

## Comparison of the Quantitative Formalin Ethyl Acetate Concentration Technique and Agar Plate Culture for Diagnosis of Human Strongyloidiasis

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**The quantitative formalin ethyl acetate concentration technique (QFEC) was compared to agar plate culture (APC) for the detection of *Strongyloides stercoralis* larvae. QFEC could substitute for APC only when the parasite load was higher than 50 larvae per g of stool. This study serves as a good reminder to those conducting stool exams about the sensitivity and specificity limitations of both techniques.**

Agar plate culture (APC) is a highly effective technique for the coprological diagnosis of human strongyloidiasis (1, 5–8, 10). But despite its high sensitivity, there are several disadvantages to APC. It is expensive, it takes a long time before results can be provided, and there is also a risk of infection for the technicians. The quantitative formalin ethyl acetate concentration technique (QFEC) is another effective and more rapid method to detect and quantify intestinal helminths, e.g., *Ascaris lumbricoides* (3) and *Opisthorchis viverrini* (4). In the present study, we compared APC results to the different intensities of *Strongyloides stercoralis* larvae determined by QFEC.

Stool samples ( $n = 1,233$ ) were collected from populations in northeast Thailand and examined by APC (6) and QFEC (3). For APC, 3-g portions of individual fecal samples were placed in the centers of nutrient agar plates during the field survey. The plates were kept in a box and transported at room temperature (30 to 35°C) to the laboratory, where they were incubated for another 5 days at room temperature. The agar plates were examined under a stereomicroscope for the presence of tracks from moving larvae or free-living adults on the 3rd and 5th days. All microscopically positive dishes were further processed by washing the surface of the agar with a 10% formalin solution to collect worms for species identification. The differentiation of *S. stercoralis* larvae from hookworm larvae was performed under microscopic magnification of  $\times 40$ . For QFEC, 2 g of individual fecal samples was weighed out and then stirred well in a vial containing a merthiolate-iodine-formalin solution (2). The preserved stools were processed by QFEC (3). Briefly, the preserved fecal suspension was filtered through two layers of wet gauze into a centrifuge tube. The volume was adjusted to 10 ml with 10% formalin. Two milliliters of ethyl acetate was added, and the tube was closed and shaken for half a second. The stopper was removed, and the

tube was centrifuged at  $600 \times g$  for 10 min. The plug of debris along with the merthiolate-iodine-formalin solution was discarded by inverting the tube, leaving only the sediment, which was resuspended. The entire suspension was examined under a microscope. The total number of larvae in the sediment was counted and is presented as the number of larvae per gram of stool (lpg). A negative result meant that no larvae were seen in the whole sediment. This study protocol was approved by the Human Research Ethics Committee of Khon Kaen University.

Of the 1,233 stool samples, 303 (24.57%) were found to be positive by at least one of the two methods: 130 (10.54%) by QFEC and 290 (23.52%) by APC (Table 1). As expected, APC's superior sensitivity was demonstrated with 173 specimens that were negative by QFEC but positive by APC. Only 13 QFEC-positive specimens were negative by APC. Compared to APC, QFEC had a statistically significantly lower positivity rate ( $P < 0.001$ ) (Pearson chi-square test, SPSS for Windows version 11.0; SPSS Inc., Chicago, Ill.). When we classified QFEC results into 10 lpg intervals, 45 (97.8%) out of 46 samples with more than 50 lpg by QFEC gave positive results by APC. This fact seems to indicate similar diagnostic values at that level of infection.

APC has consistently been found to be 1.6 to 6.0 times more effective than the formalin-ether concentration technique (1, 7). In addition, it has been found to be more sensitive than QFEC (9). Even though it is less expensive and more rapid than APC, QFEC can substitute for APC only when the stool parasite load is higher than 50 lpg. In the present study, those instances constituted only 35.38% (46 of 130 specimens) of QFEC-positive stools. Thus, in community surveys for ascariasis and opisthorchiasis, where QFEC is a useful tool for the quantification of infections, it cannot be relied upon for a determination of the prevalence of strongyloidiasis. The low detection rate by QFEC may be attributed to several factors. One is that filtration of the fecal suspension through layers of gauze may remove feces containing larvae, resulting in the loss of larvae in the final sediment, but we have no data on this issue.

Although it is widely accepted that formalin-ether sedimen-

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TABLE 1. Comparative efficacies of APC and QFEC for the detection of *S. stercoralis*

QFEC result (larval intensity) <sup>a</sup>	APC result	
	No. of positive specimens	No. of negative specimens
0	173	930
1–50	72	12
>50	45	1

<sup>a</sup> The intensity of *S. stercoralis* larvae expressed as numbers of larvae per gram of stool. "0" indicates that the stool specimens were negative by QFEC.

tation and related techniques are less sensitive than APC, one should bear in mind that a substantial portion of stools positive by these techniques were negative by APC. In the present study, 10% of QFEC-positive samples were negative by APC, while Sukhvat et al. (10) found that 20% of *Strongyloides*-positive stools examined by the formalin-ether sedimentation technique were negative by APC. No explanation was provided, and it remains to be explored which factors cause *Strongyloides* larvae not to grow and multiply under the sensitive APC technique.

This study is based upon screening a population in a field survey; thus, the subjects were not symptomatic patients. QFEC is expected to be more sensitive for symptomatic individuals, who, presumably, would have a much higher parasite burden. Thus, QFEC may be sufficient for clinical purposes, while APC can be added as a second-tier laboratory test. In conclusion, this study shows clearly that for epidemiologic purposes APC is preferable and QFEC should be used for confirming clinical diagnoses of strongyloidiasis.

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