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Evaluation of Reliability of Pooling Stool Specimens from Different Patients and Detection of *Giardia lamblia* Antigen by Microtiter Enzyme-Linked Immunosorbent Assay

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We have shown that stool samples from different patients can be pooled at a 1:2 dilution and reliably assayed for *Giardia lamblia* antigen by a commercial microtiter enzyme-linked immunosorbent assay (ELISA) system (LMD Laboratories, Inc., Carlsbad, Calif.). Laboratories can reduce reagent costs by pooling specimens submitted for the detection of *Giardia* antigen by ELISA.

Diagnostic microbiology laboratories are increasingly faced with cost constraints. Recent publications (3, 6) have demonstrated that significant cost savings can be achieved by restricting the examination of stool specimens for ova and parasites. Additionally, several researchers (1, 4) have concluded that pooling stool specimens before examination for parasites is economical and reliable. Technologists' time can also be saved by using enzyme-linked immunosorbent assays (ELISAs) for the detection of some parasite antigens. ELISAs are, however, expensive, and the yield is often low. In an attempt to reduce the cost of testing for *Giardia* antigen, we pooled consecutively isolated stool specimens from different patients before performing an ELISA.

Giardia lamblia stool antigen microtiter ELISA for in vitro diagnostic use (LMD Laboratories, Inc., Carlsbad, Calif.) was performed according to the manufacturer's instructions. Stock formalin-fixed (Alpha-Tec Systems, Irving, Calif.) stool specimens were obtained from the Scientific Laboratory Division, State of New Mexico Department of Health, Albuquerque. The stool specimens had been stored at room temperature for less than 30 days. Thirty-eight were positive for *G. lamblia* by microscopic examination, and 40 were negative. Only samples confirmed by an ELISA were included. Positive samples gave A_{450} readings of >0.25. Pooled samples diluted 1:2 or 1:5 were created from the stool specimens by drawing 1 ml from one positive specimen and 1 ml from one or four specimens negative by microscopic examination and ELISA, combining them, and mixing with a vortex mixer in plastic vials.

Of 38 stock stool specimens positive for *G. lamblia* at a 1:1 dilution by an ELISA, 28 remained positive when tested by an ELISA at a dilution of 1:5. The remaining 10 gave absorbances between 0.144 and 0.245, a gray zone, higher than any negative sample yet below the cutoff for positive specimens. At a 1:2 dilution, 37 of the 38 specimens gave absorbance readings in the range 0.255 to 1.593 and 1 gave a reading of 0.221.

Additionally, 144 stool specimens fixed in 10% formalin (Para-Pak; Meridian Diagnostics, Cincinnati, Ohio) submitted to the University of New Mexico Hospital diagnostic microbiology laboratory from 113 patients were tested individually and pooled at a 1:2 dilution (25-μl aliquots of two consecutively received specimens in the same test well). All fresh clinical specimens were tested within 7 days of collection.

Routine testing of 144 formalin-fixed stool specimens received over a 4-month period resulted in 11 (7.6%) positive specimens (absorbance range, 0.412 to >3). Two consecutively received specimens were pooled to give a 1:2 dilution. Eleven of these 72 pooled specimens yielded positive results (absorbance range, 0.356 to 2.575). All positive specimens in combination with a negative specimen gave positive results. No combination of negative specimens gave a positive result. Retesting the individual stool specimens of the 11 positive pooled specimens meant that an additional 22 tests had to be performed.

Previously, over a 19-month period, our laboratory received 2,536 stool specimens for ovum and parasite examination. Only 4.8% were positive for ova or parasites. In addition to instituting utilization controls, we investigated the reliability of pooling stool specimens before testing by an ELISA. Rosenblatt et al. (5) have recently reported that the LMD/Seradyn ELISA for detection of *G. lamblia* in stools provides a sensitivity of 97% and a specificity of 96% compared with conventional microscopic examination. Significant cost savings can be achieved by the use of an ELISA for the detection of *Giardia* antigen. Kleeman and Gilmour (2) estimated a savings of $6.12 per specimen with the Color-Vue Giardia kit (Seradyn Inc., Indianapolis, Ind.) compared with the cost of a standard ovum and parasite examination. The LMD kit used in our study, the LMD/Seradyn kit, and the Color-Vue Giardia kit are all the same LMD ELISA kit marketed by Seradyn, Inc.

Peters et al. (4) reported on the pooling of three formalin-fixed stool specimens from each of 123 patients. The pooled and individual stool specimens were examined microscopically. Of 123 sets examined, 92 were negative and 31 were positive for ova and parasites. In two cases, the pooled specimens were negative, while one or more of the individual samples were positive. Organisms were later seen in the pooled specimens on polyvinyl alcohol-preserved, stained

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slides. Aldeen et al. (1) examined three individual stool samples and pooled specimens of these three samples from 265 patients. That group detected parasites for 109 patients with the individual specimens and for 108 patients with the pooled samples. Wahlquist et al. (7) studied the detection of *G. lamblia* in pooled stool specimens. The sensitivity of microscopic detection in three pooled stool specimens from one patient was 100% compared with the microscopic detection of *G. lamblia* antigen in three individual specimens from the same patient.

In our testing of pooled stock formalin-fixed stool specimens, 37 of 38 stool specimens positive for *G. lamblia* by microscopic examination and ELISA remained positive by ELISA when diluted 1:2. When diluted 1:5, 10 of 38 gave absorbance readings in a zone between the negative and positive controls. Pooled specimens giving results in the gray zone should be tested again individually, and a final report should be based on those results. Some repeat testing would have to be performed when a pooled specimen of consecutively isolated stool specimens is determined to be positive. Eleven of 72 pooled clinical specimens received in our laboratory were positive. Retesting the positive pooled specimens individually meant that 22 additional tests had to be performed. This represents a 35% (94/144 = 65%) reduction in testing. Although the repeat testing would involve some additional labor, because the incidence of stool specimens positive for *G. lamblia* is low, the labor is minimal compared with the cost of the enzyme immunoassay kits.

Pooling of stool specimens from different patients before testing will be controversial. Laboratorians have been trained to carefully keep individual patient specimens apart. There appears to be no inherent reason not to pool specimens unless one speculates that one patient's specimen may have substances that interfere with the test being run. We did not detect any interfering substances in this ELISA. As even more expensive and extremely sensitive tests, like polymerase chain reactions, become commercially available, pooling of different patients' specimens should be investigated as a way to save kit costs. We did not test pooled stool specimens at a 1:3 dilution, although earlier studies (1, 4) demonstrated no loss of sensitivity when three stool specimens from the same patient were combined. Combining three stool specimens from different patients could lead to an even greater saving of reagents. Pooling of consecutively submitted stool specimens diluted 1:2 before testing by an ELISA is a reliable, cost-effective method for detecting *Giardia* antigen.

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