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Concentration and Identification of *Cryptosporidium* sp. by Use of a Parasite Concentrator

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A new disposable plastic tube device and technique for ovum and parasite concentration, with upper and lower chambers separated by a fine screen, was compared with the standard gauze and open-funnel filtration technique as well as with the technique of direct smearing with an acid-fast stain. The new concentrator was distinctly superior to gauze filtration in concentrating *Cryptosporidium* sp. oocysts. Processing time was reduced. The new technique was less messy and as a closed system was considered to be much safer than gauze filtration.

The FPC (Evergreen Scientific, Los Angeles, Calif.) ovum and parasite concentrator (4), the standard Formalin-ethyl acetate funnel-gauze concentration technique (1, 3), and the direct examination technique were compared for the recovery of *Cryptosporidium* sp. oocysts from fecal specimens obtained from acquired immunodeficiency syndrome patients. The concentration of ova and parasites by gauze filtration of various volumes of fecal slurry has always been a distasteful, high-risk, and time-consuming technique. The FPC was designed to obviate these disadvantages.

MATERIALS AND METHODS

Filtration-flotation. There are many variations of the conventional gauze filtration-sugar flotation technique. The technique, as used in this study, is as follows. Emulsify ca. 5 g of fresh or formalinized feces in 100 ml of tap water. Filter the mixture through cheesecloth (Curity Gauze, doubled 8 ply, 10 cm, U.S. Pharmacopeia type VIII; The Kendall Co., Hospital Products, Boston, Mass.) into a 15-ml centrifuge tube to a volume sufficient to fill the tube. Centrifuge the tube at 1,000 × *g* for 5 min, decant the supernatant, and fill the tube three-fourths full with Sheather sugar solution (500 g of sucrose, in 320 ml of H_2O, and 6.5 g of phenol). Mix the resulting solution with an applicator stick and add more sugar solution to 0.25 in. (ca. 0.64 cm) from the top. Cap and centrifuge the tube at 310 × *g* for 5 min. Using a capillary pipette, siphon 1 to 2 drops from the surface film on the top. Mount the drop(s) on a slide (1 by 3 in. [ca. 2.54 by 7.62 cm]) under a cover slip (22 by 22 mm) and scan with a 10× objective lens (100× magnification) and then with a 43× objective lens (430× magnification).

FPC concentration. The FPC is a disposable two-piece tube device with a center screen that effectively separates the bulk of fecal debris and provides a washed, compact, clean sediment. The tubes are made of clear plastic. One is a calibrated flat-bottomed tube of 12-ml capacity which is screwed onto a midpiece containing a screen especially formulated to prevent the adherence of particulate material. Through the center of the screen passes a 6-cm tube that allows air exchange between the flat-bottomed tube and a calibrated 15-ml-capacity conical-bottomed tube screwed onto the opposite side of the midpiece.

Fecal specimens were processed in accordance with the manufacturer's instructions. Briefly, 1 rounded spoonful of fresh or formalinized feces (0.5 g) is allowed to soften for 30 min (four spoonsful are used if the specimen is liquid) in 9 ml of 10% Formalin in the flat-bottomed tube, followed by thorough mixing with two applicator sticks. Then, 3 drops of 20% Triton X-100 (supplied with the FPC) and 3 ml of ethyl acetate are added to the tube. The aerator tube is extended 3.5 cm above the top of the green midpiece screen so that it extends above the fluid level when the tube is inverted. The midpiece is screwed onto the flat-bottomed tube, and both are shaken in a horizontal position for 15 s. The whole device is then held vertically, with the conical-bottomed tube down. The mixture will drain into the conical-bottomed tube. The flat-bottomed tube may be gently struck against the palm to insure passage of all the material through the screen. After all the material has passed into the conical-bottomed tube, the flat-bottomed tube and midpiece are discarded. The tube is capped and centrifuged at 310 × g for 5 min. The top plug of debris is rimmed with an applicator stick and poured off with the supernatant fluid. The sediment is mixed with a few drops of saline, Merthiolate-iodine-Formalin, or 10% Formalin for routine ovum and parasite examination.

Carbol-fuchsin staining. Approximately nine-tenths of the sediment was spread thinly over glass slides (1 by 3 in. [ca. 2.54 by 7.62 cm]). After thorough air drying, the slides were dipped in methanol, air dried, and placed in cold Ziehl-Neelsen carbol-fuchsin for 5 min, followed by a gentle tap water wash (2). The slides were decolorized for 30 s in 10% sulfuric acid, washed, and counterstained for 1 min in light-green stain (0.2 g of light-green SF yellowish, 100 ml of H_2O, and 0.2 ml of glacial acetic acid) (Gallard-Schlesinger, Carle Place, N.Y.).

Sugar flotation technique. To the remaining sediment (ca. one-tenth of the total sediment) was added 1.0 ml of Sheather sugar solution. After gentle mixing, the tube was stood upright for 3 min. With a capillary pipette a sample was skimmed from the surface onto a slide, covered with a cover slip (22 by 22 mm), and examined around the edge of the cover slip with 10× and 40× objective lenses and normal light optics. *Cryptosporidium* oocysts were seen directly under the cover slip as yeast-sized, pink, refractile spheres with sharply defined white halos. At a 40× magnification the single refractile residual body within the oocyst was seen.

RESULTS

More *Cryptosporidium* oocysts were recovered by the FPC than by the gauze filtration technique (Table 1). The FPC significantly reduced the hazard of working with infec-

TABLE 1. *Cryptosporidium* recovery from fecal specimens

Patient	No. of counts recovered per 100 high-power fields				
	Acid-fast stain of direct fresh smear	Acid-fast stain after concn		Sugar floatation after concn	
		FPC	Conventional	FPC	Conventional
A					
1[a]	2	74	7	NC[b]	NC
2	ND[c]	58	20	60	28
3	ND	68	8	NC	NC
4	ND	55	30	NC	NC
5	ND	40	11	NC	NC
6	ND	57	18	NC	NC
B					
1[a]	17	109	NC	NC	NC
2	23	NC	NC	NC	NC
C	ND	17	3	28	10
D	ND	16	1	42	28
E					
1[a]	4	570	6	TNTC[d]	TNTC
2	1	NC	NC	NC	NC
3	1	NC	NC	NC	NC
F	ND	18	2	NC	NC
G	ND	1,460	7	NC	NC
H	ND	21	2	NC	NC

[a] One to six replicate tests were done on one specimen.
[b] NC, Not counted.
[c] ND, Not done (formalinized specimens).
[d] TNTC, Too numerous to count.

tious material because the possibilities of contacting the fecal specimens during processing were greatly reduced. The technique was also considerably less messy. It is notable that the oocyst counts per high-power field were higher when the sugar flotation technique was used than when Ziehl-Neelsen-stained smears were used, even though flotation was performed on only one-tenth of the FPC sediment. Both were greatly superior to the stained slide made directly from the specimen and to the gauze filtration technique.

Processing time was greatly reduced when the FPC was used, as it was easily possible in 1 h to process 15 specimens for microscopic examination. One specimen could be processed through the FPC in 10 min. Assuming a strongly positive specimen, a report can be made in that time.

DISCUSSION

Some workers may feel that *Cryptosporidium* infections are always so heavy that no concentration technique is needed. However, it is a fact that in some cases in which a conventional concentration technique is used, only a few oocysts are found, indicating to us that some concentration technique must be used.

Our laboratory has for years recognized problems with the gauze filtration technique. The most important of these is the mass of sample on the gauze, with concomitant pressured adsorption (or entrapment) of ova and parasites within the gauze. The open-gauze technique, especially with a final sugar flotation centrifugation step with a cover slip placed on top of each tube, is untenable from a safety standpoint, and the health risk to laboratory workers increases with the number of specimens from acquired immunodeficiency syndrome patients. The reasons for the remarkable effectiveness of the FPC device are not clearly understood. However, the physical consistency given to the slurry of fecal sample by 10% Formalin, ethyl acetate, and Triton X-100 followed by passage through the screen seems to result, for unknown reasons, in a much higher oocyst concentration. The consistency of the slurry is such that ova and parasites pass through it without adsorption or mechanical entrapment and sediment as a small, dense button.

Counts were not done on all of the specimens in Table 1 because of time constraints. It was felt that the recovery of oocysts was so superior with the FPC that additional time-consuming quantitative counts by the conventional technique could not be justified. The FPC not only allows highly effective *Cryptosporidium* recovery but is notable for its safety, cleanliness, rapidity, and ease of use. A particularly advantageous aspect of the FPC is that it is a totally enclosed device which therefore significantly decreases the hazards of working with highly infectious material. *Cryptosporidium* recovery in our laboratory is 12.5% for acquired immunodeficiency syndrome patients, higher than has been communicated to us from other laboratories.

In addition to being used for the recovery of cryptosporidia, the FPC has been demonstrated to be highly efficacious for the recovery of other parasites, both protozoa and helminths (4).

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