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Comparison of Pooled Formalin-Preserved Fecal Specimens with Three Individual Samples for Detection of Intestinal Parasites

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Triplicate preserved fecal samples from 265 patients were pooled into single samples, and the recovery rate of intestinal parasites from the pooled samples was compared with that from the individual samples. Parasites were detected in 109 patients when results from the individual samples were used compared with 108 patients when results from the pooled specimens were used. Pooling preserved fecal samples is an efficient and economical procedure for the detection of ova and parasites.

When diagnosing parasitic infections, usually it is recommended that multiple specimens be collected. Fecal excretion of parasites may fluctuate; therefore, the detection rate may be increased by collecting three stool specimens at 2- to 3-day intervals over a 6- to 10-day period (2).

In 1988, Peters et al. examined the sensitivity of pooling three individual fecal samples into a single specimen (3). Of the 123 sets examined, 31 were positive for ova and parasites. Of the 31 positive pooled specimens, 8 pooled specimens were positive although each individual specimen was negative. Two pooled specimens were negative although the individual specimens were positive. Their study showed the pooling of specimens to be a useful and economical method for detecting ova and parasites. A study in 1991 by Wahlquist et al. (4) examined the feasibility of pooling 801 individual samples from children in child care centers to detect *Giardia lamblia* (4). The study showed that when two or more individual specimens were positive, the sensitivity of pooling the specimens was 100%. Sensitivity decreased to 88% when only one individual specimen was positive. This study indicated that pooling individual samples would be useful in screening for *G. lamblia*.

In an attempt to increase productivity and reduce costs in the parasitology laboratory, we likewise compared recovery of intestinal parasites from pooled formalin-preserved samples with recovery from three individual, randomly collected samples submitted to a large reference laboratory (Diagnostic Microbiology Laboratory, Associated Regional and University Pathologists, Inc., Salt Lake City, Utah). The study was performed on 795 individual randomly collected fecal samples preserved in 10% buffered formalin and polyvinyl alcohol (PVA). Only the vials containing 10% buffered formalin were used for pooling.

The patient was instructed to collect three fecal samples at 2- to 3-day intervals over a 6- to 10-day period and place each separate specimen into two vials containing 10% buffered formalin and PVA. The specimens were sent to the diagnostic laboratory in sets of three, each containing six vials (three formalin, three PVA).

All individual fecal specimens were processed by using our standard Formalin/Hemo-De (manufacturer, Scientific Safety Solvents, Keller, Tex.; distributor, Fisher Scientific Co. [catalog no. 15182507A]) concentration procedure (1). One smear was prepared from each tube by using Dobell's iodine and coverslips (22 by 40 mm). Each slide was examined in its entirety by using overlapping fields under 10× and 40× objectives.

From each individual specimen, 3 ml of sample was poured into a pooled specimen vial (9-ml total). The pooled specimen was coded and processed by the same concentration procedure. All smears from each pooled sample were made by using the same technique as described above and read in a blinded manner. A pool was not made from the PVA-containing vials. Permanent slides were prepared from each individual PVA sample and stained by the Wheatley trichrome staining procedure.

The same technologist examined all individual and pooled specimens to eliminate the possibility of inconsistent technical ability in examining the samples. The processing and preparation of the samples were done by technologists other than the examining technologist.

Of 795 individual specimens, 327 (41%) were positive in 109 patients. In 265 pooled samples, 108 (40%) were positive for protozoa and/or helminths. The pooled sample was negative in only one case in which two individual vials were positive in the set of three. The organism missed was *Blastocystis hominis*. The specificity and sensitivity showed 99% correlation between samples individually concentrated and samples pooled before concentration. Twenty-three percent of the positive pooled samples for *G. lamblia* were positive in only two of three vials. This illustrates the need for collecting multiple fecal specimens especially where a *Giardia* species is suspected. All individual samples positive for *G. lamblia* were positive after pooling. This suggests that pooling may be effective in detecting parasites for which intermittent shedding is common. With the exception of the single *B. hominis* organism that went undetected, pooling was effective for detecting helminths and amoebae in this study (Table 1).

The greatest cost of microscopic examinations is the associated labor expense. The parasitology examination is a labor-intensive procedure. By pooling specimens, there is a significant reduction in processing such that only one con-

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TABLE 1. Results from pooling individual positive fecal specimens

Organism	No. of positive pooled samples	No. of positive individual samples			
		Total	Vial 1	Vial 2	Vial 3
Ascaris lumbricoides egg	1	1	0	0	1
Blastocystis hominis	56	57	0	2	55
Chilomastix mesnili cyst	1	1	0	0	1
Endolimax nana cyst	20	20	0	2	18
Entamoeba coli cyst	4	4	0	0	4
Entamoeba hartmanni cyst	3	3	0	0	3
Entamoeba histolytica cyst	2	2	0	0	2
Giardia lamblia cyst	13	13	0	3	10
Hookworm spp. egg	1	1	0	1	0
Hymenolepis nana egg	3	3	1	0	2
Iodamoeba buetschlii cyst	3	3	0	0	3
Trichuris trichiura egg	1	1	0	0	1
Total	108	109	1	8	100

centration is done for the three specimens and the technologist evaluates the single concentration rather than three individual concentrations. Because of the natural constraints of our study, none of the three vial sets had only one vial positive for parasites. We believe that to accurately illustrate the benefits of pooling samples, there must be evidence showing that pooling did not dilute the only positive sample to a point where it would go undetected. To show that pooling three fecal specimens would not significantly dilute the specimen, 30 sets of three separate formalin vials had one vial seeded with various protozoa and/or helminths. All pooled samples were positive for protozoa and/or helminths.

This study compared the recovery of intestinal parasites from pooled formalin-preserved fecal samples with their recovery from three individual, randomly collected samples and found that pooling preserved stools is an efficient and cost-effective procedure for screening for ova and parasites. Pooling individual specimens is effective for laboratories that batch their work load or for when multiple specimens are received at one time.

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