

A Modified Formalin-Ether Concentration Technique for Diagnosis of Human Strongyloidiasis

Witthaya Anamnart^{1,*}, Pewpan M. Intapan² and Wanchai Maleewong²

¹Department of Medical Technology, School of Allied Health Sciences and Public Health, Walailak University, Nakhon Si Thammarat;

²Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand

Abstract: We compared the efficacy and applicability of a modified formalin-ether concentration technique (M-FECT) to the conventional FECT (C-FECT) and the agar plate culture (APC) method for the detection of *Strongyloides stercoralis* larvae. For this purpose, we used 600 human fecal specimens collected in an endemic area of southern Thailand. In the M-FECT, we used 2 layers of wire meshes, instead of gauze, to avoid the loss by absorption/adhesion of larvae to the gauze during filtration, and we reduced the exposure time of *S. stercoralis* larvae in stool samples to formalin. By such simple modifications, the efficacy of M-FECT has become comparable to APC and was much better than that of C-FECT for the diagnosis of strongyloidiasis.

Key words: *Strongyloides stercoralis*, formalin-ether concentration technique, agar plate culture, feces, diagnosis

In tropical and subtropical areas, strongyloidiasis is a widespread human parasitic disease that can be life-threatening to immunocompromised hosts as well as patients undergoing corticosteroid treatment or chemotherapy [1]. The most effective method of coprodetection of *Strongyloides stercoralis* larvae is the agar plate culture (APC) method as it is 1.6-6.0 times more effective than the conventional formalin-ether concentration technique (C-FECT) [2,3] or the quantitative formalin ethyl acetate concentration technique (QFEC) [4]. Unfortunately, APC is costly, time-consuming, and has a high risk of infection for laboratory technicians [3,5], and C-FECT is still commonly used in endemic areas of developing countries. Recently we made some modifications to the C-FECT to improve the recovery and detection of larvae by using 2 layers of wire meshes instead of gauze to avoid the trapping of larvae in the gauze during filtration, and by reducing the exposure time of larvae to formalin [6] to avoid the density reduction of larvae caused by long exposure to formalin. In this study, we compared the detection efficacy of the modified FECT (M-FECT) for *S. stercoralis* larvae to that of the C-FECT and APC using 600

fresh fecal specimens collected in the field.

The stool samples (n=600) were taken from villagers of Moklan, Nakhon Si Thammarat Province, Thailand. The samples were kept in a cooling box at 20°C to 25°C and were immediately carried to the laboratory 7 km away from the study field and examined by M-FECT [6], C-FECT [8], and APC [7] within 4 hr after defecation at 6 am. For APC, 3 g of each stool sample was placed at the center of a nutrient agar plate and incubated at room temperature (28° to 33°C) for up to 5 days. If tracks from movements of worms and/or larvae as well as free-living adult worms were not observed under a stereomicroscope on day 3 (48 hr) [7], the observation period was extended to days 4 and 5 [9]. Each microscopically positive dish was flooded with 10 ml of 10% formalin to collect worms for species identification using a compound microscope (×40). For M-FECT, 2 g of fresh stool sample was suspended and stirred well in a tube containing 10 ml of 0.85% saline. The fecal suspension was filtered through 2 wire meshes (4×4 cm) into a plastic centrifuge tube. The fine mesh (1.2×1.2 mm) was placed on top of the funnel while the coarse mesh (2×2 mm) was held by hand about 1 cm above the fine one. Fecal materials trapped on both meshes were washed off with 3 ml of 0.85% saline. The resulting suspension was centrifuged at 700 g for 5 min. The supernatant was decanted, the volume was adjusted to 7 ml with 10% formalin without mixing, and 3 ml of diethyl ether was then added. The centrifuge tube was closed and

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*Corresponding author (awitthay@wu.ac.th)

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Table 1. Comparative efficacies of M-FECT, agar plate culture, and C-FECT for detection of *Strongyloides stercoralis* larvae in 600 stool samples^a

Larvae /g stool (M-FECT method)	No. samples with this count (M-FECT)	Agar plate culture No. of positive (negative)	C-FECT No. of positive (negative)
0	400	10 (390)	0 (400)
1-10	67	63 (4)	2 (65)
11-25	50	44 (6)	18 (32)
26-50	45	44 (1)	43 (2)
51-75	17	16 (1)	17 (0)
76-4,000	21	21 (0)	21 (0)

^aAliquots of each sample were processed using each of the 3 methods, and the results for the M-FECT method (first column) were used as the standard, against which the other 2 were compared.

shaken vigorously by hand for 1 min and immediately centrifuged at 700 g for 5 min. The debris plug was loosened and the top 3 layers were poured off. The sediment was suspended in approximately 1 ml of 10% formalin, and the entire suspension was examined under a microscope. The number of larvae was counted and presented as the number of larvae per gram (LPG) of feces. For C-FECT, the process was the same as M-FECT except that 2 layers of wet gauze were used instead of wire mesh (fecal materials trapped on the gauze were also washed off with 3 ml of 0.85% saline) and the sediments adjusted to 7 ml with 10% formalin was mixed well and allowed to stand for 10 min before adding 3 ml of diethyl ether.

This study was approved by the Ethical Clearance Committee on Human Rights Related to Researches Involving Human Subjects, Walailak University. McNemar Chi-square test (SPSS 13.0 for Windows) was used for calculation of statistical difference.

Out of 600 stool samples, 210 were positive for *Strongyloides* larvae at least by 1 of the 3 methods. The positive detection rate was 33.3% (200/600) by M-FECT, 16.8% (101/600) by C-FECT, and 33.0% (198/600) by APC (Table 1). The positive rate by M-FECT was significantly higher ($P < 0.001$) than that by C-FECT and was comparable ($P = 0.832$) to that of APC. The detection rates of M-FECT and APC were about 2 times higher than that of C-FECT. Compared to the C-FECT, M-FECT could detect low level infections at the larval intensity of ≤ 50 LPG (Table 1). Thus, M-FECT seems to be useful for the diagnosis of asymptomatic strongyloidiasis patients with low level infections in field surveys. In the present study, the coincidence rate between M-FECT and APC was over 90% (Table 2), suggesting that M-FECT can be used as an alternative to APC. Conversely, 6% (12/200) of M-FECT positive samples were negative

Table 2. Coincidence of *Strongyloides* larvae detection by M-FECT and agar plate culture

		Agar plate culture		
		No. of positive	No. of negative	Total
M-FECT	No. of positive	188	12	200
	No. of negative	10	390	400
	Total	198	402	600

by APC and 5.1% (10/198) of APC positive samples were negative by M-FECT. Thus, to increase the sensitivity of human strongyloidiasis diagnosis, a combination of both methods is recommended whenever it is practically possible.

In this study and in our previous study [6], the increase of the efficacy of detection of *Strongyloides* larvae by M-FECT has been achieved by 2 simple modifications. Firstly, fresh stool suspensions, instead of preserved stools, was used, and the samples were exposed to formalin for as shorter time as possible. Longer exposure of larvae to formalin makes them less dense and many were trapped underneath the plug of debris after centrifugation [6]. Secondly, the use of 2 wire meshes followed by 3-ml NSS rinsing could reduce the accidental trapping of larvae in the 2 layers of wet gauze [6].

In conclusion, this study revealed that simple modifications of C-FECT markedly increased the sensitivity of the routine laboratory method for detection of *Strongyloides* larvae.

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