JOURNAL OF CLINICAL MICROBIOLOGY, June 1990, p. 1094–1097
0095-1137/90/061094-04$02.00/0

Vol. 28, No. 6

Parasite Detection Efficiencies of Five Stool Concentration Systems

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Received 24 October 1989/Accepted 12 February 1990

Fresh fecal material that was free of ova and parasites was pooled with 10% Formalin in a 1:4 ratio to prepare a standard specimen. Portions of 100 ml of this specimen were individually seeded with *Cryptosporidium* oocysts, *Entamoeba coli*, *Entamoeba histolytica*, and *Giardia lamblia* cysts; ova of *Necator americanus*; and *Strongyloides* larvae. Appropriate volumes of each parasite suspension were used to evaluate the Fecal Concentrator Kit (Remel, Lenexa, Kans.), Fecal Parasite Concentrator (Evergreen Scientific, Los Angeles, Calif.), Para-Pak Macro-Con (Meridian Diagnostics, Inc., Cincinnati, Ohio), and Trend FeKal CON-Trate (Trend Scientific, Inc., St. Paul, Minn.). A standardized gauze filtration method was used as the reference procedure. Tests were performed in triplicate with each individual parasite-concentrator combination, with three slides examined from each sediment. All of the systems effectively concentrated parasites compared with direct examination of unconcentrated fecal material. The Fecal Concentrator Kit provided the best overall performance. Clarity of sediment, lack of debris, and uniformity of background material were found to be important considerations for microscopic detection of parasites in concentrated specimens.

Parasitology has evolved much more slowly than other areas of clinical microbiology have, with there being a notable lack of instrumentation, automation, and technological development. However, commercially available preservatives packaged for home and hospital stool collection have obviated the need for rapid delivery of warm stool specimens for optimal detection of trophozoites and have reduced the incidence of leaking transport containers. Concentration procedures vary, but a modification of the original sedimentation method of Ritchie (4) is used by the majority of clinical laboratories (2, 6, 7).

Commercially available fecal concentration devices have proliferated since 1978 (3, 8, 9), helping to standardize the concentration procedure by providing consistency in methodology and subsequently improving parasite recovery and identification. Several of these concentration devices have been compared with the Formalin-ethyl acetate technique (3, 9), but detailed comparisons of individual concentration and detection efficiencies have been lacking.

In this report, we describe a protocol whereby the fecal suspension as well as the numbers and kinds of parasitic forms are standardized, providing a means of comparing the detection sensitivities of concentration systems based solely on procedural and design variations. The diversity inherent in fecal specimen consistency and content used in other comparisons could change the outcomes of specific parasite-device combinations.

MATERIALS AND METHODS

Fecal suspension and parasites. Fresh fecal material that was free of ova and parasites, with individual variations in mucus, cellular content, and consistency, was pooled in a 1:4 ratio with 10% buffered Formalin to prepare a large volume (1,800 ml) of a standardized specimen.

Formalin-fixed suspensions of *Cryptosporidium* oocysts, *Entameoba coli*, *Entamoeba histolytica*, and *Giardia lamblia* cysts; ova of *Necator americanus*; and *Strongyloides* larvae were obtained commercially (Scientific Device Laboratories, Inc., Glenview, Ill.). These organisms were selected because of their frequency of clinical occurrence and variation in size and were used to seed individually 100-ml portions of standardized fecal suspensions. This volume was adequate to perform three concentration procedures for each device and parasite combination. Parasite concentrations in stock suspensions were determined by counting and averaging the numbers of parasites present on direct examination of nine 0.01-ml samples. Sufficient volumes of these samples were separately added to respective 100-ml portions of stool specimens to allow the consistent detection of at least one parasite per cover slip upon direct examination (Table 1). No attempt was made to approximate the numbers of parasites routinely found in clinical specimens. Comparison of parasite numbers per 0.01 ml of unconcentrated and concentrated feces provided a basis for evaluating concentration efficiency. Each parasite-device combination was concentrated in triplicate, and the sediment from each trial was examined by making three separate slides for a total of nine microscopic examinations for each parasite-concentrator combination. A 10-μl pipettor (Medical Laboratory Automation, Inc., Pleasantville, N.Y.) was used for all volume measurements. Because of the heavy consistency of fecal suspensions and sediment, the distal 2 cm of each disposable pipette tip was removed with a scalpel, producing a bore size large enough to handle fecal material. The accuracy of the modified tips was endured by calibrating representative samples with an acid-base titrimetric instrument (VC-100; Streck Laboratories, Inc., Omaha, Nebr.).

Fecal concentration devices. The devices that we evaluated were the Fecal Concentrator Kit (FCK; Remel, Lenexa, Kans.), Fecal Parasite Concentrator (FPC; Evergreen Scientific, Los Angeles, Calif.), Para-Pak Macro-Con (PMC; Meridian Diagnostics, Inc., Cincinnati, Ohio), and Trend FeKal CON-Trate (TFC; Trend Scientific, Inc., St. Paul, Minn.). A standardized gauze filtration method was used as the reference procedure (5), with two layers of moistened gauze (Curity 8 ply; The Kendall Co., Boston, Mass.) and a 9-cm polystyrene funnel (American Scientific Products, McGaw Park, Ill.) used in the filtration step.

Systems were evaluated by processing the volume of

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Formalin-fixed fecal suspension specified in the instructions of the manufacturers (Table 2). The relative centrifugal force of centrifugation steps was controlled by converting specified force (gravity) into revolutions per minute on a centrifuge (model TJ-6; Beckman Instruments, Inc., Palo Alto, Calif.). Relative centrifugal force, the number and duration of centrifugation steps, reagent addition, and rimming and decanting procedures varied according to the instructions of the manufacturers. The sediment volume remaining in the tubes after the fluid was decanted varied with the initial volume of the specimen that was processed (Table 2).

Filter pore sizes were determined by averaging 10 separate measurements of representative filter material by using a dissecting microscope fitted with a calibrated ocular micrometer and was confirmed by the manufacturers.

Examination of sediment. Sediment volume was determined by premarking a set of tubes in 0.05-ml increments, using 10% buffered Formalin, to produce a template. After centrifugation and decanting, an accurate estimate of sediment volume was possible by comparison with the appropriate template. Sediment was thoroughly mixed with wooden applicator sticks with no diluent added. Triplicate 0.01-ml samples were pipetted onto microscope slides; and 1 drop of Dobell and O'Conner iodine solution was added (Spot Test; Difco Laboratories, Detroit, Mich.), mixed, and covered with a cover slip (22 by 22 mm). Cover slips were sealed by using equal parts of heated paraffin and petroleum jelly, which preserved the specimens for multiple examinations. Sediment containing *Cryptosporidium* oocysts was similarly pipetted onto slides and spread in a uniform thin layer of approximately 20 by 40 mm contained within the lines scribed on the undersides of the slides. A modified Kinyon acid-fast stain procedure (9) was performed, and the entire smear was examined at ×400 magnification. The sediment of each parasite-concentrator combination was subjected to nine microscopic examinations of the entire cover slip by using low power (×100) and low light intensity (1); suspicious objects and small parasites were examined at ×400 or ×1,000 when appropriate. The average number of organisms counted per 0.01 ml of fecal sediment was used to compare the parasite detection efficiency with the efficiency of parasite detection in 0.01 ml of the unconcentrated fecal suspension.

RESULTS

All systems effectively concentrated parasites compared with direct observation of unconcentrated material. Notable differences in the volume of feces processed, sediment volume, and speed and duration of centrifugation steps were evident (Table 2). The volumes of ethyl acetate added were similar, but the percentage of the total volume in that procedural step varied significantly.

Smaller parasite forms (cysts of *Entamoeba* and *G. lamblia*) were concentrated more effectively by all methods than the larger forms (hookworm ova and *Strongyloides* larvae) were. Devices with large filter pore sizes appeared to produce better results with the large parasite forms.

Combined averages of all parasites detected per 0.01 ml of sediment were compared with a similar average of parasites counted by direct examination (Table 1) and provided an overall detection rate for each fecal concentrator (Fig. 1). Although Fig. 1 does not reflect variations with individual parasite-concentrator pairings, it demonstrates the overall numbers of all parasite forms that were detected microscopically. These numerical differences should be taken as indi-

TABLE 1. Comparison of fecal concentration procedures with direct microscopic examination of seeded feces

Organism	Direct examination[a]		FPC		PMC		TFC		FCK		Gauze filtration	
	No./cover slip (range)	No./ml of feces	No. into device[b]	No./cover slip[c] (range)	No. into device	No./cover slip (range)	No. into device	No./cover slip (range)	No. into device	No./cover slip (range)	No. into device	No./cover slip (range)
E. coli cysts	1 (0–4)	408	408	3 (0–6)	6,120	7 (6–10)	1,428	6 (4–8)	2,448	6 (4–8)	2,856	7 (4–12)
E. histolytica cysts	1 (1–3)	510	510	16 (8–24)	7,650	19 (13–26)	1,785	25 (12–26)	3,060	27 (18–34)	3,750	26 (20–38)
G. lamblia cysts	16 (16–18)	7,580	7,580	92 (64–106)	113,700	50 (30–74)	26,530	97 (64–160)	45,480	113 (86–178)	53,060	104 (92–110)
Strongyloides larvae	1 (0–1)	55	55	1 (0–3)	825	2 (0–4)	193	2 (1–3)	330	3 (0–6)	385	3 (2–6)
Cryptosporidium oocysts	11 (8–14)	3,830	3,830	33 (16–80)	57,450	56 (34–74)	13,405	40 (26–74)	22,980	47 (32–66)	26,810	18 (14–24)
N. americanis ova	1 (0–2)	28	28	0.2 (0–1)	420	1 (0–2)	98	1 (0–2)	168	1 (0–2)	196	1 (0–2)

[a] Average number seen per cover slip (22 by 22 mm) from nine examinations of 0.01 ml of seeded feces prior to processing by fecal concentrators.
[b] Total number of organisms placed into concentrator (numbers of organisms per milliliter of fecal suspension × milliliter of suspension processed).
[c] Average number seen from microscopic examination of nine 0.01-ml samples of sediment.

TABLE 2. Comparison of fecal concentrators

Device	Vol (ml) of feces processed	Sediment vol (ml)	Filter pore size (μm)	Total processing time (min)	List price ($) each[a]	Ease of microscopic examination[b]	Ease of use[b]
FPC	1.0	0.10	600	6.0	0.81	1	1
PMC	15.0	3.5	600	7.5	1.50	3	1
TFC	3.5	0.25	600	8.5	1.39	2	2
FCK	6.0	0.5	750	6.5	1.30	2	2
Gauze filtration	7.0	0.5	600–2,000	8.0	0.41[c]	2	2

[a] List price for purchase of smallest quantities.
[b] 1, Easiest; 2, easy; 3, difficult.
[c] Cost for funnel, gauze, and tube.

cators of concentration efficiency and not as statistically absolute values.

DISCUSSION

We carefully considered the negative aspects of using a seeded fecal suspension for this study rather than positive patient specimens. However, the greater variability in fecal specimens, extent of mixing, potential of multiple infection and carrier states, and lack of sufficient volume of feces containing an appropriate range of parasite types and sizes prompted us to eliminate the positive patient specimen. The standardized specimen contained stool specimens with various consistencies, some of which had mucus, yeasts, vegetable and meat fibers, and other debris typical of stool specimens encountered in microbiological laboratories for parasite examination.

The number of parasites in 100-ml portions of fixed fecal material was determined empirically by counting the number of organisms available in commercial preparations. Parasite numbers in these unconcentrated portions varied from 28/ml (*N. americanus* ova) to 7,580/ml (*G. lamblia* cysts), with averages of direct microscopic examination of 0.01-ml samples ranging from 0.3 ova to 17 cysts, respectively.

For concentration methods except the gauze filtration method, we used a surfactant to help reduce adhesive forces in mucus and fecal lumps. Surfactants purportedly free helminth eggs and parasites and reduce the filtration time. Although we did not use a surfactant in the gauze filtration method, the results of this method were comparable to those of the other methods for all parasites except *Cryptosporidium* spp. Zierdt (9) has reported similar results and theorized that filtration problems with gauze and no surfactant were partially responsible for the low numbers of *Cryptosporidium* parasites that were observed.

The effect of varying the ethyl acetate concentrations appears to be most applicable to the second centrifugation step that is inherent in open systems. When hazy, chalky material was removed by initial centrifugation with saline, the application of increased ethyl acetate volumes was most effective. Under these conditions, the addition of ethyl acetate up to but not in excess of 30% of the total volume increased parasite detection (data not shown). Similar increases in ethyl acetate volume in single centrifugation systems (FPC and PMC) did not provide better microscopic detection rates.

Overall detection rates were determined by averaging the results of thorough microscopic examination of nine cover slips (22 by 22 mm) for each parasite-concentrator combination (Fig. 1). It was apparent that subjective analysis of sediment quality must be considered, with the amount of debris and the distribution and clarity of fecal material being important variables that influence parasite detection.

The cleanest, easiest to read slides in this study were made from sediment of the FPC, and the heaviest, layered debris with an opaque background were produced by PMC sediment. These two extremes were results of devices in which

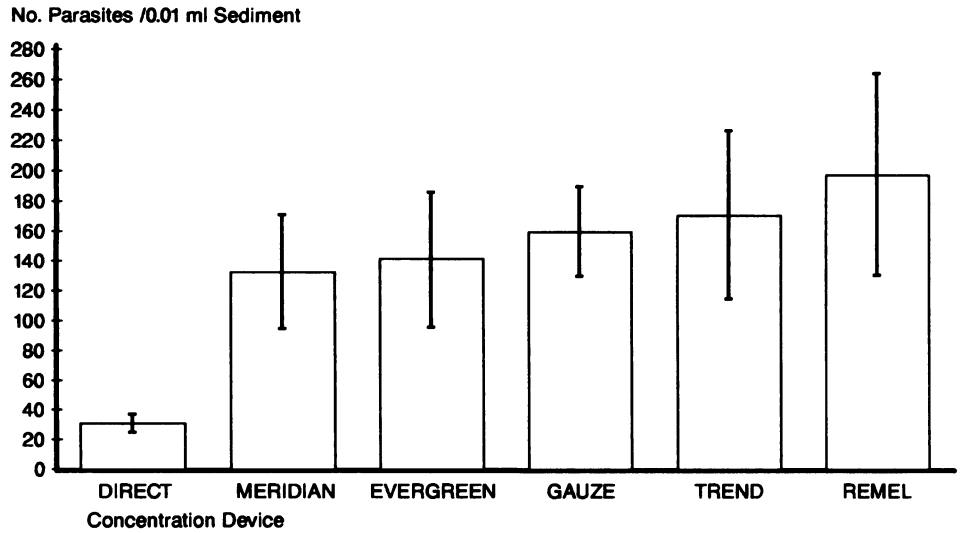

FIG. 1. Detection rate by the different systems for all parasite forms combined compared with that by direct examination.

a single centrifugation step was used and in which the smallest and largest volumes of fecal specimens were processed, respectively. Similarly, sediment volumes ranged from the smallest to the largest with these same devices. TFC sediment was ranked slightly easier to read overall than FCK sediment was. Specimens obtained by the gauze filtration method contained more microscopic debris than did TFC or FCK sediment and tended to produce microscopic material that appeared to be layered but with a bright background that allowed easy recognition of parasite forms, unlike the opaque background of PMC sediment.

PMC and FPC were the easiest systems to use because they used mating centrifuge tubes attached to the fecal suspension tube, with the filter being between the tube and the specimen. After filtration, one tube was discarded and reagents were added, mixed, and centrifuged. In the TFC, FCK, and the gauze filtration methods, an initial filtration step was used, with saline used as the suspension medium. The remaining steps were similar for the TFC and FCK devices. The initial centrifugation step appeared to remove heavy debris and brightened the background of the final sediment. The clean appearance of FPC sediment was probably a function of a small specimen volume in a relatively large volume of suspension medium.

FCK provided the best overall performance in this study and had the highest detection rate coupled with clean, easily readable sediment. All of the methods effectively concentrated parasites compared with direct examination. No device failed to detect any of the parasites that were studied. The gauze filtration method required the most manipulation, with additional steps involving folding and wetting of the gauze; however, the procedural steps for all methods that we studied required less than 10 min. The time needed for filtration was not considered because it was dependent on the consistency of the fecal specimen being processed. Costs varied from $0.41 for the gauze filtration method to $1.50 for the PMC.

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