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Comparison of Clinical Results for the Use of Ethyl Acetate and Diethyl Ether in the Formalin-Ether Sedimentation Technique Performed on Polyvinyl Alcohol-Preserved Specimens

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One hundred fecal specimens preserved in polyvinyl alcohol fixative were examined by the Formalin-ether sedimentation technique with ethyl acetate substituted for diethyl ether. Technical performance of the procedures, appearance and amount of sediment obtained, and organism morphology were comparable. Also, ethyl acetate is less flammable and, therefore, less dangerous to use than diethyl ether. Results of parasite recovery when diethyl ether or ethyl acetate was used revealed few clinically relevant differences, most of which could also have been attributed to other variables inherent in this type of diagnostic testing.

The introduction of ethyl acetate (EAc) as a substitute for diethyl ether (DE) in the Formalin-ether sedimentation concentration procedure provides a much safer chemical for use in the clinical laboratory. Results comparing the use of these two compounds on Formalin-preserved fecal material indicate that the differences in organism recovery and identification are minimal and probably do not reflect differences which are clinically relevant (5).

This study compares the effectiveness of EAc and DE in the sedimentation concentration of polyvinyl alcohol fixative-preserved specimens. Our main objective was to determine if there were any differences in organism recovery which would be clinically relevant to the physician in terms of therapy, etc. Since intestinal protozoa are not quantitated on laboratory report forms, the critical information would be the answers to these questions: (i) are the organisms detected by using either DE or EAc, and (ii) how does the use of DE or EAc affect the recovery of the organisms as compared with the recovery resulting when neither compound is used? Recovery of helminth ova and larvae would have to be reviewed not only in terms of their presence or absence, but also in terms of quantity, since this information may directly affect the use of therapeutic agents.

We were also interested in determining, for each specimen, how many additional organisms were recovered and identified on the basis of the trichrome permanent stained smear.

MATERIALS AND METHODS

From patient samples received in the laboratory, we selected 100 positive specimens for routine ova and parasite examinations which consist of a Formalin-ether sedimentation procedure and a trichrome permanent stained smear on each specimen (2). Fecal specimens were collected in 15 ml of polyvinyl alcohol preservative; the recommended ratio of stool to preservative is approximately 1/4 to 1/3. Positive specimens were selected on the basis of parasite recovery without the use of either EAc or DE in the Formalin concentration procedure, and did not include specimens which were found to be negative for organisms by concentration but were subsequently found to be positive by trichrome staining of polyvinyl alcohol-fixed films.

After thorough mixing, approximately 3 ml of each fecal specimen was placed in each of two tubes. Formalin (9 ml) was added to each tube and the contents were well mixed and strained through gauze. The volume of Formalin in each tube was adjusted to 12 ml, and 3 ml of DE or EAc was added to each tube. The tubes were thoroughly shaken for 30 s and centrifuged at a relative centrifugal force of 560 in a 45° angle head for 2 min. After centrifugation, four layers were evident in each preparation: a layer of sediment in the bottom of the tube, a Formalin layer, a small layer or plug of debris, and the DE or EAc layer at the top of the tube (4). The layer of debris was rimmed with an applicator stick, and the fluid contents of the tube (Formalin, debris layer, and top layer of DE or EAc) were decanted and discarded.

The amount of sediment left in the tube after decanting the fluid was approximately the same for each specimen, regardless of whether DE or EAc had been used for the procedure. The same person performed the concentration technique with both DE and EAc on the same specimen at the same time. Both were

centrifuged at the same time, and the tubes were randomized in the racks before examination. Usually, 6 to 10 specimens (12 to 20 concentrate sediments) were examined each day. Specimens containing the same organisms were usually processed at the same time.

Examination of the sediment was performed by another technologist on coded specimens, so that there was no indication on the tube as to which specimen was being examined or which compound (DE or EAc) had been used. The coding of the specimens was not revealed until all 100 specimens had been concentrated with both DE and EAc and examined and the data had been recorded. All specimens were examined within 2 weeks after collection in polyvinyl alcohol preservative.

After thorough mixing of the sediment, one unstained wet preparation from each tube was examined (10× and 40× objectives), and all organisms present on a cover slip (22 by 22 mm) were quantitated with each magnification. The criteria used for quantitation (5) are shown in Table 1.

The results obtained with DE and EAc were compared with original results from the examination of concentration sediments which were obtained by rinsing 3 ml of fecal material with 9 ml of 10% Formalin and spinning down the specimen (centrifuged at a relative centrifugal force of 560 in a 45° angle head for 2 min) without the use of either DE or EAc.

TABLE 1. *Quantitative criteria*

Frequency of occurrence	No. of cysts observed[a]	No. of eggs observed per cover slip
Rare	2–5 (c)	≤2
Few	1 (hpf)	3–9
Moderate	2 (hpf)	10–19
Many	≥3 (hpf)	≥20[b]

[a] Number observed on cover slip (22 by 22 mm) (c) with low power field of magnification (×10) or with high power field (hpf) of magnification (×40).
[b] Number of eggs or larvae.

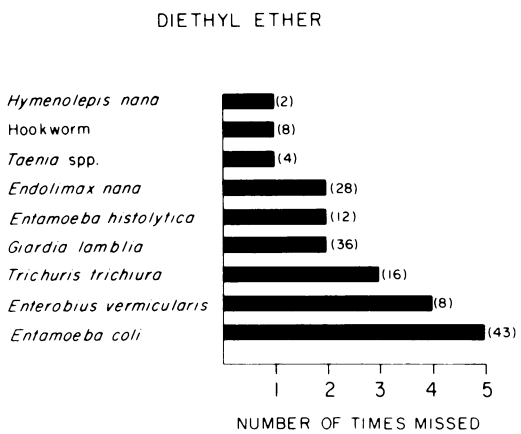

FIG. 1. *Organism recovery when DE is used in the Formalin-ether sedimentation technique, as indicated by the number of times missed per number of challenges (numbers in parentheses).*

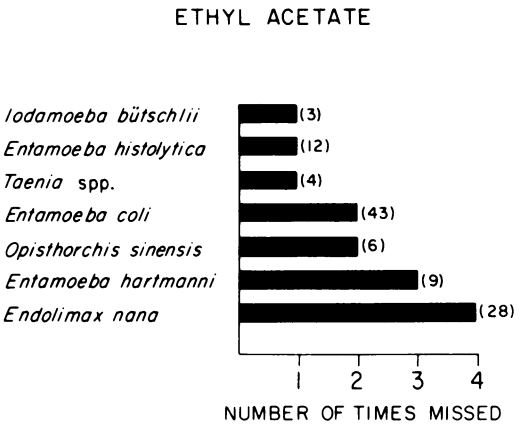

FIG. 2. *Organism recovery when EAc is used in the Formalin-ether sedimentation technique, as indicated by the number of times missed per number of challenges (numbers in parentheses).*

RESULTS

As can be seen from the results shown in Fig. 1, the failure to recover *Entamoeba histolytica* cysts, *Giardia lamblia* cysts, *Taenia* spp. eggs, and hookworm eggs when using DE would be significant in terms of clinical relevance from the standpoint of diagnostic testing. However, in the case of *Trichuris trichiura*, the original sedimentation yielded only rare *T. trichiura* eggs. As the results in Fig. 2 show, the failure to recover rare (original specimen results) *Opisthorchis* eggs and nonpathogenic intestinal protozoa when using EAc would not be as critical.

In Fig. 3, we have indicated those organisms that were not recovered when either DE or EAc was used. Each time an organism is missed represents a possible false-negative and, in terms of pathogens, could be significant. Although *T. trichiura* eggs were missed in nine different specimens, egg quantities varied from rare to few in the original specimen concentrates. Significant findings would include failure to recover *Ascaris* eggs, *Hymenolepis nana* eggs, and *G. lamblia* cysts.

If we review the organisms that were recovered when either DE or EAc or both were used but that were missed on the original concentration examination, we find that *T. trichiura* eggs were missed on a single specimen but were recovered when using both DE and EAc (rare eggs), and that *Chilomastix mesnili* cysts, *Entamoeba hartmanni* cysts, and *Endolimax nana* cysts were each missed on a single specimen but were recovered when EAc only

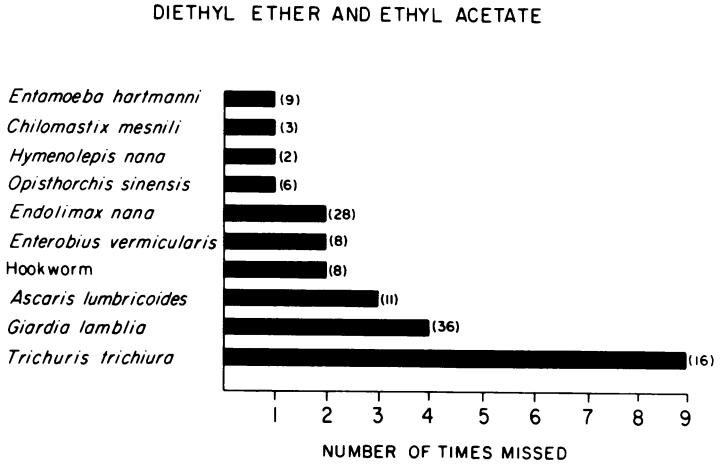

FIG. 3. *Organism recovery when DE and EAc are used (duplicate specimens) in the Formalin-ether sedimentation technique, as indicated by the number of times missed per number of challenges (numbers in parentheses).*

TABLE 2. *Quantitative comparison of organism recovery when DE and EAc are used in the Formalin-ether sedimentation technique*

Organism	No. of quantitative changes	Occurrence with DE	Effective recovery[a]	Occurrence with EAc	Effective recovery[a]
Entamoeba histolytica	2	Negative	−	Rare	+
	1	Rare	+	Negative	−
Entamoeba coli	5	Negative	−	Rare	+
	5	Rare	−	Few	+
	1	Rare	−	Moderate	+
	2	Rare	+	Negative	−
	1	Few	+	Rare	−
	1	Many	+	Few	−
Entamoeba hartmanni	2	Rare	+	Negative	−
	1	Few	+	Negative	−
Endolimax nana	3	Moderate	+	Few	−
	2	Many	+	Moderate	−
	3	Few	+	Rare	−
	2	Few	+	Negative	−
	2	Rare	+	Negative	−
	1	Negative	−	Rare	+
	1	Negative	−	Moderate	+
	1	Rare	−	Few	+
Giardia lamblia	2	Negative	−	Rare	+
	1	Rare	−	Few	+
	1	Few	−	Moderate	+
	2	Moderate	−	Many	+
	1	Moderate	+	Few	−
	1	Many	+	Few	−

[a] +, More effective organism recovery; −, less effective organism recovery.

was used. *Ascaris* eggs were not recovered in a single specimen on the original concentrate examination, but were recovered when DE was used on the same specimen.

Although quantitation of intestinal protozoa was not appropriate to include on laboratory report forms due to the tremendous variability in numbers from day to day, it is interesting to

TABLE 3. *Comparison of organism recovery when either DE or EAc is used in the Formalin-ether sedimentation technique*[a]

Organism	Organism recovery resulting in same quantitation from both solvents	Organism recovery resulting in different quantitation from both solvents	Total recovery	Organisms missed with:		
				DE	EAc	DE and EAc
Entamoeba histolytica	9/12 (75)		9/12 (75)	2/12 (17)	1/12 (8)	
Entamoeba coli	28/43 (65)	8/43 (19)	36/43 (84)	5/43 (12)	2/43 (5)	
Entamoeba hartmanni	5/9 (56)		5/9 (56)		3/9 (33)	1/9 (11)
Endolimax nana	11/28 (39)	9/28 (32)	20/28 (71)	2/28 (7)	4/28 (14)	2/28 (7)
Iodamoeba bütschlii	2/3 (67)		2/3 (67)		1/3 (33)	
Giardia lamblia	24/36 (67)	6/36 (17)	30/36 (83)	2/36 (6)		4/36 (11)
Chilomastix mesnili	2/3 (67)		2/3 (67)			1/3 (33)
Enterobius vermicularis	2/8 (25)		2/8 (25)	4/8 (50)		2/8 (25)
Ascaris lumbricoides	7/11 (64)	1/11 (9)	8/11 (73)			3/11 (27)
Strongyloides stercoralis	2/2 (100)		2/2 (100)			
Trichuris trichiura	1/16 (6)	3/13 (19)	4/16 (25)	3/16 (19)		9/16 (56)
Taenia spp.	1/4 (25)	1/4 (25)	2/4 (50)	1/4 (25)	1/4 (25)	
Hymenolepis nana				1/2 (50)		1/2 (50)
Opisthorchis sinensis		3/6 (50)	3/6 (50)		2/6 (33)	1/6 (17)

[a] Data given as number of organisms/number of specimens tested (percent).

examine different organisms and the variation in quantity recovered when either DE or EAc was used. This information is found in Tables 2 and 3. In most cases, the quantitation remained the same when DE and EAc were used and was not entered in Table 2.

The final laboratory report, based on the inclusion of the permanent, stained slides, indicated the presence of *E. histolytica* trophozoites in 7 of 100 specimen smears, of *G. lamblia* trophozoites in 6 of 100 smears, and of *Dientamoeba fragilis* trophozoites in 23 of 100 smears. *D. fragilis* was consistently missed on the wet concentration sediment examination, and any laboratory report should be considered complete only after examination of the permanent, stained smear.

DISCUSSION

We felt it would be important to use patient specimens with all the inherent variables (collection, mixing, etc.) rather than specimens which were artificially mixed to obtain high numbers of organisms in a small number of samples. There is great variability in fecal specimens; these variables are an unavoidable part of clinical testing and should be included in any study of diagnostic techniques.

We also felt that it was inappropriate to complicate the results by assigning values to quantitation or by applying statistical analysis to the data and, instead, presented actual figures representing the number of challenges and number of times a particular organism was not recovered under different conditions. It is important to remember that studies of this kind should reflect as closely as possible the actual working conditions and variables found in a working laboratory situation.

The data as presented indicate that EAc provides a good substitute for DE as a solvent in the Formalin-ether sedimentation technique performed on polyvinyl alcohol-preserved fecal specimens, and the minor differences seen would probably not be clinically relevant in terms of patient care. There were no apparent differences in organism morphology between the two solvents.

The only significant findings would be those where a pathogen had been missed completely and the specimen is considered negative for that particular organism. However, on examination of the data obtained in this study, it appears that the use of DE or EAc tends to provide approximately the same organism recovery rates, and neither would appear to influence organism recovery any more than many other variables present in this type of testing, e.g., the specimen/preservative ratio, adequate mixing and fixation, adherence to testing procedure, proper calibration of centrifuge speed, etc.

Nonpathogens which would be missed would not be as significant in terms of therapy; however, these protozoan organisms are a definite indicator of fecal/oral transmission. Patients who are found to be infected with those nonpathogens should be thoroughly checked with a minimum of three ova and parasite examina-

tions to insure that they are not also infected with pathogenic protozoa.

Although the results obtained when neither DE nor EAc was used provided excellent organism recovery, the use of neither is not recommended as a routine method for any laboratory unless the personnel are extremely experienced in parasitological diagnostic testing. The concentrate sediments obtained with Formalin alone contain more debris and must be examined by experienced personnel who routinely examine several hundred clinical specimens per month. Sediments obtained after the use of DE or EAc provide a cleaner preparation, and this approach is definitely recommended for routine use in the clinical laboratory. Any laboratory which decides to try this method should do so by performing duplicate examinations on patient specimens: one examination with neither compound, and another examination of the same patient material with the technique presently in use. The introduction of any new or modified technique should always include comparisons with the method currently being used.

Based on previous studies in our laboratory, we strongly recommend that all laboratories performing ova and parasite examinations confirm intestinal protozoan identification on the permanent, stained slide (2). When performed correctly, this procedure is one of the most important techniques in the recovery and identification of intestinal protozoa. The examination of both concentrates and permanent, stained smears will lead to the highest number of positive specimens and, ultimately, the highest quality patient care (1, 3).

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