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Use of Hemo-De To Eliminate Toxic Agents Used for Concentration and Trichrome Staining of Intestinal Parasites

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In a blind comparison, 465 randomly collected clinical fecal specimens were examined. Hemo-De was found to be an excellent replacement for ethyl acetate in the concentration procedure and for carbol-xylene and xylene in the trichrome staining procedure. Elimination of toxic reagents, combined with its lower cost, makes Hemo-De the preferred choice in routine parasitology examinations.

Clinical parasitology has evolved slowly toward elimination of toxic reagents used in parasitologic concentration and trichrome staining procedures. A problem of present-day parasitology is the need to identify suitable cost-effective replacements for ethyl acetate, carbol-xylene, and xylene while maintaining the recovery rate of intestinal parasites. Elimination of these reagents would help to ensure the safety of the technologist performing the examination as well as other laboratory personnel (Table 1).

An increasing concern with the use of toxic agents has prompted several studies by Neimeister (5, 6) and Gubash (3) evaluating xylene substitutes as possible replacements in parasitologic procedures. The two studies by Neimeister found Hemo-De to be an excellent replacement for ethyl acetate and xylene in the concentration and trichrome staining techniques, respectively. In a later study, Gubash also found xylene substitutes to be excellent replacements for xylene in the trichrome staining procedure. Both studies were performed in public health laboratories on small numbers of known positive samples (25 and 39, respectively). Unlike the previously published studies, our study evaluated the effect of Hemo-De, a xylene substitute, on a large number of randomly collected samples in a clinical microbiology setting. The larger sample size confirms Hemo-De's performance as a substitute for standard reagents used in parasitologic procedures.

In 1948, the Formalin-diethyl ether concentration procedure was developed (8). Hazards reported with the use of diethyl ether (11) led to the introduction of the Formalin-ethyl acetate procedure in 1979. This helped to reduce one of the risks, fire in the laboratory, but did not reduce or eliminate exposure to reagents that have been shown to be neurotoxic and toxic upon contact with skin and mucous membranes during concentration (9).

In 1951, the Wheatley trichrome staining procedure, in which carbol-xylene and xylene are used as clearing agents, was introduced (10). These reagents, used by the majority of clinical laboratories, are potentially neurotoxic and carcinogenic after repeated exposure (1).

There has been no suitable substitute for these reagents until Hemo-De, a biodegradable, nontoxic, less flammable terpene-based clearing agent (7) (manufacturer, Scientific Safety Solvents, Fort Worth, Tex.; distributor, Fisher Scientific Co. [catalog no. 15182507A]) became commercially available.

The purpose of this blinded study was to evaluate the effectiveness of Hemo-De as a replacement for ethyl acetate in the concentration procedure and carbol-xylene and xylene in the trichrome staining procedure.

To eliminate the possibility of matching results between each reagent, the method of concentration and trichrome staining was disguised. To eliminate bias, each of the specimens was read by the examining technologist as individually labelled specimens, not as paired samples. Since Hemo-De has a noticeable citrus fragrance, 3 drops of Hemo-De was added to each centrifuge tube after being processed with ethyl acetate. This prevented the examining technologist from recognizing the samples processed with Hemo-De. The same technologist examined all specimens to eliminate inconsistent technical ability in examining the samples. One other technologist processed all samples in the concentration and trichrome staining procedures.

A total of 465 randomly collected clinical fecal specimens were examined by using the sedimentation centrifugation and trichrome staining methods (4). Three hundred forty specimens preserved in 10% buffered Formalin and 125 preserved in polyvinyl alcohol were accepted for concentration with Hemo-De and ethyl acetate (2). Samples from both preservatives were treated identically. Five milliliters of fecal specimen was poured into each of two centrifuge tubes. Both specimen tubes were filtered through a Meridian CONtrate (Meridian Diagnostics, Inc., Cincinnati, Ohio) conical wire mesh screen filtering device. Each tube was washed twice with 0.85% saline and centrifuged for 2 min at 400 × *g*. The last and third wash mixed 9 ml of Formalin to one specimen tube processed with 3 ml of Hemo-De and one specimen tube processed with 3 ml of ethyl acetate. One smear was prepared from each tube by using Dobell's iodine and coverslips (22 by 40 mm), smears were entirely examined by using overlapping fields under 10× and 40× objectives, and all parasites were counted. Each specimen was examined for pellet size, number of each parasite present, organism morphology, and ability to remove debris.

Only fecal specimens preserved in polyvinyl alcohol were accepted for staining by the Wheatley trichrome staining method (11). For all specimens except one, two slides were prepared and stained by the standard trichrome staining technique; for the exception, the standard clearing agents carbol-xylene and xylene were replaced with Hemo-De. Each trichrome smear was entirely examined by using

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TABLE 1. Physical characteristics of ethyl acetate, phenol, xylene, and Hemo-De

Solvent	Flash point (°C)	Boiling point (°C)	Flammable limits (°C)	Sp gr	Presence of toxicity	Solubility in water
Hemo-De	57.8	177.8	0.7–6.1	0.841	No	None
Ethyl acetate	−4.0	77.0	1.4–7.6	0.9	Yes	8.7%
Phenol[a]	78.0	182.0	1.7–8.6	1.07	Yes	11.1%
Xylene	26.2	137.0–140.0	1.0–7.0	0.86	Yes	None

[a] Active ingredient in carbol-xylene.

overlapping fields under a 100× oil objective, and all parasites were counted. Each smear was examined for clarity, organism morphology, and number of each parasite present.

Sample correlation analysis was used to compare the total numbers of organisms present after using Hemo-De or ethyl acetate in the concentration procedure and Hemo-De or carbol-xylene and xylene in the trichrome staining procedure. The paired t test was utilized to determine whether Hemo-De compromised the rate of recovery of intestinal parasites in the concentration and trichrome staining procedures to a statistically significant level.

Specificity correlated 100% between Hemo-De and ethyl acetate in the concentration procedure and between Hemo-De and carbol-xylene and xylene in the trichrome staining procedure (Table 2). Sensitivity with Hemo-De was greater in both the concentration and trichrome staining procedures (Table 3). Positivity rates in both procedures with either reagent was 26.9%. There was no distortion of organisms observed with Hemo-De in the concentration procedure. The lower specific gravity of Hemo-De compared with that of ethyl acetate resulted in more debris observed in the sediment following concentration. This did not interfere with the detection of parasites. After the study, the excess debris that remained in the sediment following concentrating with Hemo-De could be reduced twofold if the number of washes with 0.85% saline was increased from two to three. A smaller average pellet size (0.5 ml) was noted with Hemo-De, compared with 0.75 ml with ethyl acetate.

TABLE 2. No. of positive specimens with intestinal parasites in concentrates with Hemo-De and ethyl acetate and in trichrome smears with Hemo-De, carbol-xylene, and xylene

Organism	No. of positive specimens in 465 concentrates with:		No. of positive specimens in 465 trichrome smears with:	
	Hemo-De	Ethyl acetate	Hemo-De	Carbol-xylene + xylene
Ascaris lumbricoides egg	1	1	0	0
Blastocystis hominis	56	56	56	56
Chilomastix mesnili	1	1	1	1
Cryptosporidium sp.	1	1	0	0
Dientamoeba fragilis	0	0	1	1
Endolimax nana	45	45	45	45
Entamoeba coli	30	30	30	30
Entamoeba hartmanni	30	30	6	6
Entamoeba histolytica	6	6	3	3
Giardia lamblia	27	27	27	27
Hymenolepsis nana egg	2	2	0	0
Iodamoeba buetschlii	11	11	11	11
Isospora sp.	1	1	0	0
Trichuris trichiura egg	9	9	0	0
Total	220[a]	220[a]	180[a]	180[a]

[a] Actual number of positive specimens (125) was lower than the actual number of total individual positive specimens observed.

TABLE 3. Total no. of intestinal parasites in concentrates with Hemo-De and ethyl acetate and in trichrome smears with Hemo-De, carbol-xylene, and xylene

Organism	Total no. of parasites in 465 concentrates with:		Total no. of parasites in 465 trichrome smears with:	
	Hemo-De	Ethyl acetate	Hemo-De	Carbol-xylene + xylene
Ascaris lumbricoides egg	5	4	0	0
Blastocystis hominis	2,512	2,029	1,513	1,256
Chilomastix mesnili	15	12	21	23
Cryptosporidium sp.	21	18	0	0
Dientamoeba fragilis	0	0	6	5
Endolimax nana	1,227	1,152	1,084	861
Entamoeba coli	563	516	489	462
Entamoeba hartmanni	177	136	100	82
Entamoeba histolytica	35	29	15	12
Giardia lamblia	1,283	1,107	696	589
Hymenolepsis nana egg	6	7	0	0
Iodamoeba buetschlii	261	273	161	168
Isospora sp.	3	3	0	0
Trichuris trichiura egg	36	32	0	0
Total	6,144	5,318	4,085	3,458

In the trichrome staining procedure, evaporation of Hemo-De is necessary. Slides processed with Hemo-De require a longer drying time (approximately 30 min) than those processed with carbol-xylene and xylene before examining. There was no difference in clarity of the smears with either reagent. Hemo-De did not distort the morphology of the parasites.

No statistical difference between total numbers of each type of parasite detected with either reagent in the concentration and trichrome staining procedures was found by using the paired t test. Hemo-De performed better than ethyl acetate ($P < 0.009$) in recovering intestinal parasites in the concentration procedure and better than carbol-xylene and xylene ($P < 0.009$) in the trichrome staining procedure. Strong linear relationships exist between Hemo-De and ethyl acetate in the concentration procedure and between Hemo-De and carbol-xylene and xylene in the trichrome staining procedure, evident by the sample correlation coefficients of 0.995 and 0.997, respectively.

Hemo-De is both nontoxic and 25% less expensive than ethyl acetate, carbol-xylene, and xylene combined. Our evaluation found, by replacing the standard reagents with Hemo-De, that specificities were equivalent and that the sensitivity of Hemo-De was better than that of the standard reagents yet did not prevent the identification and recovery of intestinal parasites. Therefore, Hemo-De is an acceptable and preferred substitute in the concentration and trichrome staining procedures used to identify and recover intestinal parasites.

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