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Original Article

Diagnostic Performance of Mini Parasep® Solvent-Free Foecal Parasite Concentrator for the Diagnosis of Intestinal Parasitic Infections

Abhishek Mewara, Sumeeta Khurana, Swati Gupta[1], Vimal S. Munda[1], Shreya Singh[1], Rakesh Sehgal

Departments of Medical Parasitology and [1]Medical Microbiology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

Abstract

Introduction: Various stool concentration techniques have been used to increase the microscopic detection of parasites. We assessed the enclosed, single-vial, Mini Parasep® technique in comparison to the currently used coprodiagnosis procedures. **Materials and Methods:** A total of 150 stool samples were collected. Samples concentrated by formol–ether method (FEM) and Mini Parasep® SF foecal concentrator and unconcentrated samples were subjected to wet mount, iodine mount microscopy and smear examination by modified acid-fast staining. **Results:** Direct wet mount detected 72 positive samples (48.6%), whereas 77 (51.3%) and 80 (53.3%) samples were detected by FEM and Mini Parasep® SF methods, respectively. The sensitivity of detection of parasites was 98.7%, 95% and 90.1% with Mini Parasep®, FEM and direct microscopy, respectively. A clearer background with less foecal debris and a better yield of *Hymenolepis nana*, *Trichuris trichiura*, *Entamoeba coli* and *Giardia lamblia* were noted with Mini Parasep®. **Conclusion:** Mini Parasep® SF technique is simple, rapid and less cumbersome than conventional diagnostics, making it suitable for routine use. In addition, it offers higher sensitivity and better background clearance than both direct stool examination and FEM.

Keywords: Mini Parasep®, parasites, stool concentration

Introduction

Diarrhoeal diseases are the second-most common cause of mortality in children under the age of 5 years and is responsible for nearly 5 million deaths per year in developing countries.[1] Although several pathogens including bacteria, parasites and viruses can cause diarrhoea, intestinal parasites are present in 3.5 billion people and clinically manifest in 450 million, accounting for 20%–30% of diarrhoeal diseases.[2,3] Apart from acute manifestations, prolonged intestinal colonisation, particularly by soil-transmitted helminthes, also results in growth stunting and reduced physical fitness, in addition to impairments in memory and cognition in affected children.[4] Other adverse health consequences include poor performance in school and reduced school attendance.[5]

The high clinical, educational, social and economic burden of intestinal parasitic infections provides an important rationale for the prompt diagnosis of such cases. Unfortunately, diagnostic modalities for detection of these agents in stool have stagnated over the past few decades, and microscopy remains the mainstay of diagnosis in several laboratories.[6] Although the demonstration of parasitic agents on direct microscopy of stool is certainly specific for diagnosis, it has a low sensitivity and requires experienced microscopy for parasite detection and identification. To increase the microscopic detection of parasites, various concentration techniques have been introduced which separate parasites from the foecal debris and enable the detection of parasitic agents even when present in small numbers,[7,8] including flotation-based methods such as simple flotation, zinc

Access this article online

Quick Response Code:

Website: www.ijmm.org

DOI: 10.4103/ijmm.IJMM_19_44

Address for correspondence: Dr. Sumeeta Khurana, Department of Medical Parasitology, Postgraduate Institute of Medical Education and Research, Chandigarh - 160 012, India.
E-mail: sumeetakhurana@hotmail.com

Received: 04-02-2019 **Revised:** 05-08-2019
Accepted: 05-11-2019 **Published Online:** 05-12-2019

How to cite this article: Mewara A, Khurana S, Gupta S, Munda VS, Singh S, Sehgal R. Diagnostic performance of mini parasep® solvent-free foecal parasite concentrator for the diagnosis of intestinal parasitic infections. Indian J Med Microbiol 2019;37:381-6.

sulphate centrifugal flotation and Sheather's sugar flotation method and sedimentation-based methods such as formol–ether centrifugation method (FEM). While flotation-based methods provide a cleaner background than sedimentation-based methods, the latter causes less morphological alteration of organisms and also increases the recovery of operculated eggs. The formol–ether concentration method uses 10% formalin as fixative, and ether is added for fat and debris extraction, followed by filtration and centrifugation.[9] The labour-intensive procedure and hazards associated with the use of inflammable lipid solvents have encouraged the development of commercial products such as Parasep® which is a disposable, single-vial, enclosed foecal concentration system. It is a solvent-free foecal parasite concentrator without ether or ethyl acetate for clean and efficient concentration of helminth ova, larvae, protozoal cysts and oocysts. In this study, we assessed the single-vial Mini Parasep® technique in comparison to the currently used coprodiagnosis procedures i.e., direct microscopy and formalin-ethyl acetate sedimentation technique for the detection and comparative morphology of cyst/ova in stool.

Materials and Methods

Study details

This prospective study was conducted at the Department of Medical Parasitology at the Postgraduate Institute of Medical Education and Research, Chandigarh, from August to November 2018, and a total of 150 stool samples were collected.

Sample processing

Simple wet mount and iodine-stained mount were prepared from all unprocessed stool samples and examined. In addition, a smear was prepared, and modified (Kinyoun) acid-fast stain was performed as per the standard operating procedures for the detection of coccidian parasites.

For concentration of stool samples, FEM and the Mini Parasep® SF foecal concentrator (DiaSys, Berkshire, England) were used. For concentration by FEM, 5 ml of foecal suspension was strained through a wet gauze and 0.85% saline was added to bring it to a volume of 15 ml. Following centrifugation, the supernatant was discarded, and 10 ml of 10% formalin was added to the sediment and mixed thoroughly with a wooded applicator. Four millilitre of ether was added, and the tube was capped and shaken vigorously. The tube was then centrifuged at 500 g × 10 min. The free plug formed at the top of the tube was dislodged and the supernatant was discarded. The concentrated specimen was resuspended with few drops of 10% formalin and used. The Mini Parasep® SF foecal concentrator was used as per the manufacturer's instructions. Following concentration, the foecal samples were subjected to simple wet mount, iodine wet mount examination and modified (Kinyoun) acid-fast staining, and microscopic evaluation of the mount/smear was performed by two microbiologists independently who were blinded to the method used for sample preparation. All smears were graded on background clearing, detection of parasites and retention of morphological integrity of the detected parasites.

Statistical analysis

The results of all the three methods used were compared by Chi-square test, and $P < 0.05$ was taken as statistical significance. The sensitivity, specificity, positive predictive value and negative predictive value of the three methods were estimated. The interrater agreement for all the three methods was assessed by the Cohen's kappa test.

Results

Of the 150 samples examined, a total of 80 (53.3%) were positive for parasites (by any technique). Direct wet mount could detect 72 positive samples (48.6%), whereas 77 (51.3%) and 80 (53.3%) positive samples were detected by FEM and Mini Parasep® SF methods, respectively [Table 1]. Thus, an additional five and eight positive samples were detected by FEM and Mini Parasep® SF methods, respectively. The difference between the detection of parasites using direct wet mount and Mini Parasep® SF test was statistically significant ($\chi^2 = 117.2$, $P < 0.05$, by Yates correction, $\chi^2 = 113.7$, $P < 0.05$).

On analysing the profile of parasites, a total of 12 types of parasites could be detected in the entire study. Mini Parasep® SF technique could detect all the 12 parasites, whereas FEM and direct microscopy could both reveal 11 parasitic agents each. Amongst all the tested samples ($n = 150$), the following parasites were identified on direct wet mount: *Giardia lamblia* ($n = 39$, 26%), *Entamoeba histolytica*/dispar ($n = 20$, 13.33%), *Entamoeba coli* ($n = 7$, 4.6%), *Enterobius vermicularis* ($n = 2$, 1.33%), *Isospora* spp. oocyst ($n = 2$, 1.33%) and others. With FEM, *G. lamblia* ($n = 39$, 26%), *E. histolytica*/dispar ($n = 23$, 15.3%), *E. coli* ($n = 7$, 4.6%), *Hymenolepis nana* ($n = 2$, 1.33%), *E. vermicularis* ($n = 2$, 1.33%), *Isospora* spp. oocyst ($n = 2$, 1.33%) and others were detected. With Mini Parasep® SF, *G. lamblia* ($n = 40$, 26.6%), *E. histolytica*/dispar ($n = 23$, 15.3%), *E. coli* ($n = 9$, 6.0%), *H. nana* ($n = 3$, 2.0%), *E. vermicularis* ($n = 2$, 1.33%), *Isospora* spp. oocyst ($n = 2$, 1.33%) and others were detected [Table 2].

The comparative detection of various parasites by direct wet mount, FEM and Mini Parasep® SF revealed that two samples positive for *H. nana* were missed by direct mount and one was missed by FEM; *Trichuris trichiura* was only

Table 1: Results of direct microscopy and microscopy after concentration with formol-ether technique and Mini Parasep® solvent-free technique#

Technique	Positive, *n* (%)	Negative, *n* (%)	Types of parasites detected
Direct mount	72 (48.6)	77 (51.3)	12
Modified formol-ether	77 (51.3)	73 (48.6)	11
Mini Parasep® SF	80 (53.3)	70 (46.6)	12

#Total number of samples tested was 150. SF: Solvent free

detected by Mini Parasep® SF technique; three *E. histolytica*/dispar-positive samples missed by direct wet mount were detected following concentration by both methods and two additional *E. coli* and one *G. lamblia* were detected by Mini Parasep® SF technique compared to both the other methods. *Hymenolepis diminuta* was detected only in one sample by direct wet mount and was not seen following concentration procedures. A better clearing of the background in samples processed by Mini Parasep® SF method compared to FEM and direct microscopy was observed [Figure 1]. The morphology of all the parasites detected was unaltered in all the three methods except *G. lamblia* cyst which showed mild distortion following concentration by Mini Parasep®. The images of all the parasites detected following Mini Parasep® are depicted in Figure 2. The nomogram shown in Figure 3 displays the probability that a patient has parasitic infection after a positive (dotted line) or a negative (solid line) microscopic examination after processed by all the three methods. Dotted line indicates the positive likelihood ratio (LR+) for positive test which was 0.99 for all the three methods. Solid line indicates the negative likelihood ratio (LR−) for negative test result which was: 0.0, 0.04 and 0.1 for Mini Parasep®, FEM and direct microscopy, respectively. The sensitivity of direct wet mount method, FEM and Mini Parasep® SF method was 90.1%, 95% and 98.7%, respectively, whereas the specificity of all the three methods was 100% [Table 3]. The assessment of interrater reliability revealed a very good agreement (0.96) between Mini Parasep® and FEM by Cohen's kappa test [Table 4].

Discussion

The use of concentration methods to examine faeces for intestinal parasitic infections has been known to increase the likelihood of detecting cysts, ova and larvae, especially in specimens where they are present in insufficient numbers. The FEM technique by Ridley–Allen is the method of choice employed by most clinical laboratories and is also the method on which most commercial tests are based. The Mini Parasep® is a recently developed commercial method for foecal concentration which does not use hazardous solvents such as ether and can be performed in a single enclosed system. In this study, we evaluated the Mini Parasep® in comparison to the routine formol–ether stool concentration method and direct wet mount microscopy from unconcentrated stool.

Table 2: The spectrum of intestinal parasites detected by direct wet mount microscopy and microscopy after concentration with formol-ether technique and Mini Parasep® solvent-free technique

Intestinal parasite	Positive, *n* (%)		
	Direct wet mount	Formol-ether technique	Mini Parasep® SF technique
Hymenolepis nana	1 (0.66)	2 (1.33)	3 (2.0)
Hymenolepis diminuta	1 (0.66)	0 (0.0)	0 (0.0)
Hookworm	1 (0.66)	1 (0.66)	1 (0.66)
Ascaris lumbricoides	1 (0.66)	1 (0.66)	1 (0.66)
Trichuris trichiura	0 (0.0)	0 (0.0)	1 (0.66)
Strongyloides stercoralis	1 (0.66)	1 (0.66)	1 (0.66)
Enterobius vermicularis	2 (1.33)	2 (1.33)	2 (1.33)
Tapeworm	1 (0.66)	1 (0.66)	1 (0.66)
Entamoeba histolytica/dispar	20 (13.33)	23 (15.3)	23 (15.3)
Entamoeba coli	7 (4.6)	7 (4.6)	9 (6.0)
Giardia lamblia	39 (26)	39 (26)	40 (26.6)
Isospora spp. oocyst	2 (1.33)	2 (1.33)	2 (1.33)
Cryptosporidium spp. oocyst	1 (0.66)	1 (0.66)	1 (0.66)

The total number of samples tested was 150. SF: Solvent free

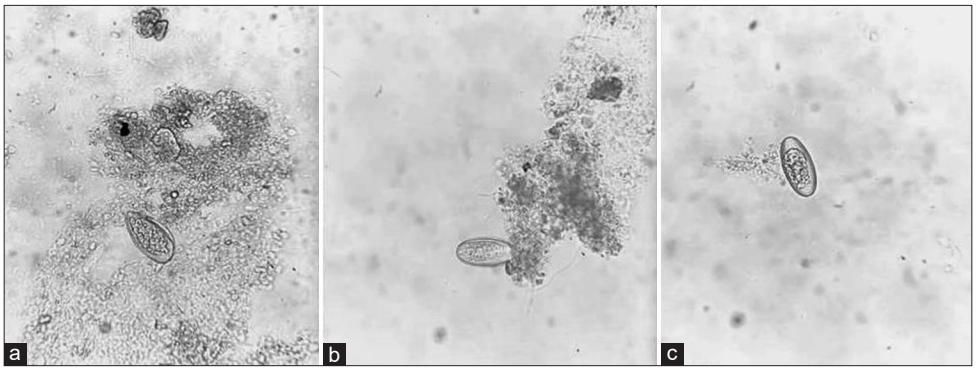

Figure 1: Comparison of background clearing on wet mount microscopy of unprocessed stool sample (a) and stool after concentration by formol–ether method (b) and Mini Parasep® technique (c) in a sample positive for *Enterobius vermicularis*

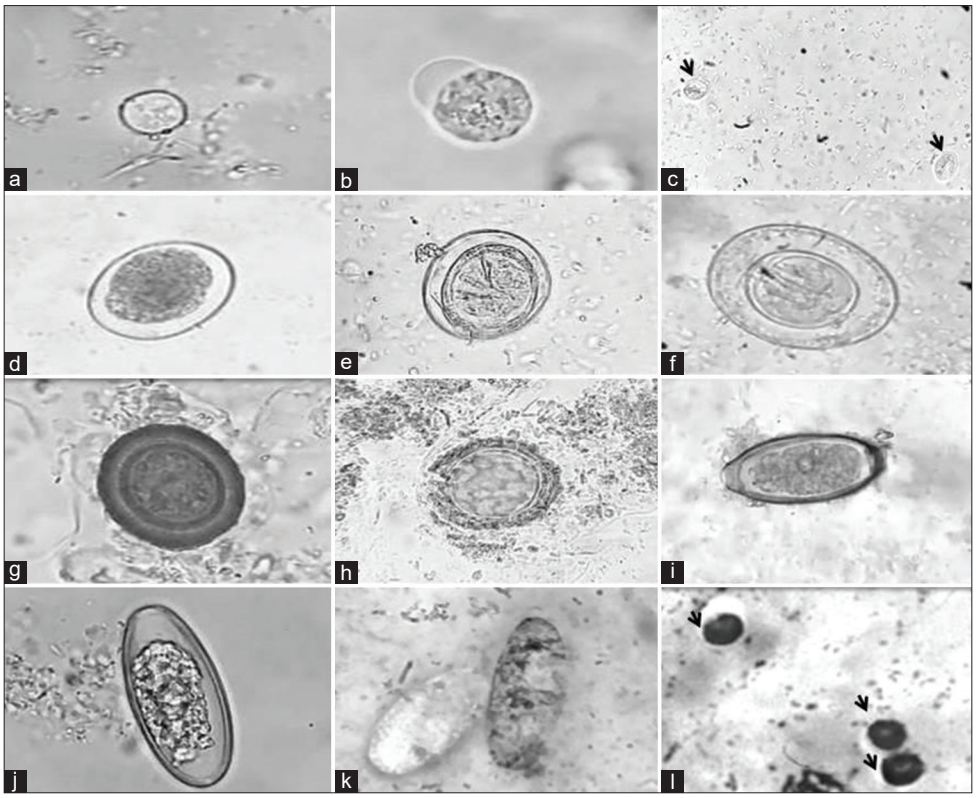

Figure 2: Wet mount microscopy and Kinyoun-stained smear images showing the intestinal parasites observed in the study: (a) *Entamoeba histolytica*/dispar cyst, (b) *Entamoeba* trophozoite, (c) *Giardia lamblia* cyst, (d) hookworm ova, (e) *Hymenolepis diminuta,* (f) *Hymenolepis nana,* (g) *Taenia* species, (h) *Ascaris lumbricoides* fertilised ova, (i) *Trichuris trichiura,* (j) *Enterobius vermicularis,* (k) *Isospora* spp. Oocysts and (l) *Cyclospora* oocysts

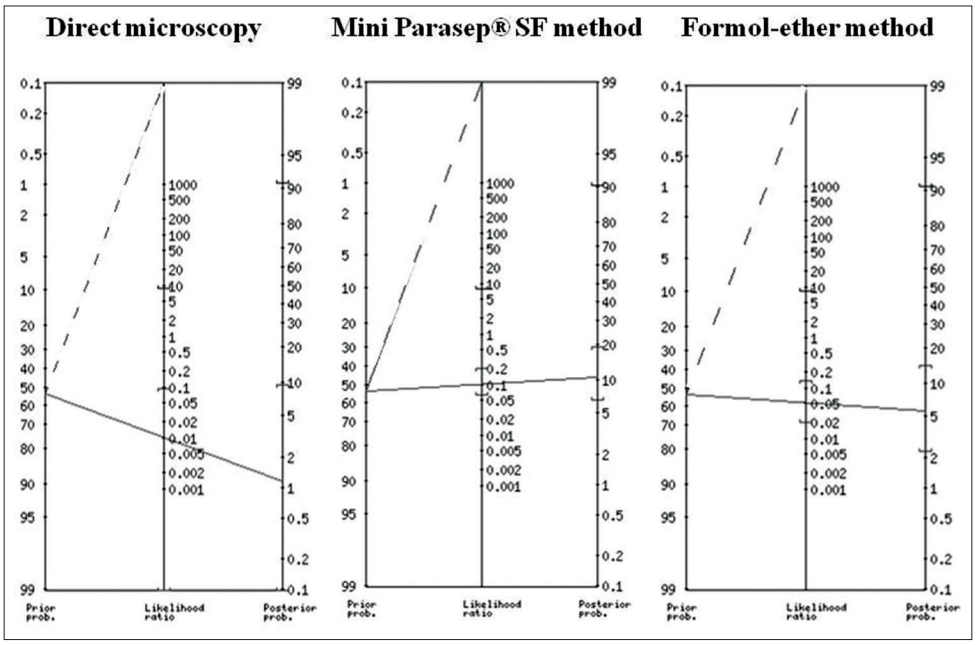

Figure 3: The nomogram depicting the probability of infection after positive (dotted line) and negative (solid line) results on Mini Parasep® SF, formol–ether concentration method and direct microscopy

A higher parasite detection rate was noted with Mini Parasep® SF (53.3%) compared to direct wet mount (48.6%) and FEM (51.3%) ($P < 0.05$). Amongst all the three techniques, the highest sensitivity for the detection of parasites was noted with Mini Parasep® (98.7%), followed by FEM (95%) and direct microscopy (90.1%), while the specificity of all the three methods was 100%. The evaluation of Mini Parasep® in a recent study revealed a sensitivity of 90.2%, which is

Table 3: Sensitivity, specificity, positive predictive value, negative predictive value and negative likelihood ratio of the three foecal microscopy techniques used

Parameter (%)	Direct wet mount microscopy	Formal-ether technique	Mini Parasep® SF technique
Sensitivity (95% CI)	90.1 (0.81-0.95)	95 (0.87-0.98)	98.7 (0.93-0.99)
Specificity (95% CI)	100 (0.94-1.00)	100 (0.94-1.00)	100 (0.94-1.00)
NPV (95% CI)	89.6 (0.81-0.94)	94.5 (0.86-0.97)	98.5 (0.90-0.99)
PPV (%)	100	100	100
Negative likelihood ratio (95% CI)	0.10 (0.05-0.19)	0.05 (0.02-0.13)	0.01 (0.0-0.09)

CI: Confidence interval, NPV: Negative predictive value, PPV: Positive predictive value, SF: Solvent free

Table 4: Interrater reliability of Mini Parasep® solvent-free technique, formol-ether concentration technique and direct wet mount techniques in stool samples

	Formal ether test +	Formal ether test −	Interrater reliability (κ)
Direct microscopy +	72	1	0.92 (good)
Direct microscopy −	5	72	
Mini Parasep® SF +	72	3	0.96 (very good)
Mini Parasep® SF −	0	70	
	Mini Parasep® SF +	**Mini Parasep® SF −**	**Interrater reliability (κ)**
Direct microscopy +	72	1	0.88 (good)
Direct microscopy −	8	69	

SF: Solvent free

comparable to our results.[10] Another study revealed a better accuracy of the commercial stool concentrator kit in field settings as compared to the direct smear and FEM.[11] In HIV patients in Thailand, a higher diagnostic yield for parasites was reported with Parasep® (10.5%), compared to direct microscopy (8%) and FEM (4%).[12] A higher sensitivity of 56.38% was noted in school-age children,[13] while a sensitivity of 55.2% has been reported in adults working as gardeners.[14] Apart from a better parasitic yield, a lower turnaround time and a better workflow capacity on using Mini Parasep® along with a better clearing of the background with less distortion of parasite morphology has also been reported, similar to our experience.[15] This method has also been reported to be more sensitive particularly for the detection of *Ascaris lumbricoides, Schistosoma mansoni* and *H. nana* infections.[10] We observed that in samples processed by Mini Parasep®, a better yield of *H. nana, T. trichiura, E. coli* and *G. lamblia* was noted, whereas *E. histolytica*/dispar cyst detection was better that direct mount but comparable to FEM. The interrater reliability was very good ($\kappa = 0.96$) between the examination of samples processed by Mini Parasep® and FEM and good ($\kappa = 0.88$) between Mini Parasep® and direct microscopy, indicating consistency amongst the individual observations.

The self-contained design of the Mini Parasep® in addition to its long storage time after sample preparation provides a considerable advantage over other methods, particularly for field conditions and remote primary care settings. This method also has a lower turnaround time compared to the FEM (approximately 4 min vs. 15 min per sample).[15] Not only is the Mini Parasep® more sensitive, rapid and less cumbersome, but is also more cost-effective in high-burden regions and high-throughput laboratories.[16] Although a rough estimate of cost suggests that FEM is much cheaper than Mini Parasep®, the cost may prove to be comparable after accounting for the limitations of FEM such as longer processing time and need for trained laboratory personnel.

Conclusion

The Mini Parasep® SF technique is simple, efficient and rapid compared to the conventional stool examination protocols. It provides a better background clearance for the detection of intestinal parasites and concentrates the sample effectively. This method holds potential for application as a routine concentration procedure in clinical parasitology laboratories with a heavy sample load as it is less cumbersome, does not involve the use of inflammable reagents and has a high turnaround time.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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