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Short Communication

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

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Abstract

Background: Tween as a substitute for diethyl ether in the formalin-ether sedimentation technique was evaluated for parasite detection.

Methods: Fresh fecal material free of parasites with 10% formalin to prepare standardized specimen was thoroughly pooled. This specimen was divided into 5 equal portions; one was without infection, and each of the others was individually seeded with *Entamoeba coli*, and *Giardia lamblia* cysts, ova of *Ascaris lumbericoides*, and *Hymenolepis nana.* Six hundred and eighty four slides including 228 stool samples for each of formalin-tween, formalin-ether and direct wet mount procedures were examined.

Results: The sensitivity of above mentioned procedures were computed 72.1%, 55% and 30 %; their negative predictive value were 69.3%, 58.3% and 47.3%; and their false negative error rate were 27.9%, 45% and 70%, respectively. There were no false-positive results among the 264 specimens previously identified as negative for the presence of intestinal parasites. Therefore, specificity for each technique was 100%.

Conclusion: In the range of our study, formalin-tween method proved to be equivalent to or better than formalin-ether technique in concentrating parasite eggs, and cysts, as well as in maintaining characteristic morphology. Tween is more stable, safer, cheaper, and less flammable than that of ether; and promises to be a useful alternative to ether.

Keywords: *Parasites, Formalin, Tween, Formalin, Ether*

Introduction

Parasitic infections are endemic and represent a major public health problem in developing countries (1). The formalin-ether sedimentation technique (2) has been the method of choice for many laboratories performing parasitologic examinations to concentrate parasite eggs, cysts, and larvae in stool specimens. This technique is not only reliable for the detection of helminthic eggs, larvae and protozoan cysts, but is also thought to provide certain advantages, including less distortion of organisms and enhanced recovery of parasites. However, the formalin-ether method may be suboptimal for the detection of *Hymenolepis nana* and *Iodamoeba* (3). In addition, the use of diethyl ether, an essential reagent of this technique, may be hazardous to laboratory personnel; because it is explosive, contains anaesthetic vapours, has potential toxicity such as respiratory irritation, and causes cardiovascular depression and narcosis (4, 5). Therefore, a replacement for diethyl ether has been sought for this technique.

In the present study, we have evaluated the sensitivity, specificity, negative predictive value, and false negative error rate of the formalin-tween technique with that of the conventional direct wet mount and formalin-ether method for detection of stool specimens for intestinal parasites.

Materials and Methods

Fresh fecal material free of parasites, with individual variations in mucus, cellular content, and consistency, was pooled with 10% formalin to prepare standardized specimen. This suspension was divided into 5 equal portions, one was without infection, and each of the other four portions of this specimen were individually seeded with *Entamoeba coli*, and *Giardia lamblia* cysts, ova of

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Ascaris lumbericoides, *H. nana*. These organisms were selected because of their frequency of clinical occurrence and variation in size. Suspension specimens with and without intestinal parasitic infections were examined; 684 slides including 228 stool samples for each of formalin-tween, formalin-ether and direct wet mount procedures. In each procedure, 88 negative stool samples and 140 infected stool samples with *E. coli*, and *G. lamblia* cysts; ova of *A. lumbericoides*, *H. nana*, i.e. 35 samples for each mentioned parasite in every procedures were studied.

In addition, to testing 228 direct wet mounts of un-concentrated materials, two similar sets of conical 10 ml centrifuge tubes were separately considered for formalin-tween and formalin-ether concentration procedures.

Approximately 7 ml of each suspension specimens with and without intestinal parasitic infections were strained through two layers of gauze into each of two set 10 ml conical tubes and the 3 ml of diethyle ether were then added to a set of tubes, and the same volume of 7.5% tween 20™ were added to the other set. Both sets of tubes were then closed with a stopper, inverted, and shaken vigorously for 30 s and the tubes were centrifuged together at 1500 r.p.m. for 2 min (3, 6). The plug of debris was loosened with an applicator stick and, together with the liquid, was carefully decanted. The remaining pellets, was re-suspended with a drop of Lugol's iodine solution in residual water by gentle stirring, and 50 ul of material was transferred onto a clean glass slide, and covered with a cover slip (20 by 20 mm). Both concentrated and un-concentrated preparations were examined blindly. The slides were examined under light microscope at the magnifications of 100 and 400, respectively.

Sensitivity, specificity, negative predictive value, and false negative error rate were evaluated by using the results of slides with and without intestinal parasitic infections for each procedure. Statistical analysis was made using the x^2 test, and a $P< 0.05$ was considered statistically significant.

Results

A total of 684 slides including 264 negative stool samples, and 420 positive stool samples were examined by the use of formalin-tween, formalin-ether and direct wet mount procedures; and the results of each parasite species seen for each procedure have been given in Table 1. The recovery of *H. nana* eggs from formalin-tween sedimentation method was significantly more than formalin-ether sedimentation procedures ($P< 0.01$). The recovery of *G. lamblia* cysts and *A. lumbericoides* eggs was slightly better with the former method, but the detection of *E. coli* cysts was somewhat better with the latter procedure. Sensitivities, negative predictive values and false negative error rates of formalin-tween, formalin-ether and direct wet mount procedures for the identification of studied intestinal parasites are showed in Table 2. There were no false-positive results among the 264 specimens previously identified as negative for the presence of intestinal parasites. So, specificity for each technique was 100%. Morphology of the cysts and eggs of studied parasites, found in the formalin-tween and formalin-ether sedimentation methods, were similar, but clarity of sediment in formalin-ether method was slightly better than formalin-tween method.

Table 1: Recovery of parasite ova and cysts by the formalin-tween, formalin-ether, and direct wet mount procedures in stool specimens previously identified as positive or negative for the presence of intestinal parasites.

Parasite species	No. of samples tested		Detection of positive samples by different methods		
	Negative*	Positive	Formalin-tween	Formalin-ether	Direct wet mount
G.lamblia (cysts)	22	35	24	18	9
E.coli (cysts)	22	35	24	31	16
Ascaris (ova)	22	35	22	17	9
H.nana (ova)	22	35	31	11	8
Total (%)	88	140	101(72.1)	77(55.0)	42(31.0)

*Total of stool specimens previously identified as negative for the presence of intestinal parasites were not positive in studied different procedures .

Table 2: Sensitivity, specificity, positive and negative predictive values, and false negative error rate of formalin-tween, formalin-ether and direct wet mount procedures for the detection of parasites in stool specimens.

Parasite species	Procedure	Sensitivity (%)	Specificity (%)	False negative error rate (%)	Negative predictive value (%)	Positive predictive value (%)
Giardia lamblia	Wet mount	25.7	100	74.3	45.8	100
	Formalin tween	68.6	100	31.4	66.7	100
	Formalin ether	51.4	100	48.6	56.4	100
Entamoeba coli	Wet mount	45.7	100	54.3	53.7	100
	Formalin tween	68.6	100	31.4	66.7	100
	Formalin ether	88.6	100	11.4	84.6	100
Ascaris lumbricoides	Wet mount	25.7	100	74.3	45.8	100
	Formalin tween	62.9	100	37.1	62.9	100
	Formalin ether	48.6	100	51.4	55.0	100
Hymenolepis nana	Wet mount	22.9	100	77.1	44.9	100
	Formalin tween	88.6	100	11.4	84.6	100
	Formalin ether	31.4	100	68.6	47.8	100
All parasites	Wet mount	30	100	70	47.3	100
	Formalin tween	72.1	100	27.9	69.3	100
	Formalin ether	55	100	45	58.3	100

Discussion

The standardized suspension specimen was free of parasites, but it contained fresh fecal material with some of which had mucus, vegetables and meat fibers, and other debris typical of stool specimens encountered in microbiological laboratories for parasite examination. This suspension was divided into several portions, and was individually seeded with cysts and ova of more common parasites for providing positive suspension specimens. Several reagents, such as ether, ethyl acetate, detergent and acetone have been employed in stool concentration techniques (2, 3, 5, 7). Of these, ether is one of the more commonly useful fat solvents in the concentration of stool for parasites (8). However, when ether is exposed to light, it is extremely flammable and highly volatile, produces anaesthetic vapours, and forms explosive peroxides. It therefore needs careful handling in a Parasitology laboratory and must be kept away from sources of ignition, oxidizers, iodine and chlorine (9-11).

Because of these problems, several other chemicals have been evaluated as substitute for diethyl ether (5, 7, 9, 11). Young et al. (5) introduced ethyl acetate as a replacement. Even though ethyl acetate appeared to be an effective reagent for concentrating parasite organisms as well as in maintaining characteristic morphology, the formalin-ethyl acetate method also had some drawbacks. The thickest interface plugs of ethyl acetate were difficult to remove and they sometimes remixed with the concentrated sediment. In addition, they included a confluence of small liquid bubbles, probably composed of remaining insoluble ethyl acetate under the cover slips, or they obscured small parasite organisms (9).

Recently, a replacement for diethyl ether with another solvent has been developed for the formalin-detergent technique (7). Although, this tech-

nique was evidently an efficacious method for intestinal helminthes detection, however, has been found to be unreliable for protozoan cysts. Moreover, this method required an overnight sedimentation time and the amount of fine precipitate in the sediment concealed parasite observation. Thus, such stronger surfactant as tween 20™ may yield greater parasite detection, and shortening the procedure and reducing these fine particles. Tween 20™, commercially known as polysorbate 20, has been widely used in serological work. Its property is mostly different from diethyl ether. Tween is stable, less expensive and as it does not produce anaesthetic vapour, it is also safer (10). Ether used in the formalin-ether technique was employed to dissolve fats and float fecal debris, producing the parasite organisms that were separated from fecal debris during centrifugation (3, 8, 11). The surfactant property of tween 20™ may cause the slippery surface of parasites that were excluded easily from fecal debris and subsequently settled on the precipitate.

According to the result of this study, the formalin-tween technique gave more parasitic detection than the formalin-ether technique. However, clarity of sediment in formalin-ether method was slightly better than formalin-tween method. The formalin-tween method detected *G. lamblia* cysts, *H. nana* eggs, and, *Ascaris* eggs more efficiently than did the formalin-ether procedure, and whereas for *E. coli* cysts was reversed. In this study, we found the formalin-tween method to be as sensitive as or more sensitive than formalin-ether technique for concentration of parasite ova and cysts in stool specimens (Table 2). There were no false-positive results among the 264 specimens previously determined to be negative. Both helminthic and protozoan parasitic elements were demonstrated in stool and the morphology of the individual parasites was well retained.

In conclusion, our results indicate that tween promises to be a stable, safe and effective alternative to ether in the concentration of stools for intestinal parasites. Moreover, the use of tween does not require any additional training of the staff performing the formalin-tween technique. Evaluation of the formalin-tween technique compared with formalin-ether method for detection of intestinal parasites is suggested with a larger sample size in the field.

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The authors declare that they have no Conflict of Interests.

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