

COMPARISON OF PARASITE AND OVA IDENTIFICATION BY FORMALIN-ETHYL ACETATE CONCENTRATION AND AUTOMATIC METHOD (SCIENDOX 50®)

Nuntiya Sawangkla, Pisith Chinabut, Jerapas Thongpiya* and Kosol Roongruangchai*

Received: April 01, 2021; Revised: April 21, 2021; Accepted: April 22, 2021

Abstract

Intestinal parasitic infections in Thailand remain a significant healthcare burden. To expose the presence of parasites, concentration technique is associated with higher detection rates of intestinal ova and parasites even in small numbers, when compared with the direct wet mount preparation. The most commonly used concentration technique is formalin-ethyl acetate (FECT), however this method is complex, time consuming, and posing hazard due to chemical reagents involved. The Sciendox 50® automatic machine is a newly developed machine processing feces using a sedimentation concentration technique similar to FECT. This study aims to evaluate the detection of ova and parasites of positive specimens including a variety of nematodes, trematodes, cestodes, and protozoa, of FECT and precipitate processed from Sciendox 50® machine. Kappa inter-rater reliability value yielded a perfect agreement (Kappa = 1). In conclusion, the Sciendox 50® machine is suitable for clinical use as a concentration technique device with additional benefits of higher safety to laboratory technicians and shorter processing times.

Keywords: Automatic stool examination, FECT, Formalin-ethyl acetate concentration, Sciendox 50®, Stool concentration technique

Introduction

Despite improvement of personal hygiene and sanitation, gastrointestinal parasitic infections remain significant healthcare burden, especially in low- resource setting in some rural areas of Thailand. A nationwide survey revealed that 18.1% of stool samples collected from 75 provinces in Thailand tested positive for *Opisthorchis viverrini*, hookworm and *Strongyloides stercoralis*, which are the three most prevalent helminths in Thailand. (Wongsaroj *et al.*, 2014) Another recent survey yielded consistent result of parasitic contamination

in fresh vegetable samples from Southern region of Thailand, which was high with hookworm and *Strongyloides stercoralis* predominance. (Punsawad *et al.*, 2019) The pathogenic organisms cause a variety of gastrointestinal manifestations, ranging from mild forms i.e., colitis, dysentery, malabsorption, to more invasive forms i.e., pneumonia, and peritonitis, eventually resulting in healthcare burden.

Microscopic examination of ova and parasites in fecal specimen is the gold standard method.

Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Wanglang Road, Bangkok Noi, Bangkok 10700, Thailand. Tel: (+66)2-419-6468, Fax: (+66)2-419-6470, E-mail: jerapas.tho@mahidol.edu; kosol.run@gmail.com

* Corresponding author

Direct wet-mount microscopy is a fast and simple procedure for identifying species of ova and parasites, together with approximation of the parasitic burden. Moreover, motility of protozoan trophozoites remains active during microscopic observation, allowing species identification. In case of low parasitic burden, gastrointestinal parasitic infections may be underdiagnosed when using the direct wet-mount microscopy. Thus, a larger amount of feces is subjected to a concentration step prior to microscopic examination, allowing species identification. Formalin-ethyl acetate sedimentation (FECT), the most commonly used concentration technique, allows detection of low burden of parasites, addressing low sensitivity of the direct wet mount microscopy. (Oguoma and Ca, 2006; Khanna *et al.*, 2014; Garcia *et al.*, 2017) The fecal concentration procedure separates parasites from fecal debris and increases the chances of detecting parasites when these are in small numbers. However, the FECT has several limitations, including exposure to highly hazardous chemicals of laboratory technicians, flammability, requirement of proper elimination of environment toxic agents long period required to carry out the test, and distortion of trophozoites or cysts. (Young, 1979; Garcia and Shimizu, 1981; CDC, 2016; NCBI, 2020)

Therefore, there is a need for a tool to automatically perform the FECT to avoid such hazard and shorten the time for large-scale examination, while sensitivity and species identification remain consistent. Sciendox 50® is a newly developed automatic instrument that performs a formalin-ethyl acetate-free sedimentation in a closed system. Diagnostic performance of the Sciendox 50® is unknown in a real laboratory use. Thus, this study aims to examine the diagnostic performance in comparison to conventional FECT.

Materials and Methods

Sample collection and preparation

The stool samples, which were positive for ova and parasites (species identified using direct wet mount), were collected after submission from patients who visited the Faculty of Medicine, Siriraj Hospital, Mahidol University. The examiners primarily used 10% formalin to preserve specimens if ova and parasites were observed. There are ninety-five formalin-preserved and sixteen fresh, non-preserved samples recruited in this study. An average time for direct wet smear is 30 seconds by 2 laboratory technicians. The cost of direct wet smear at Siriraj hospital is 100 Thai bahts.

Formalin-ethyl acetate sedimentation concentration technique (FECT)

The FECT was adopted from previously described technique with the following steps. (Allen and Rideley, 1970) Briefly, in a biosafety cabinet class 2, a 1-2 gram of feces was mixed with 10 mL of 10% formalin. The suspension passed through a 2-layer gauze pre-soaked with normal saline. Then, a 1 mL of ethyl acetate was added into the filtered feces in 8 mL conical tube. The mixture was rigorously shaken by hand for 1 minute, and centrifuged at 2,500 rpm for 4 minutes. The precipitate at the bottom of the tube was collected for microscopic examination. An average time for FECT process is 15 minutes by 2 laboratory technicians. The cost of FECT at Siriraj hospital is 250 Thai bahts.

Automatic stool processing machine (Sciendox 50®)

The fecal specimen, up to 1 gram, was loaded into Sciendox 50® sample container. The machine operates with the following steps of liquidation and filtration. Briefly, 0.9% NaCl was added into Sciendox 50® sample container for liquidation. After mixing, the liquidized samples were passed through a 2-layer of filters: 2 mm and 275 µm in diameter respectively. The filtrates are final products, and composed of supernatant solution and precipitate, as depicted in Figure 1 and Figure 2. The precipitates were subjected to morphological examination. An average time for Sciendox 50® automatic stool processing is 2 minutes by 2 laboratory technicians. The cost of Sciendox 50® machine Figure 3 is approximately 1,000,000 Thai bahts. The cost to operate one stool sample including container and processing, is 60 Thai bahts. Two independent reviewers (laboratory scientists:



Figure 1. Sciendox 50® container.



Figure 2. Schematic process by the machine.

NS, PC) blindly examined the positive specimens processed by both methods for intestinal ova and parasites under compound microscope.



Figure 3. Sciendox 50® machine.

Statistical analysis

A sample size calculation of 100 samples was carried out. Kappa was 0.7 with a 95% confidence interval with an acceptable error of 0.07.

To compare the detection rate of FECT and Sciendox 50® machine, SPSS 18.0 software was used for statistical analysis. An interrater reliability analysis using the Kappa statistic was performed to determine consistency among raters (FECT and Precipitate from Sciendox 50®). Intraclass correlation coefficient or Kappa is considered to be significant to least 0.7 (substantial agreement) within 95% confidence interval. (McHugh, 2012).

Results

The randomly selected 111 specimens were processed by both FECT and Sciendox 50® and examined under compound microscope. Ninety-five formalin-based specimens including 7 cestode, 50 nematode, 20 protozoa, 5 trematode and 13 mixed infection, were identically discovered in specimens processed by both methods. Of sixteen fresh specimens including 8 nematode, 6 protozoa, 1 trematode, and 1 mixed infection, parasites were identically discovered in specimens processed by both methods. Mixed infection consisted of two-species combination between larva, egg, and cyst. The result demonstrated the identical findings between the two methods, regardless of the types of specimen either formalin-infused or fresh specimen. A variety of phyla of parasites was consistently uncovered including cestodes, nematodes, protozoa, trematodes, and mixed infection without missing organisms. The list of all parasites included in this study is in the supplemental data. The Kappa value in all phyla is 1 (95% CI: N/A), which is a perfect agreement, as described in Table 1 and Table 2.

The preparation time by direct wet smear, FECT, and Sciendox 50® was half, fifteen, and two

minute(s) respectively. FECT exposed laboratory technicians with formalin and ethyl acetate, whereas, direct wet smear and Sciendox 50® were chemical-free. The cost of each method was compared as described in Table 3.

Table 1. Detection of parasites from formalin-based feces processed by FECT and Sciendox 50® with Kappa value.

Phylum of parasites	FECT	Sciendox 50®	Kappa value (95% CI)
Cestodes	7	7	1(N/A)
Nematodes	50	50	1(N/A)
Protozoa	20	20	1(N/A)
Trematodes	5	5	1(N/A)
Mixed infection	13	13	1(N/A)
Total	95	95	1(N/A)

Table 2. Detection of parasites from formalin-based feces processed by FECT and Sciendox 50® with Kappa value.

Phylum of parasites	FECT	Sciendox 50®	Kappa value (95% CI)
Nematodes	8	8	1(N/A)
Protozoa	6	6	1(N/A)
Trematode	1	1	1(N/A)
Mixed infection	1	1	1(N/A)
Total	16	16	1(N/A)

Table 3. Preparation time, chemical exposure, and cost of each method.

	Direct wet smear	FECT	Sciendox 50®
Preparation time (minutes)	0.5 (30 s)	15	2
Chemical exposure			
Ethyl acetate	-	+	-
Formalin	-	+	-
Cost per sample (Thai Baht)	100	250	60*

*Sciendox 50® machine was not included.

Discussion

Recent advances in science and technology have led to the development of commercial kits that have comparatively similar accuracy with the conventional technique. (Soares, 2020) Several studies have been conducted to evaluate the clinical use of commercial kits, and demonstrated the promising results when compared with FECT. For instance, study on Feconomics®, and Mini Parasep®. (Kurt *et al.*, 2012; Sanprasert *et al.*, 2016) This is the first study to investigate the clinical use of Sciendox 50®. The Sciendox 50® machine processes fecal specimens through the

sedimentation concentration technique, which resembles FECT.

From the results, the Sciendox 50® machine is reliable to process automatic stool concentration technique for detecting the presence of parasites commonly endemic in Thailand. Both preserved and fresh specimens are suitable for the Sciendox 50® machine. Accordingly, our findings revealed similar reliability as of other published studies on commercial kits as mentioned earlier. The machine is suitable for laboratory use especially in the area that lacks specialized personnel.

In terms of safety, the specimen loaded into the container is processed within the machine, there is no hazard to the operating staff. The machine runs in a standardized manner leading to negligible errors when compared against human errors. Moreover, turnaround time for specimens processed by Sciendox 50® was comparatively shorter than conventional method mentioned above. However, a concerning disadvantage of this machine is the cost, the specimen container, as well as the training required to operate the machine.

Further study should be conducted to elicit the reliability of Sciendox 50® comparing with the use of other stool concentration technique, as our study compared only to formalin-ethyl acetate concentration technique. In addition, the clinical specimen should emphasize on using the fresh specimen to further validate the reliability of this machine.

Conclusions

The Sciendox 50® automatic machine can be an alternative to formalin-ethyl acetate stool concentration technique (FECT) with comparable yield of ova and parasites detection, better safety for laboratory technicians, and shorter processing times.

Acknowledgement

This study was supported by Meditop Co., Ltd. for the Sciendox 50® machine use. This study has not been involved by any personnel nor related entities of Meditop Co.Ltd. We would like to thank to Assoc.Prof.Dr. Anchalee Tuntrongjit and Dr. Kasem Kulkaew, faculty of the Department of Parasitology, Faculty of Medicine, Siriraj Hospital, Mahidol University, for their contributions in support and manuscript editing. and laboratory technicians of the Parasitology lab, Siriraj Hospital, for their help during the study period. Authors

declare that there is no conflict of interest in this research.

Supplemental data

Species of parasites included in this study as follows:

Cestodes: *Hymenolepis nana* egg, *Taenia* spp. egg
Nematodes: *Ascaris lumbricoides* egg, *Capillaria philippinensis* egg, Hookworm egg, *Strongyloides stercoralis* L1 larva, *Trichuris trichiura* egg
Protozoa: *Blastocystis hominis* cyst, *Cystoisospora belli* oocyst, *Endolimax nana* cyst, *Entamoeba coli* cyst, *Entamoeba histolytica* cyst, *Giardia lamblia* cyst, *Giardia lamblia* trophozoite
Trematodes: *Fasciolopsis buski* egg, *Opisthorchis viverrini* egg, *Paragonimus westermani* egg

References

- Allen, A.V. and Rideley, D.S. (1970). Further observations on the formol-ether concentration technique for faecal parasites. *J. of clinical pathology.*, 23(6):545-546.
- CDC-DPDx. (2016). Diagnostic Procedures-Stool Specimens. Available from: <https://www.cdc.gov/dpdx/diagnosticprocedures/stool/specimenproc.html>. Accessed date: Apr 01, 2021.
- Garcia, L.S. and Shimizu, R. (1981). Comparison of clinical results for the use of ethyl acetate and diethyl ether in the formalin- ether sedimentation technique performed on polyvinyl alcohol- preserved specimens. *J. of clinical microbiology.*, 13(4):709-713.
- Garcia, L., Arrowood, M., Kokoskin, E., Paltridge, G., Pillai, D., and Procop, G. (2017). Practical guidance for clinical microbiology laboratories: laboratory diagnosis of parasites from the gastrointestinal tract. *Clinical Microbiology Reviews.*, 31(1).
- Khanna, V., Tilak, K., Rasheed, S., and Mukhopadhyay, C., (2014). Identification and preservation of intestinal parasites using methylene blue-glycerol mount: a new approach to stool microscopy. *J. of Parasitology Research.*, 2014
- Kurt, O., Akyar, I., Görgün, S., Kocagöz, T. and Özbilgin, A. (2012). Feconomics®: a simple, novel and fast technique for stool concentration in parasitology laboratory. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi.*, 18
- McHugh, M.L. (2012). Interrater Reliability: The Kappa Statistic. Available from: <https://pubmed.ncbi.nlm.nih.gov/23092060/>. Accessed date: Apr 01, 2021.
- NCBI, National Center for Biotechnology Information. (2020). Pubchem Compound Summary For CID 8857, Ethyl Acetate. Available from: <https://pubchem.ncbi.nlm.nih.gov/compound/Ethyl-acetate>. Accessed date: Apr 01, 2021.
- Oguoma, V. and Ca Ekwunife. (2006). The need for a better method: comparison of direct smear and formol-ether concentration techniques in diagnosing intestinal parasites. *The Int. J. of Tropical Medicine.*, 3
- Punsawad C, Phasuk N, Thongtup K, Nagavirochana S. and Viriyavejakul P. (2019). Prevalence of parasitic contamination of raw vegetables in Nakhon Si Thammarat province, southern Thailand. *BMC Public Health.*, 19(1):34.

- Sanprasert, V., Charuchaibovorn, S., Srirungruang, S., Nuchprayoon, S. (2016). Comparison between direct smear, formalin-ethyl acetate concentration, and Mini Parasep® Solvent-Free Concentrator for screening of intestinal parasitic infections among school-age children. *Chula Med J.*, 60:255-269.
- Soares, F., Benitez, A., Santos, B., Loiola, S., Rosa, S. and Nagata, W. (2020). A Historical Review Of The Techniques Of Recovery Of Parasites For Their Detection In Human Stools *Rev. Soc. Bras. Med. Trop.* Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0037-86822020000100203&lng=en. Accessed date: Apr 01, 2021.
- Wongsaroj, T., Nithikathkul, C., Rojkitikul, W., Nakai, W., Royal, L. and Rammasut, P. (2020). Brief communication (Original). National Survey of Helminthiasis In Thailand. *Asian Biomedicine.*, 8(6):779-783.
- Young, K., Bullock, S., Melvin, D. and Spruill, C. (1979). Ethyl acetate as a substitute for diethyl ether in the formalin-ether sedimentation technique. *J. of Clinical Microbiology.*, 10(6):852-853.