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Hemo-De as Substitute for Ethyl Acetate in Formalin-Ethyl Acetate Concentration Technique

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In comparative studies, Hemo-De (PMP Medical Industries, Inc., Irving, Tex.) was found to be a suitable replacement for ethyl acetate in the Formalin-ethyl acetate concentration technique. With essentially equivalent recovery rates for both procedures, the Formalin–Hemo-De concentration technique is considered to be the preferred technique because Hemo-De is less toxic and less flammable and does not present disposal problems, and its cost is approximately one-fourth that of ethyl acetate.

Since its introduction in 1948, the Formalin-ether concentration procedure (3) has been the method of choice for many laboratorians performing parasitologic examinations on fecal specimens. Because of the recognized hazards of diethyl ether, i.e., fire and explosion, coupled with the lack of a comparably effective concentration procedure, the Formalin-ethyl acetate procedure (4) was developed. This procedure does not possess the hazards of the Formalin-ether procedure and does not compromise the recovery rate of parasites. Unfortunately, ethyl acetate is considered a hazardous chemical by the U.S. Environmental Protection Agency (1) and many state environmental agencies. Ethyl acetate is toxic upon contact with the skin and mucous membranes and possesses neurotoxicity. Because of these problems, we attempted to find a suitable replacement for ethyl acetate.

Hemo-De (produced by PMP Medical Industries, Inc., Irving, Tex., and distributed by Fisher Scientific Co., Pittsburgh, Pa. [catalog no. 15-182-507A]), a solvent with a specific gravity and solubility in water similar to those of ethyl acetate, recently became available. A comparison of the physical properties of ether, ethyl acetate, and Hemo-De is shown in Table 1. Hemo-De is relatively nonflammable (Table 1). Furthermore, Hemo-De is nontoxic, biodegradable, and classified by the U.S. Food and Drug Administration as GRAS (generally regarded as safe). Hemo-De, which has successfully replaced xylene in the trichrome staining technique (2) for intestinal protozoans, was evaluated as a possible replacement for ethyl acetate in the formalin-ethyl acetate concentration procedure.

Thirty formalinized fecal specimens which were thoroughly mixed and immediately decanted into duplicate vials were used to compare the efficiency of the standard Formalin-ethyl acetate concentration procedure with that of the modified concentration procedure. The modified technique incorporates the same steps and times as the standard technique, except Hemo-De is used in place of ethyl acetate. To eliminate any bias, one of us (R.N.) concentrated and coded matched specimens. Two experienced parasitologists (A.L.L. and B.G.), who were not aware of the concentration procedures used, examined and evaluated the specimens. Both unstained and iodine-stained wet mounts were examined to identify the organisms present and to note their physical appearance. The entire Vaspar-sealed mount (25 by 25 mm) was systematically examined using overlapping

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

Solvent	Flash point (°C)	Boiling point (°C)	Flammable limits (%)	Sp gr	Solubility in water
Ether (diethyl)	−45	34.5	1.9–48	0.714	Yes
Ethyl acetate	−4	77	1.4–7.6	0.9	8.7%
Hemo-De	57.8	177.8	0.7–6.1	0.841	No

TABLE 2. Comparison of total specimen scores obtained with the ethyl acetate and Hemo-De procedures

Specimen no.	Total score		Method advantage[a]	
	Ethyl acetate	Hemo-De	Ethyl acetate	Hemo-De
1	6	10		+
2	9	9		
3	7	8		+
4	11	11		
5	3	3		
6	5	5		
7	3	3		
8	6	5	+	
9	5	4	+	
10	3	4		+
11	2	3		+
12	6	6		
13[b]	4	6		+
14	3	3		
15[b]	4	3	+	
16	1	1		
17	6	7		+
18	9	9		
19	5	6		+
20	4	6		+
21[b]	6	8		+
22	9	10		+
23	5	6		+
24	5	5		
25	9	11		+
All	136	152		

[a] Three and twelve specimens showed ethyl acetate and Hemo-De advantages, respectively.

[b] Slightly heavier amount of fecal debris seen in the Hemo-De-concentrated specimen.

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TABLE 3. Comparison of total scores for organisms identified using ethyl acetate and Hemo-De

Organism	Score obtained with:	
	Ethyl acetate	Hemo-De
Ascaris lumbricoides eggs	22	22
Trichuris trichiura eggs	19	18
Hookworm eggs	9	12
Taenia species eggs	5	5
Strongyloides stercoralis larvae	7	9
Diphyllobothrium latum eggs	0	1
Schistosoma mansoni eggs	4	1
Hymenolepis nana eggs	5	4
Clonorchis sinensis eggs	5	6
Entamoeba histolytica cysts	8	14
Entamoeba hartmanni cysts	4	2
Entamoeba coli cysts	13	15
Endolimax nana cysts	11	13
Giardia lamblia cysts	23	30
Chilomastix mesnili cysts	1	0
All	136	152

microscopic fields, and all organisms present were counted. The numbers of organisms were noted as rare (1 to 5), few (6 to 20), moderate (20 to 40), and many (>40) per cover glass wet mount preparation. To compare the efficiency of the two methods, values were assigned to each density as follows: none, 0; rare, 1; few, 2; moderate, 3; many, 4.

A total of 25 of the 30 specimens processed by both methods contained one or more species of parasites. When the total specimens scores are reviewed (Table 2), it can be seen that the method incorporating Hemo-De had higher scores for 12 of the 25 specimens; the ethyl acetate procedure had higher scores for only 3 of the 25 specimens. Three specimens showed slightly heavier amounts of fecal debris when they were processed by the modified technique than when they were processed by the standard technique (Table 2). The comparability between the two methods is also shown in Table 3, in which the organisms identified and their total scores per method are shown.

The physical characteristics of Hemo-De make it an attractive ethyl acetate substitute for concentrating intestinal parasites. The slightly lower specific gravity of Hemo-De when compared with ethyl acetate may have permitted the increased amount of fecal debris seen in three specimens. The additional debris did not interfere with recognition of the parasites.

This study showed the Formalin–Hemo-De concentration procedure to be equivalent to the Formalin-ethyl acetate procedure in its ability to concentrate intestinal parasites. However, the fact that Hemo-De is nontoxic, does not present disposal problems, and has a cost approximately one-fourth that of ethyl acetate makes the Formalin–Hemo-De concentration method the preferred method.

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