Comparison of Formalin-Ethyl Ether Sedimentation, Formalin-Ethyl Acetate Sedimentation, and Zinc Sulfate Flotation Techniques for Detection of Intestinal Parasites

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Formalin-ethyl ether sedimentation, Formalin-ethyl acetate sedimentation, and zinc sulfate flotation techniques were compared using over 250 clinical parasitology specimens. Fifty positive specimens were identified, and a variety of parasites, including amoebae, flagellates, cestodes, nematodes, and trematodes, were encountered. The Formalin-ether and Formalin-ethyl acetate sedimentation procedures gave identical results for the detection of cysts, ova, and larvae, and these methods offered an advantage over the flotation procedure for the detection of selected ova. However, the zinc sulfate procedure was more effective for the detection of protozoan cysts, Hymenolepis nana, and hookworm eggs. The results indicate that the Formalin-ethyl acetate procedure provides a suitable alternative to the Formalin-ether method, and they demonstrate the value of using both flotation and sedimentation procedures in the analysis of fecal specimens for parasites.

Detection of parasites in fecal specimens is enhanced by the use of concentration procedures. The concentration procedures in general use in most clinical parasitology laboratories are zinc sulfate flotation (2) and Formalin-ether sedimentation (4). The zinc sulfate procedure is reliable for detection of most eggs, cysts, and larvae, but the Formalin-ether method is thought to provide certain advantages, including less distortion of organisms and enhanced recovery of Schistosoma eggs and operculated eggs. However, the Formalin-ether method may be suboptimal for the detection of Hymenolepis nana and Iodamoeba. In addition, a potential explosion hazard exists with the use of ether.

Recently, a promising alternative sedimentation procedure was reported (6). This procedure substituted ethyl acetate for diethyl ether in the development of a Formalin-ethyl acetate method. A preliminary evaluation of this method suggested that it provided results comparable to the Formalin-ether procedure (6). This report describes an evaluation of the Formalin-ethyl acetate procedure in comparison with the Formalin-ether and zinc sulfate procedures for detection of parasites in more than 250 clinical specimens.

MATERIALS AND METHODS

Specimens. Consecutive fecal specimens received in the Clinical Parasitology Laboratory at The University of Texas Medical Branch and specimens preserved in Formalin for up to 2 months were processed by zinc sulfate flotation, Formalin-ethyl ether sedimentation, and Formalin-ethyl acetate sedimentation on the same day. All specimens were initially examined macroscopically, and a direct wet mount of unconcentrated material was examined in both iodine-stained and unstained samples. Both iodine-stained and unstained preparations were made from a sample of each concentrate, and they were examined by a microscopist who did not know the procedure used for each preparation. The slides were examined systematically, and the number of each species of organism was determined. The results are expressed as a score (Table 1) determined by the method of Young et al. (6).

Sedimentation procedures. Approximately 5 ml of each formalinized specimen was strained through wet gauze into a conical 15-ml centrifuge tube. This amount was adjusted to obtain a sediment of 0.5 to 0.75 ml after initial centrifugation. Distilled water was added to a volume of 10 ml. The suspension was thoroughly mixed and centrifuged at 300 × g (1,500 rpm with a table model centrifuge) for 2 min. The supernatant was decanted, and distilled water was added to a volume of 10 ml. A 3-ml amount of solvent (either ethyl ether or ethyl acetate) was added to each tube. The tube was stoppered and shaken vigorously for 30 s, and the mixture was centrifuged at 300 × g (1,500 rpm with a table model centrifuge) for 2 min. After loosening the debris plug, the top three layers were decanted, the pellet was suspended in residual water and homogenized with gentle stirring, and slides were prepared for examination.

Flotation procedure. Approximately 4 ml of each
formalinized specimen was placed in a test tube (13 by 100 mm). This amount was adjusted to obtain a sediment of 0.5 to 0.75 ml. Distilled water was added to within 1 cm of the top of the tube, and the suspension was thoroughly mixed with applicator sticks. The suspension was centrifuged at 500 × g for 1 min (2,000 rpm with a table model centrifuge), the supernatant was decanted, and zinc sulfate (specific gravity, 1.190) was added to the tube to within 1 cm of the top. The tube was placed in the centrifuge, and zinc sulfate was added to the rim of the tube. A cover slip was placed gently on the top of the tube and in contact with the suspension. The tube was centrifuged with the cover slip in place at 500 × g for 1 min (2,000 rpm with a table model centrifuge). After centrifugation, the cover slip was gently removed and placed on a small drop of saline or iodine solution on a slide. The flotation procedure was performed in duplicate so that both iodine-stained and unstained preparations could be examined.

**RESULTS**

A total of 280 fecal specimens were analyzed by three concentration procedures. Only slight differences in the detection of parasites were found for the three methods, and the results obtained with the Formalin-ether and Formalin-ethyl acetate procedures were identical (Table 2). The recovery of *Entamoeba coli* and *Giardia lamblia* cysts and *H. nana* and hookworm eggs was slightly better with the zinc sulfate procedure, but the detection of *Taenia*, *Clonorchis*, *Trichuris*, and *Ascaris* eggs was somewhat better with the sedimentation procedures. The sedimentation procedures failed to detect small numbers of *E. coli* cysts in one of six specimens and small numbers of *Giardia* cysts in 3 of 22 specimens in which they were detected by the zinc sulfate procedure. On the other hand, the zinc sulfate procedure failed to detect *Taenia* eggs in one specimen and *Trichuris* eggs in one of two specimens. In addition, the morphology of *Giardia* cysts was better preserved with the sedimentation procedures than with the flotation procedure.

In addition to the concentration procedures included in this study, all specimens were examined macroscopically. Iodine-stained and unstained direct wet mounts were prepared and examined, a permanent Trichrome stain was prepared and examined, and in suspected cases of extraintestinal *Entamoeba histolytica*, a counterimmunoelectrophoresis test was performed on a serum specimen. Briefly, these examinations showed the following: (i) 11 specimens were found to be positive for trophozoites by direct or Trichrome stain or both, (ii) two specimens contained adult *Enterobius vermiformis*, (iii) two specimens contained *Taenia saginata* proglottids, (iv) in two specimens *G. lamblia* cysts were detected only in Trichrome-stained preparations, and (v) two specimens were positive for *E. histolytica* by counterimmunoelectrophoresis.

**DISCUSSION**

A variety of sedimentation procedures have been used for concentrating parasites from fecal specimens, including gravity, centrifugation, and chemical procedures (3). Gravity sedimentation has been found to be acceptable for operculated eggs and schistosome eggs. A major advantage of this technique is that the organisms remain viable. Disadvantages that accrue from this technique are that the concentration is time
consuming and confusing debris may remain in the concentrate. Centrifugal sedimentation may be used and can be of particular aid in the diagnosis of schistosome eggs in urine. Chemical procedures such as acid ether may be good for schistosome eggs and most helminth eggs; however, they are not acceptable for protozoa since the acid destroys the organisms. Formalin-ether sedimentations (i.e., chemical) are acceptable for both operculated and non-opperculated eggs, larvae, and cysts. In addition, schistosome eggs may be recovered with high efficiency. Some advantages of the Formalin-ether technique include (i) little distortion of the organisms, (ii) recovery of both helminths and protozoa, and (iii) recovery of both schistosome and operculated eggs. Disadvantages of the Formalin-ether technique include (i) concentration of granular material, (ii) suboptimal concentration of *H. nana* and *Iodamoeba*, and (iii) greater expense and significant fire and explosion hazard.

Both zinc sulfate and brine techniques have been used in flotation concentration procedures. The brine technique has been found to be useful for the recovery of helminth eggs, with the exception of operculated and schistosome eggs (3, 5). The zinc sulfate flotation technique, however, has been found to be a very useful routine procedure in the clinical parasitology laboratory. This procedure can be used for helminth eggs, larvae, and protozoan cysts. The zinc sulfate procedure, however, has been found to be unreliable for schistosome eggs and will not concentrate operculated eggs well unless they are Formalin preserved (1).

Due to the flammability of diethyl ether, attempts have been made to find a suitable alternative, and a recent study suggested that ethyl acetate would provide an adequate substitute (6). Our findings confirm and extend those reported previously, and they indicate that the Formalin-ethyl acetate procedure is a suitable alternative to the traditional Formalin-ether method. The recovery of cysts, eggs, and larvae was exactly the same for each specimen analyzed by the two methods. Since ethyl acetate does not pose the fire hazard associated with diethyl ether, the Formalin-ethyl acetate procedure can now be recommended for wide use.

The question of performance of sedimentation procedures versus flotation procedures for the detection of parasites in fecal specimens has been debated for several years, but it is generally believed that sedimentation procedures should be used because *Schistosoma* and operculated eggs may not be detected efficiently by flotation procedures. Our results demonstrate the value of using both sedimentation and flotation procedures. The zinc sulfate method detected protoskoan cysts, *H. nana* eggs, and hookworm eggs more efficiently than did the sedimentation procedures, and the Formalin-ethyl acetate (and Formalin-ether) method detected *Taenia, Ascaris, Trichuris*, and *Clonorchis* eggs more effectively than did the flotation procedure. These results emphasize the importance of using both flotation and sedimentation procedures to achieve maximum detection of parasites in fecal specimens.

In conclusion, our results indicate that the Formalin-ethyl acetate procedure provides a safe and effective alternative to the Formalin-diethyl ether sedimentation procedure. Our findings also demonstrate the value of using both sedimentation and flotation procedures in the analysis of fecal specimens for parasitic infection, in addition to macroscopic examination, direct wet mounts (stained and unstained), Tri-

chromic staining of unconcentrated specimens, and selected counterimmunoelectrophoresis testing for *E. histolytica*. Based on these results, we currently employ both the zinc sulfate flotation and Formalin-ethyl acetate sedimentation procedures in our laboratory.

**LITERATURE CITED**