because of the toxic nature of the chemicals. As a result, some new non-toxic preservatives and methods of processing specimens have reached the marketplace. Ecofix (EFX), (Meridian Diagnostics) preservative is alcohol-based, contains no mercury and requires no special disposal. In addition, because the EFX system uses a single-solution/single-vial method the patient is required to collect and manipulate only one collection container. The Mini-Parasep (MP, Diasys) concentrator uses a multi-directional filter and centrifugation to provide a stool concentrate without the use of ethyl acetate (ETAC). Additionally, EFX used with the MP provides concentrates with the added bonus of high numbers of trophozoites which do not survive concentration

methods using ETAC. We compared these new preservative and concentration methods to traditional methods in the detection of intestinal parasites. In addition, we compared the detection of *Giardia* and *Cryptosporidium* from stools preserved in EFX or formalin using antigen detection assays (Prospect, Remel).

METHODS AND MATERIALS

PHASE 1- FORMALIN-PRESERVED SPECIMENS:
Fecal Parasite Concentrator (FPC) with ETAC vs. Mini-Parasep (MP) Concentrator

59 specimens collected in formalin and submitted for routine ova and parasite (OAP) exam were used to compare the two concentrators. 4-6 mLs of the formalin samples were placed into an additional 9 mLs of 10% formalin in the FPC tube with 3 drops of 10% Triton surfactant. The FPC tube was then inverted and the sample was allowed to drain through the filter by gravity. 3 mLs ETAC was added, the tube was then centrifuged, and the fecal pellet was used for wet prep examination. For the MP tube, 2-3 mLs sample were placed into the concentrator tube which was then assembled and centrifuged. The resulting fecal pellet was used for wet prep examination. The concentrate sediments were compared for visual identification characteristics and quantity of organisms present.

PHASE 2- MP VS. FPC WITH ETAC:
EFX-preserved Specimens vs. Formalin-preserved Specimens

24 specimens previously determined to contain parasites were collected in duplicate (both EFX vial and formalin vial) and compared using the two concentration procedures. Specimens collected in formalin were processed with the FPC procedure as described above in Phase 1. Specimens collected in EFX were processed with the MP procedure as described above in Phase 1. The concentrate sediments were compared for visual identification characteristics and quantity of organisms present.

PHASE 3- GIARDIA AND CRYPTOSPORIDIUM ANTIGEN ASSAYS:
EFX-preserved Specimens vs. Formalin-preserved Specimens

EFX preserved specimens were compared to formalin-preserved specimens for use with two stool EIA antigen detection kits (PROSPECT GIARDIA MICROPLATE ASSAY and CRYPTOSPORIDIUM MICROPLATE ASSAY, Remel Diagnostics.) Samples were collected in duplicate (both EFX and formalin vials) and tested directly from the preservative vial following package insert instructions.

RESULTS

PHASE 1- FORMALIN-PRESERVED SPECIMENS:
FPC with ETAC vs. MP

- 26 specimens negative by both methods
- 33 specimens positive by both methods, with equivalent quality and quantity of organisms present:

EXCEPTIONS:

- *Hookworm, Ascaris,* and *Trichuris* each found in one specimen ONLY with FPC with ETAC.
- *Chilomastix* and *E. coli* each found in one specimen ONLY with MP

Parasites found using both MP and FPC with ETAC included:

Giardia lamblia	*Cryptosporidium species*
Entamoeba coli	*Endolimax nana*
Iodamoeba butschlii	*Blastocystis hominis*
Entamoeba hartmani	*Dientamoeba fragilis*
Entamoeba histolytica/dispar	

PHASE 2-MP VS. FPC WITH ETAC:
EFX-preserved Specimens vs. Formalin-preserved Specimens

- 24 specimens positive by both methods, with equivalent quality and quantity of organisms present:

EXCEPTIONS:

- *Hookworm, Ascaris, E. coli,* and *Trichuris* each found in one specimen ONLY with FPC with ETAC.
- *Giardia* and *Blastocystis* each found in one specimen ONLY with MP

Parasites found using both MP and FPC with ETAC included:

Ascaris lumbricoides	*Giardia lamblia*
Cryptosporidium species	*Entamoeba coli*
Endolimax nana	*Isospora belli*
Iodamoeba butschlii	*Trichuris trichiura*
Strongyloides lumbricoides	*Blastocystis hominis*
Entamoeba hartmani	*Dientamoeba fragilis*
Entamoeba histolytica/dispar	*Chilomastix mesnili*
Hymenolepis nana	*Diphyllobothrium latum*

PHASE 3: GIARDIA AND CRYPTOSPORIDIUM ANTIGEN ASSAYS:
EFX-preserved Specimens vs. Formalin-preserved Specimens

	FORMALIN POS	FORMALIN NEG
EFX POS	7	2*
EFX NEG	1**	249

*Confirmed negative for Giardia by microscopy and subsequent antigen testing
**Negative by DFA (Merifluor) for Cryptosporidium

SUMMARY

- Stools preserved in EFX, when used with the MP, provide parasite detection results comparable to stools preserved in formalin processed with the FPC with ETAC. EFX/MP also provides the additional advantage of observing trophozoite forms in the concentrate.

- The EFX preserved specimens are also suitable for both *Giardia* and *Cryptosporidium* antigen detection EIAs.

- Additional studies in our laboratory (not described here) have demonstrated that EFX-preserved stools (when used with ECOSTAIN) provide permanently stained smears comparable in quality to those prepared using PVA, obviating the need for that mercury-containing reagent.