

Detection of *Giardia lamblia* and *Cryptosporidium parvum* Antigens in Human Fecal Specimens Using the ColorPAC Combination Rapid Solid-Phase Qualitative Immunochromatographic Assay

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The ColorPAC *Giardia/Cryptosporidium* (Becton Dickinson) is a solid-phase qualitative immunochromatographic assay that detects and distinguishes between *Giardia lamblia* and *Cryptosporidium parvum* in human stool. Agreement between the Alexon-Trend ProSpecT *Giardia* Rapid EIA and the ColorPAC assay was 166 of 172 (96.5%). Agreement between the Alexon-Trend ProSpecT *Cryptosporidium* Rapid EIA and the ColorPAC assay was 169 of 171 (98.8%). No cross-reactions were seen with other parasites or human cells.

With the increasing interest in potential waterborne outbreak situations, fewer well-trained microscopists, and confirmation that both *Giardia lamblia* and *Cryptosporidium parvum* can cause severe symptoms in humans, laboratories are reviewing their options with regard to immunoassay kits that can be incorporated into their routine testing protocols (4, 14, 15, 20). Not only must these methods be acceptable in terms of sensitivity and specificity but they must provide clinically relevant, cost-effective, rapid results, particularly in a potential waterborne outbreak situation (1, 2, 7, 13). Antigen detection assays for *G. lamblia* and *C. parvum* have proven to be very useful in the diagnosis of these infections (5, 10–12, 16–19). Advantages of these assays include labor, time, and batching efficiencies that may lead to reduced costs. These reagents offer alternative methods to the routine “ova and parasite examination” (O&P) method and provide the added sensitivity required to confirm infections in patients with low parasite numbers.

Most commercially available immunoassays use the enzyme immunoassay (EIA) format that requires multiple reagent additions, washing steps, and incubations. A nonenzymatic rapid immunoassay for *Giardia* and *Cryptosporidium* antigens has been developed. This test (Genzyme Diagnostics Contrast *Giardia/Cryptosporidium*) is marketed commercially (Becton Dickinson ColorPAC *Giardia/Cryptosporidium*). The assay can be performed in approximately 12 min on formalin-fixed (5 or 10% formalin or sodium acetate-acetic acid-formalin [SAF]) or unfixed stool specimens. In the current environment of managed care and cost containment, this new device may provide diagnostic testing that is more accurate, rapid, and cost-effective than traditional methods.

Human fecal specimens were collected in 10% formalin or SAF and polyvinyl alcohol-mercuric chloride base fixatives by the patients and submitted to the laboratory from various clinics affiliated with the UCLA Medical Center. Different parasites (nine protozoa, including both trophozoites and cysts and 1 helminth; 74 positive specimens) and stools containing human cells (21 positive specimens) were included in the negative

specimens; all specimens were patient specimens, not seeded specimens. Specific organisms included *Blastocystis hominis* (23), *Chilomastix mesnili* (3), *Dientamoeba fragilis* (6), *Endolimax nana* (14), *Entamoeba coli* (6), *Entamoeba hartmanni* (3), *Entamoeba histolytica/E. dispar* (10), *Iodamoeba bütschlii* (5), *Trichomonas hominis* (1), and *Hymenolepis nana* eggs (3). Specific human cells seen in fecal specimens included red blood cells (1), polymorphonuclear leukocytes (19), and macrophages (3); these specimens also contained normal fecal debris, including various yeast cells. All specimens were tested as indicated below; additional testing was performed on discrepant specimens.

The following immunoassay diagnostic kits were used according to the manufacturer's directions: (i) Alexon-Trend ProSpecT *Giardia* Rapid EIA (Alexon-Trend-Seradyn, Ramsey, Minn.), (ii) Alexon-Trend ProSpecT *Cryptosporidium* Rapid EIA, (iii) Alexon-Trend ProSpecT microwell EIA (for *Giardia* or *Cryptosporidium*), and (iv) ColorPAC *Giardia/Cryptosporidium* (Becton Dickinson, Sparks, Md.). The Alexon-Trend-Seradyn rapid EIA methods were selected as being the most comparable to the ColorPAC method in terms of time required for performing the procedure. All immunoassay kits were used with unconcentrated, preserved stool specimens (5 or 10% formalin or SAF). Specimens were tested using the following combinations: (i) Alexon-Trend ProSpecT *Giardia* Rapid EIA and ColorPAC *Giardia/Cryptosporidium* or (ii) Alexon-Trend ProSpecT *Cryptosporidium* Rapid EIA and ColorPAC *Giardia/Cryptosporidium*. Discrepant results were tested using the Alexon-Trend ProSpecT microwell EIA tests (for *Giardia* or *Cryptosporidium*); this test was selected in order to maintain consistency with a single manufacturer.

The ColorPAC *Giardia/Cryptosporidium* assay procedure involves the addition of 2 drops of sample treatment buffer to a tube, the pipetting of 60 μ l of uncentrifuged stool specimen into the tube, the addition of 2 drops of a *Giardia* capture antibody conjugate, and the addition of 2 drops of a colloidal carbon-conjugated detection reagent for *Giardia* and *Cryptosporidium*. After the sample is mixed, it is immediately poured into the test device. Assay results are read after 10 min. Positive results are visualized as gray-black lines in the appropriate position in the results window (Fig. 1). The tubes, pipettes, devices, and all reagents are provided with the kit.

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FIG. 1. ColorPac devices demonstrating positive results: left, positive control; middle, positive *Giardia*; right, positive *Cryptosporidium*.

By the Merifluor *Cryptosporidium*/*Giardia* direct fluorescent-antibody assay (the reference method), the O&P examination (concentration, trichrome stain), and modified acid-fast staining (concentration, modified acid-fast stain), 47 specimens were positive for *Giardia*, 41 specimens were positive for *Cryptosporidium*, and 256 were negative for both organisms. Positive *Giardia* specimens ($n = 47$) and negative specimens ($n = 125$) were tested using two different kits. Agreement between the Alexon-Trend ProSpecT *Giardia* Rapid EIA and the Becton Dickinson ColorPAC was 166 of 172 (96.5%); the six positives by microscopy, the Merifluor direct fluorescent-antibody assay, and the ColorPAC were false negatives in the Alexon-Trend ProSpecT *Giardia* Rapid EIA (Table 1). There were no cross-reactions with other organisms present in the fecal specimens; specificity was 100%. Positive *Cryptosporidium* specimens ($n = 41$) and negative specimens ($n = 131$) were tested using two different kits. Agreement between the Alexon-Trend ProSpecT *Cryptosporidium* Rapid EIA and the Becton Dickinson ColorPAC was 169 of 171 (98.8%); the three negatives were false negatives using the Alexon-Trend ProSpecT *Cryptosporidium* Rapid EIA. The one false-negative *C. parvum* (ColorPAC) was confirmed by immunofluorescence (Table 1). There were no cross-reactions with other organisms present in the fecal specimens; specificity was 100%.

In patients with giardiasis or cryptosporidiosis, the use of routine diagnostic methods such as concentration and trichrome or modified acid-fast staining may be insufficient to demonstrate the presence of these organisms (3, 4, 6, 9, 11, 21). Based on the O&P examination with trichrome stain, as well as the Meridian *Giardia*/*Cryptosporidium* Merifluor combination reagent, the sensitivity and specificity, respectively, using the

TABLE 1. Comparison of rapid test methods for identification of *Giardia* and *Cryptosporidium*^a

Organism	Specimens	Results with:			
		ColorPac rapid test		ProSpecT <i>Giardia</i> Rapid EIA	
		Pos	Neg	Pos	Neg
<i>Giardia</i>	Pos ($n = 47$)	47	0	41	6
	Neg ($n = 125$)	0	125	0	125
<i>Cryptosporidium</i>	Pos ($n = 41$)	40	1	38	3
	Neg ($n = 131$)	0	131	0	131

^a Pos, positive; Neg, negative.

ColorPAC device were as follows: *G. lamblia*, 100% and 100%; *C. parvum*, 97.6% and 100%. The Alexon-Trend ProSpecT Rapid EIAs did not perform as well, but results were within previous published expected values regarding sensitivity and specificity. The ability to concurrently detect and distinguish between *Giardia* and *Cryptosporidium* antigens in formalin-fixed or unfixed fecal specimens with a 10-min nonenzymatic immunoassay provides the user with another very useful diagnostic kit, the Becton Dickinson ColorPAC. The rapid immunoassays do not take the place of routine O&P examinations, but they are very useful when trying to confirm *Giardia* and *Cryptosporidium* infections (8).

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