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Ethyl Acetate as a Substitute for Diethyl Ether in the Formalin-Ether Sedimentation Technique

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Ethyl acetate appears to be a satisfactory substitute solvent for diethyl ether in the Formalin-ether sedimentation technique. In comparative studies, concentration of organisms with ethyl acetate was equal to or greater than that with diethyl ether. No distortion or alteration of morphology was observed with either solvent, and preparations were comparable in appearance and ease of examination. In addition, ethyl acetate is less flammable and less hazardous to use than diethyl ether.

The Formalin-ether (F-E) sedimentation technique (1), widely used for concentrating eggs, larvae, and cysts in fecal specimens, is an efficient procedure and is relatively easy to perform. Since diethyl ether is both flammable and explosive, however, many laboratories are reluctant to take the risks involved in using this procedure.

The zinc sulfate centrifugal flotation technique is an alternate concentration method but does not recover as wide a range of organisms as does the F-E procedure. Thus, finding a substitute solvent for diethyl ether has become an important consideration in diagnostic parasitology.

Several solvents were screened for their ability to concentrate organisms. Their flammability, density, cost, and carcinogenicity were compared. Of the solvents tried, ethyl acetate appeared to be the most satisfactory. The studies reported here compare the effectiveness of diethyl ether and ethyl acetate in the F-E technique.

MATERIALS AND METHODS

Formalinized specimens containing a variety of eggs, larvae, and cysts and unpreserved human and monkey feces containing several species of protozoa were used to determine the relative efficiency of diethyl ether and ethyl acetate. Direct wet mounts and concentrated wet mounts were prepared from each test specimen by the same person and examined by two other experienced microscopists. The microscopists were not informed of the solvent used or the organisms present before examining the specimens.

All specimens were concentrated by the procedure used in the Center for Disease Control laboratories for the F-E sedimentation technique. Approximately 10 ml of the well-mixed fecal suspension was strained into a 15-ml conical centrifuge tube and centrifuged for 2 min at 500 × *g* to 650 × *g* (2,000 to 2,500 rpm with a table model centrifuge). After the supernatant was decanted, approximately 0.5 ml of sedimented feces for formalinized material and 0.75 ml for unpreserved specimens were left. If the amount of sediment was greater or less than 0.5 or 0.75 ml, it was adjusted to this amount. A 9-ml amount of neutral buffered 10% Formalin was added to the tube and thoroughly mixed with the sediment. A 4-ml amount of either ethyl acetate or diethyl ether was added to each tube. The tube was stoppered, shaken in an inverted position for 30 s, and then centrifuged for 2 min at 450 × *g* to 500 × *g* (1,800 to 2,000 rpm with a table model centrifuge). The usual four layers resulted: solvent, a plug of debris, Formalin, and sediment. The plug of debris was loosened by ringing with an applicator stick, and the top three layers were decanted. Unstained and iodine-stained mounts were prepared. The relative efficiency of the solvents was evaluated by the quantity of organisms recovered by concentration. The entire mount was examined systematically, and all organisms present were counted. Densities of each species were expressed as "many" (>three cysts per high-power field; >20 eggs or larvae per mount); "moderate" (two cysts per high-power field; 10 to 19 eggs or larvae per mount); "few" (one cyst per high-power field; three to nine eggs or larvae per mount); and "rare" (two to five cysts and <two eggs or larvae per mount). For simplification, numerical values were assigned to each density: many, 4; moderate, 3; few, 2; rare, 1; and none, 0.

RESULTS

Since a given organism was not usually present in all of the test specimens, a total score value for each specimen was used for comparing the effectiveness of the solvents. This total specimen score is the sum of the averages of the scores obtained by the two microscopists for each parasite species in the sample concerned.

Table 1 shows the total specimen scores obtained with each solvent for 10 formalinized

specimens. In general, ethyl acetate recovered more organisms than did diethyl ether. The data were analyzed by using a modified nonparametric Friedman two-way analysis of variance which indicated that the difference in recovery rates was significant at the 1% probability level.

The recovery rates of selected species of cysts, eggs, and larvae in five formalinized specimens are shown in Table 2. The total scores for each organism indicate that the recovery rates of *Entamoeba histolytica* cysts, *Giardia lamblia* cysts, *Hymenolepis nana* eggs, and *Strongyloides stercoralis* larvae are higher with ethyl acetate than with diethyl ether. Other helminth eggs were recovered in about the same numbers with both solvents.

Results obtained with fresh, unpreserved feces were essentially the same as with formalinized specimens.

No distortion or alteration of morphology of organisms was observed with either solvent. Except that the plug of debris after the final centrifugation was sometimes thicker and "fluffier" with ethyl acetate, there were no apparent differences in the concentration. Wet mounts, either unstained or iodine stained, were comparable in appearance and ease of examination.

TABLE 1. *Comparison of total score values obtained with ethyl acetate and diethyl ether on formalinized specimens*

Specimen	Total score with solvent[a]:	
	A	B
1	25.5	31.0
2	27.5	45.0
3	44.5	40.5
4	36.5	33.0
5	25.0	34.0
6	22.0	34.5
7	29.0	41.0
8	30.0	34.5
9	30.5	32.5
10	28.5	43.0

[a] A, Diethyl ether; B, ethyl acetate.

DISCUSSION

Ethyl acetate compares favorably with diethyl ether as a solvent in the F-E sedimentation procedure. In our study, no distortion or alteration of parasite morphology occurred with ethyl acetate, and the concentration of organisms was equal to or greater than that with diethyl ether. In general, ethyl acetate seemed to increase the efficiency of the procedure both in numbers of organisms and in range of species recovered. It was more effective than diethyl ether in concentrating *Giardia* cysts and *H. nana* eggs which are often not recovered by the original F-E method.

Not only is ethyl acetate equally effective in recovering organisms, but it is also less hazardous to use than diethyl ether. Both solvents are flammable; however, ethyl acetate is less flammable, with a flash point of −4°C and a boiling point of 77°C, compared with −45°C and 34.5°C, respectively, for diethyl ether. Ethyl acetate also has narrower flammability limits: 1.4 to 7.6% per unit volume compared with 1.9 to 48% per unit volume for diethyl ether. Both solvents are noncarcinogenic.

On the basis of these studies, ethyl acetate appears to be a satisfactory and efficient solvent for use in the F-E concentration procedure for intestinal parasites. In laboratories where the use of diethyl ether is prohibited or hazardous, ethyl acetate is suggested as a substitute.

LITERATURE CITED

1. **Ritchie, L. S.** 1948. An ether sedimentation technique for routine stool examination. Bull. U.S. Army Med. Dep. 8:326.

TABLE 2. *Comparison of score values for selected species obtained with ethyl acetate and diethyl ether*

Species	Score with specimen no.[a]:										Total score	
	2		5		6		7		10			
	A	B	A	B	A	B	A	B	A	B	A	B
Entamoeba histolytica cysts	0.5	1.0	1.0	1.0	0.5	0.5	0	1.0	0	2.0	2.0	5.5
Giardia lamblia cysts	0	2.5	3.0	3.5	1.5	2.0	0.5	2.5	2.0	3.5	7.0	14.0
Trichuris trichiura eggs	4.0	4.0	3.0	2.5	3.5	2.0	4.0	4.0	1.0	2.0	15.5	14.5
Ascaris lumbricoides eggs	3.5	4.0	2.0	3.5	2.0	2.0	2.0	3.0	2.0	3.5	11.5	16.0
Hookworm eggs	2.0	3.0	4.0	4.0	3.5	4.0	1.0	2.5	2.0	4.0	12.5	17.5
Strongyloides stercoralis larvae	0	3.0	0	0	1.0	2.0	1.0	2.5	1.0	2.0	3.0	9.5
Hymenolepis nana eggs	0.5	2.0	0	3.0	1.5	2.0	1.5	2.0	2.0	2.5	5.5	11.5
Diphyllobothrium latum eggs	4.0	3.5	4.0	4.0	4.0	4.0	3.5	3.0	4.0	4.0	19.5	18.5
Schistosoma mansoni eggs	2.0	3.0	0	0	0.5	0	3.0	3.0	1.0	1.0	6.5	7.0

[a] A, Diethyl ether was used; B, ethyl acetate was used. Each value represents the average of scores obtained by two microscopists.