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Clinical Comparison of Ethyl Acetate and Diethyl Ether in the Formalin-Ether Sedimentation Technique

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A substitute for the volatile solvent diethyl ether has been actively sought for the Formalin-ether sedimentation technique. Ethyl acetate has recently been shown to be a comparable substitute. In an effort to verify these findings and evaluate ethyl acetate under clinical conditions, comparison studies with 62 fresh human stool specimens were performed. Parallel concentrates with diethyl ether and ethyl acetate were prepared for each specimen, and the quantity and appearance of recovered parasite species were determined. Ethyl acetate was found comparable to diethyl ether in the quantitative recovery of parasite eggs, cysts, and larvae; no distortion or alteration of parasite morphology was observed with either solvent. More care was required, however, to completely remove the interface plugs of ethyl acetate and prevent their remixing with the concentrate sediment. In addition, wet mounts prepared from ethyl acetate concentrates were occasionally obscured by liquid bubbles probably composed of remaining insoluble ethyl acetate. Clinical laboratories considering substituting ethyl acetate for diethyl ether in the Formalin-ether sedimentation technique should be aware of these problems and take the appropriate precautions to avoid them.

The Formalin-ether sedimentation technique (1) is commonly used in laboratories to concentrate parasite eggs, cysts, and larvae in stool specimens. Unfortunately, diethyl ether (DE), an essential component in the technique, is both flammable and explosive, thus adding a hazardous element to this procedure.

Efforts by Young et al. to replace DE with a less volatile solvent have proven successful (2). By using a total of 10 human and monkey stool specimens containing parasite eggs, cysts, or larvae, parallel concentrates were prepared, using either DE or ethyl acetate (EAc) as the solvent. Within the range of their study, EAc proved to be as good as or better than DE in concentrating parasite eggs, cysts, and larvae, as well as in maintaining characteristic morphology. A confirmation of their results under more typical clinical laboratory conditions appears warranted.

MATERIALS AND METHODS

In this study, a comparison of DE and EAc in the Formalin-ether sedimentation technique was made on stool specimens from Indochinese refugees referred to the Long Beach City Health Department. The study data were collected by the investigator simultaneously with the diagnostic examination of the patients' stools by the facility's personnel.

Unpreserved human stool specimens were examined within 24 h of passage by the Formalin-ether sedimentation technique for the presence of parasite eggs, cysts, and larvae. Each specimen was from a different individual, thus providing a wide range in stool consistency and composition. The investigator prepared a DE and EAc concentrate for each specimen and submitted them for clinical evaluation. Once the clinical data were obtained, the laboratory personnel code-labeled each concentrate tube. The investigator then prepared and examined a wet-mount pair for each specimen, recorded the results, and was subsequently informed as to the solvents involved.

All specimens were concentrated by a modification of the procedure recommended by the California Microbial Disease Laboratory for the Formalin-ether sedimentation technique. Approximately 4 g of stool was emulsified, using applicator sticks in 15 ml of physiological saline solution in a 160- by 15-mm tube. Half of this suspension was then filtered through two thicknesses of gauze into each of two 12-ml conical tubes and centrifuged at 425 × *g* for 2 min. The supernatant was then discarded. An 8-ml amount of 10% Formalin was added to each tube, and the sediment was emulsified and allowed to fix for at least 5 min. A 3-ml amount of DE was then added to one tube, and the same volume of EAc was added to the other. Both tubes were then stoppered, inverted, shaken vigorously for 30 s, and recentrifuged at 425 × *g* for 1.5 min. Four layers were formed: solvent, a plug of debris, Formalin, and sediment. The plug of debris was loosened by ringing with an applicator stick, and the top

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three layers were decanted. Sediment was mixed and removed with a disposable pipette, and 1 drop was covered with a 22-mm^2 cover slip. A single unstained wet mount was prepared from each concentrate. Low power (×10) was used to examine the entire cover slip area; high power (×40) was used in a similar fashion to identify and enumerate cysts. All parasites were counted. Parasite densities were expressed as follows: "many" (>20 eggs or larvae per mount; three or more cysts per high-power field); "moderate" (6 to 20 eggs or larvae per mount; two cysts per high-power field); "rare" (1 to 5 eggs or larvae per mount; one or fewer cysts per high-power field). For statistical purposes, numerical values were assigned to each density: many = 3, moderate = 2, rare = 1, and none = 0.

RESULTS

A total of 93 specimens were examined, and 62 were found positive for parasites in one or both concentrates; the 31 specimens found negative for parasites in both concentrates were not included in the analysis. Multiple infections were common. Of the 62 positive stool specimens, hookworms were found in 38 specimens; *Ascaris lumbricoides*, in 12; *Trichuris trichiura*, in 12; *Hymenolepis nana*, in 3; *Opisthorchis* sp., in 5; *Giardia lamblia*, in 7; *Entamoeba coli*, in 8; and *Strongyloides stercoralis*, in 12. In total, 97 comparisons of parasite species were made with the two concentration techniques.

The score results for each of the species present were analyzed by using a nonparametric sign test. There was no significant difference in recovery rates for all parasites between the two techniques at the 5% probability level. The average score values and the observed number of false-negatives and totals for both techniques are shown in Table 1. There was no significant difference in the efficiency of recovery between the two techniques for most of the species found. An adequate assessment of differences between *H. nana* and *Opisthorchis* sp. egg values for the two techniques was prevented by sample size restrictions.

No distortion or alteration of morphology of the parasites was observed with either solvent.

The characteristic plug of debris at the aqueous solvent interface was consistently thicker in the EAc concentrates than in the tubes with DE. EAc plugs were also more adherent to the sides of the concentrate tubes and were therefore more difficult to remove. EAc concentrates with the thickest plugs of debris often left more debris behind after decantation than when DE was used. Approximately 11% (10 of 93) of the EAc concentrates were obscured by small liquid bubbles formed beneath the cover slip of the wet mounts; six of these EAc-DE pairs were positive for parasites in one or both concentrates and were included in the final 62 specimens used for comparisons. Similar liquid bubbles were not observed in wet mounts prepared from DE concentrates. For all other stool specimens, unstained wet mounts with either solvent were comparable in appearance and ease of examination.

TABLE 1. *Relative effectiveness of parasite recovery with EAc and DE*

Parasite species	No.[a]	Avg score[b]		False negatives[c]	
		DE	EAc	DE	EAc
Helminths (eggs)					
A. lumbricoides	12	2.4	2.5	0	0
H. nana	3	1.3	2.0	1	0
Hookworms	38	2.3	2.4	1	0
Opisthorchis sp.	5	2.2	2.2	0	0
T. trichiura	12	2.1	1.9	0	1
Helminths (larvae)					
S. stercoralis	12	1.8	1.8	0	1
Protozoa (cysts)					
Entamoeba coli	8	1.7	1.6	0	0
G. lamblia	7	2.3	2.3	0	0

[a] Number of comparisons made with each species.
[b] Average scores were determined by dividing the total score for each species by the number of specimens containing each species. Scores were determined as described in the text.
[c] The number of times a parasite species was missed by one concentration method although detected by the other.

DISCUSSION

As a previous study has shown (2), EAc appears to be an effective substitute for DE in the Formalin-ether sedimentation technique in terms of both the quantitative recovery and the appearance of parasite eggs, cysts, and larvae from human stool. In this study, the efficiency of recovery of those species present was similar for both solvents.

Occasionally, wet mounts prepared from EAc concentrates contained more stool debris and were therefore more difficult to read than the DE pairs. This problem was resolved when additional care was taken to dislodge and decant the thicker EAc plugs to prevent their remixing with the remaining sediment.

About 11% (10 of 93) of the EAc concentrates developed a confluence of small liquid bubbles under the cover slips of the wet mounts. The origin and composition of these bubbles was unclear, but they probably consisted of remaining insoluble EAc. Possible explanations for this observation include: (i) the inadequate removal of the EAc concentrate plugs; (ii) excess EAc

added at the beginning of the sedimentation procedure; and (iii) some quality of these stool specimens that promotes retention of the EAc. This observation was not found with DE concentrates. Although no attempt was made to repeat the entire sedimentation procedure on these concentrates, repeated wet mounts produced similar results. Of the six positive specimens in which bubbles were found in the EAc concentrate pair, only parasite eggs were identified. Because of their similar size, however, cysts would probably be difficult to locate and identify in such preparations. As a result, clinical laboratories encountering this problem should be prepared to repeat the sedimentation procedure with EAc, substitute an alternative concentration technique, e.g., zinc sulfate flotation, or reintroduce DE to ensure an adequate diagnosis.

In terms of its effectiveness in recovering parasites and its added safety benefits (EAc has a flash point of −4°C and a boiling point of 77°C, compared with −45 and 34.5°C, respectively, for DE), EAc could be a useful replacement for DE in the Formalin-ether sedimentation technique. However, clinical laboratories should be aware of the problems that might arise with EAc concentrate wet mounts and be prepared to take the appropriate precautions necessary to prevent them.

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