

## Use of Pooled Formalin-Preserved Fecal Specimens To Detect *Giardia lamblia*

S. P. WAHLQUIST,<sup>1\*</sup> R. M. WILLIAMS,<sup>1</sup> H. BISHOP,<sup>1</sup> D. G. ADDISS,<sup>1</sup> J. M. STEWART,<sup>1</sup> R. J. FINTON,<sup>2</sup>  
D. D. JURANEK,<sup>1</sup> AND J. J. SULLIVAN<sup>1</sup>

Parasitic Diseases Branch, Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333,<sup>1</sup> and Fulton County Health Department, Atlanta, Georgia 30303<sup>2</sup>

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**Three formalin-preserved fecal specimens from the same child attending a child-care center were pooled and compared with the three separate individual specimens by a single microscopic examination of concentration sediment for *Giardia lamblia*. The sensitivity of the pooled system was 100% when two or more individual specimens were positive and 88% when only one individual specimen was positive. The organism density in a single specimen was not a factor of whether the pool of specimens was positive or negative. Nearly half of the pools that contained positive specimens had only one of three specimens with positive results, reinforcing the need for multiple stool examinations when diagnosing *G. lamblia* infections.**

Examination of multiple stool specimens increases the probability of diagnosing an intestinal parasitic infection (1, 6, 7). A single specimen may recover only some of the parasites infecting the host because of the intermittent passage of some helminths and protozoa. For example, *Giardia lamblia* cysts may be passed at 2- to 3- or even 7- to 8-day intervals (2, 5). To ensure the maximum recovery of parasites, two or three normally passed stool specimens spaced 2 to 3 days apart are commonly examined (3).

To reduce the labor cost of processing and examining individual stool specimens, a study done by Peters et al. (4) recently compared results for pooled stool specimens with those for three individual specimens. Their study indicated that the pooled system is efficient, practical, and cost-effective.

In this study, we compared pools of three formalin-preserved fecal specimens collected from the same person on different days with the three individual specimens that made up the pool by microscopic examination for *G. lamblia*. We also examined the effect of the density of positive organisms on the results.

Fecal specimens were collected from the diapers of children attending 17 randomly selected child-care centers and 3 child care-centers with outbreaks of diarrheal illness in Fulton County, Ga., from February 1989 to March 1990. Fecal material was removed from the soiled diaper and placed in vials of 10% formalin (Parapak; Meridian Diagnostics). Each vial contained 15 ml of preservative; fecal material was added to the fill line to complete the volume to 20 ml.

The data manager at the Centers for Disease Control assigned codes to blind the laboratory to the source of the specimens. Stools fixed in 10% formalin were concentrated by the formalin-ethyl acetate sedimentation technique (3, 8) by using 5 ml of preserved stool per concentrate. The concentrate was placed on a slide and covered with a 22-mm<sup>2</sup> coverslip; the entire slide was examined microscopically for the presence of *G. lamblia* and other parasites. Slides were read by one of two microbiologists, who recorded the presence and density of organisms. All positive or questionable specimens and a 10% subset of randomly

selected negative specimens were confirmed by an independent reviewer.

From specimens collected from children on 3 different days, we selected those with at least 3 ml of formalin-fixed stool remaining after they were processed for the pooled specimen study. The data manager gave the three laboratory specimens to be pooled to a person who processed the specimens but who was not involved in the microscopic examination. After each specimen was thoroughly mixed, 3 ml of each specimen was placed in a new "pooled specimen" vial by using a plastic pipet whose tip had been removed to facilitate the flow of the fecal material. This pooled specimen was, likewise, assigned a new code by the data manager to blind the laboratory to the source of the individual samples in the pool. The pooled specimens were then processed as described above for the individual specimens.

The data presented in Tables 1 and 2 were from one initial examination. However, to determine whether individual *G. lamblia*-negative specimens within a positive pool might convert to positive if they were retested because of low numbers of organisms, we retested individual negative specimens if enough specimen remained to be concentrated.

The sensitivity of the pooled system for identification of *G. lamblia* was evaluated in relation to the positivity of the three separate components in the pool. The association between the density of the organisms in the individual and pooled specimens was evaluated statistically by the Fisher exact test (two-tail).

We were able to identify a total of 35 infected persons. The pooled system detected 33 (94%) of all infected individuals, while 34 (97%) infected individuals were detected by examining three individual stool specimens (Table 1). The sensitivity of the pooled system was 100% when two or more individual specimens were positive and 88% when one of three individual specimens was positive. To determine how the density of *G. lamblia* organisms in the individual components might affect the positivity of a pooled specimen, we reviewed results for pooled specimens containing only one positive component (Table 2). The density of organisms in the single positive component in a pooled specimen was not a significant predictor ( $P = 1.000$ ) of whether the pool was positive or negative.

\* Corresponding author.

TABLE 1. Results of pooled fecal specimens tested for *G. lamblia* after combining three individual specimens with various results

Pooled results	No. of pools tested	Individual specimen results for pools with the following no. of positive specimens/three pooled specimens:			
		3	2	1	0
Positive	33	12	5	15	1
Negative	234	0	0	2	232
Total	267	12	5	17	233

Of 13 negative individual specimens retested, 7 remained negative and 6 converted to positive, with the densities of organisms in these specimens being only rare to few. Of those individual specimens that converted from negative to positive on retesting, one was contained in the positive pool in which none of the three specimens was positive on the first examination (Table 1). This seems to indicate that when the density of the organisms is low in number, those few organisms may or may not be on a particular slide that has been prepared for examination. It is possible that a pooled specimen could be positive when its three components had organisms just below the threshold of detection that became detectable after they were combined. The pooled sample study of Peters et al. (4) likewise concluded that organisms were probably present in insufficient numbers in the eight instances in which the three individual specimens were negative but the pool was positive. A puzzling observation was made regarding the densities of organisms in pools that had only one positive component. The single positive component of two negative pools contained moderate to many organisms, while the single positive component of two positive pools contained few organisms.

TABLE 2. Density of *G. lamblia* organisms in pools containing only one positive component

Density <sup>a</sup> of organisms in pooled specimen	No. of pools with the following densities of organisms in individual specimens			
	Few	Moderate	Many	Total
Negative	0	1	1	2
Few	1	1	1	3
Moderate	1	2	3	6
Many	0	1	5	6
Total	2	5	10	17

<sup>a</sup> Density indicates category for number of organisms per 10 400× magnification fields, as follows: few, ≤2 organisms; moderate, 3 to 9 organisms; many, ≥10 organisms.

The fact that 49% of the pools with positive components had only one of three specimens with positive results reinforces the need for multiple stool examinations when diagnosing *G. lamblia* infections. The use of one pooled sample of three multiple specimens is not only efficient in diagnosing *G. lamblia* infection but also saves labor and money, as the study by Peters et al. (4) indicated. However, we disagree with the suggestion of Peters et al. (4) that patients pool all their own specimens into one container to save time. Of 35 *G. lamblia*-infected children with negative pools, 2 (6%) were confirmed to be positive only after examination of an individual component of the pool; thus, individual specimens need to be available for further diagnostic testing in cases in which *G. lamblia* is suggested clinically and the pool is negative.

Future studies need to examine situations in which specimens from patients with acute diarrhea are likely to contain a greater proportion of protozoan trophozoites than cysts (3); in those situations, the stained polyvinyl alcohol slide might be more useful than pooled formalin specimens.

Although our study was limited to children with *G. lamblia* infections in child-care centers, the pooled system could be useful in clinical laboratory screening for other ova and parasites, as suggested by the study of Peters et al. (4). In addition, this pooled system could be used in large-scale surveys of stool specimens and outbreaks of parasitic disease to obtain the benefits of examining three multiple stool specimens in less time with reduced personnel and financial resources.

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