Rev. Inst. Med. trop. S. Paulo
45(5):289-291, September-October, 2003

TECHNICAL REPORT

EVALUATION OF THE FORMALIN-TWEEN CONCENTRATION TECHNIQUE FOR PARASITIC DETECTION

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SUMMARY

The formalin-Tween sedimentation method was compared with the formalin-ether sedimentation for parasitic detection. Of a total 297 fecal specimens examined, 72.1% were positive. The formalin-tween technique was effective for ascertaining helminths, particularly *Ascaris lumbricoides, Trichuris trichiura* and hookworm eggs; however it has less capability for protozoa detection. This method is simple, inexpensive, less time consuming and highly sensitive when detecting the parasitic infection, particularly when focusing on helminth eggs.

KEYWORDS: Formalin-tween; Concentration; Detection; Parasite.

Parasitic infections are endemic and represent a major public health problem in developing countries[5]. Detection of parasite products in fecal specimens is enhanced by the use of concentration procedures, of which formalin-ether sedimentation[7] is generally used in most clinical parasitology investigation. Not only is this technique reliable for the detection of helminth eggs, larvae and protozoan cysts, but is also thought to provide certain advantages, including less alteration to organisms and increased recovery of *Schistosoma* and operculated eggs[8]. However, the use of diethyl ether, an essential reagent of this technique, may be hazardous to laboratory personnel, since it is explosive, of potential toxicity such as respiratory irritation, and a cause of cardiovascular depression and narcosis[2,9]. Moreover, it may yield residues of mutagenic peroxides in *Salmonella typhimurium* strains TA100 and TA102[3]. Therefore, a replacement for diethyl ether has been sought for this technique. YOUNG *et al*.[9] 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[4].

Recently, an alternative sedimentation procedure was reported[6]. A locally common dish-washing detergent was used to replace diethyl ether in this formalin-detergent method, and it gave a slightly better result than the formalin-ether procedure. This method, however, required an overnight sedimentation time and the amount of fine precipitate in the sediment concealed parasite observation. Thus, the other stronger surfactants may help in shortening the procedure and reducing these fine particles. Herein, we developed the formalin-tween sedimentation technique for the detection of parasites in the stool.

A total of 297 freshly passed fecal specimens were received from hill-tribe students of the Somdet Prabhudhachinnawong School, San-Sai District of Chiang Mai Province, northern Thailand. All specimens were kept in refrigerator set at 4 ^{o}C for not more than 7 days before examination. These specimens were used for both formalin-ether and formalin-tween sedimentation. Approximately 2 g of stool were emulsified in a wax-paper cup using applicator sticks in 20 ml of distilled water. Half of this suspension was then filtered through two layers of gauze into each of two 15 ml-conical tubes and centrifuged at 1,500 r.p.m. for 2 min[1]. The supernatant was then discarded. The 10 ml of 10% formalin was added to each tube, and the sediment was thoroughly mixed and allowed to fix for at least 10 min. The 1 ml of diethyl ether was then added to one tube, and the same volume of 5% Tween 20™ was added to the other. Both tubes were then closed with a stopper, inverted, shaken vigorously for 30 s and the mixture was re-centrifuged at 1,500 r.p.m. for 2 min. After loosening the debris plug, the top three layers were discarded. The sediment was mixed using a wooden stick and transferred onto a clean glass slide after placing 1 or 2 drops of iodine solution. A cover slip was placed over the slide. An unstained preparation was also carried out from the same sediment of each tube,

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without applying the iodine solution. Both iodine-stained and unstained preparations were examined by a parasitologist, who did not know the procedure used for each preparation. The slides were examined under light microscope at the magnifications of 100× and 400×, respectively. Statistical analysis was made using the non-parametric McNemar test in Epistat programme, and a P value of < 0.05 was deemed significant.

Of a total 297 specimens examined, 72.1% (214/297) were positive for parasites in one or both concentration techniques. Morphology of the cysts, eggs or larvae of these parasites, found in both methods, were similar. The comparison of parasites detected by using the formalin-ether (FE) and formalin-tween (FT) concentration technique are shown in Table 1. A statistically significant difference between the two methods was noticed in the detection of *Ascaris lumbricoides*, *Endolimax nana* and *Blastocystis hominis* (P = 0.000, P = 0.031 and P = 0.016, respectively).

As shown in Table 1, for most helminth infections, the FT technique detected a higher percentage of positive samples. The positive samples of *A. lumbricoides*, hookworm and *Trichuris trichiura* examined by the FT technique were 98.2% (55/56), 80.3% (110/137) and 83.6% (61/73), whereas, those processed by the FE technique were 69.6% (39/56), 69.3% (95/137) and 72.6% (53/73), respectively.

A replacement for diethyl ether with another solvent has been developed for the formalin-detergent technique[6]. Although, this technique was evidently an efficacious method for intestinal helminth detection, the protozoa detection has not been documented. Thus, such stronger surfactant as Tween 20™ may yield greater parasite detection. Tween 20™, commercially known as polysorbate 20, has been widely used in serological work. Its property is mostly different from diethyl ether. Ether used in the FE technique was employed to dissolve fats and float fecal debris, producing the parasite organisms that were separated from fecal debris during centrifugation[5,8]. 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.

With particular attention to helminths, the FT technique gave more parasitic detection than the FE technique, according to the result of this study. However, the recover of protozoa, either non-pathogenic (e.g. *Entamoeba coli)* or of medical importance (e.g. *Entamoeba histolytica*, *Giardia lamblia*), resembled each other. Otherwise, these protozoa were detected more in the FE than FT technique. Since protozoan parasites are very tiny, they may be concealed in fecal specimens. On the other hand, the relatively low concentration of Tween 20™ employed in this study may not be suitable for the detection of the tiny protozoa, thus an increased concentration of this substance merits for further investigation.

RESUMO

Avaliação da técnica de concentração de formalina-Tween 20™ na detecção de parasitas

O método de detecção de parasitas por meio de sedimentação com Tween 20™ foi comparado com o de formalina-éter. De um total de 297 amostras fecais examinadas, 72,1% foram positivas. A técnica de formalina-Tween 20™ foi eficaz para demonstrar a presença de helmintos, particularmente ovos de *Ascaris lumbricoides*, *Trichuris trichiura* e ancilostomídeos. Entretanto, foi menos capaz de revelar protozoários. Este método é simples, de rápida execução e altamente sensível; revela infecções por parasitas, especialmente por meio da identificação de ovos de helmintos.

Table 1

Quantitative recovery of parasite eggs, cysts and larvae by the formalin-ether (FE) only, formalin-Tween (FT) only and both concentration techniques

Parasites	No. of positive samples by FE or FT	No. of positive FE samples (%)	No. of positive FT samples (%)	No. of both positive FE&FT samples (%)	P
Helminths (eggs)					
Hookworm	137	95 (69.3)	110 (80.3)	68 (49.6)	0.092
Trichuris trichiura	73	53 (72.6)	61 (83.6)	41 (56.2)	0.216
Ascaris lumbricoides	56	39 (69.6)	55 (98.2)	38 (67.9)	0.000*
Taenia sp.	6	5 (83.3)	4 (66.7)	3 (50.0)	1.000
Opisthorchis viverrini	3	3 (100.0)	0 (0.0)	0 (0.0)	0.250
Helminths (larvae)					
Strongyloides stercoralis	1	1 (100.0)	0 (0.0)	0 (0.0)	1.000
Protozoa (cysts)					
Entamoeba coli	63	57 (90.5)	47 (74.6)	41 (65.1)	0.052
Giardia lamblia	24	19 (79.2)	19 (79.2)	14 (58.3)	1.000
Endolimax nana	13	13 (100.0)	7 (53.9)	7 (53.9)	0.031*
Entamoeba histolytica/ Entamoeba dispar	11	7 (63.6)	6 (54.5)	2 (18.2)	1.000
Blastocystis hominis	7	7 (100.0)	0 (0.0)	0 (0.0)	0.016*
Sarcocystis sp. (sporocyst)	3	3 (100.00)	0 (0.0)	0 (0.0)	0.250

* Significant difference between FE and FT techniques (McNemar test; P < 0.05).

ACKNOWLEDGMENT

We thank the Faculty of Medicine Endowment Fund for Medical Research, Chiang Mai University, for financial support.

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Received: 07 July 2003
Accepted: 08 August 2003