Detection of *Giardia lamblia* Antigens in Human Fecal Specimens by a Solid-Phase Qualitative Immunochromatographic Assay

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The SIMPLE-READ *Giardia* rapid assay (Medical Chemical Corporation) is a solid-phase qualitative immunochromatographic assay that detects *Giardia lamblia* in aqueous extracts of human fecal specimens. Testing 106 *Giardia*-positive and 104 *Giardia*-negative stool specimens yielded a sensitivity of 97.2% and a specificity of 100% for the SIMPLE-READ *Giardia* rapid assay.

Revised ordering options related to diagnostic parasitology testing include ova and parasite (O&P) examinations, the newer fecal immunoassays, and special stains for the coccidia and microsporidia. Laboratories are incorporating these procedures into their test menus, and each of the three test options has very specific benefits in terms of clinically relevant testing. While the use of routine diagnostic methods such as fecal concentration, permanent stained smears, and modified acid-fast staining may be insufficient to demonstrate the presence of intestinal parasites, antigen detection assays for *Cryptosporidium* spp. and *Giardia lamblia* have proven to be very useful (1–9, 12–15).

As acute infections with *G. lamblia* resolve, the number of organisms shed in the stool varies dramatically and the number of cysts passed by patients, including those who may be compromised, varies from day to day and week to week, particularly if they do not have diarrhea (10).

The nonenzymatic rapid immunoassay for *Giardia* antigens (SIMPLE-READ *Giardia* rapid assay; Medical Chemical Corp.) was performed in approximately 10 min on formalin-fixed (5 or 10% formalin or sodium acetate-acetic acid-formalin) stool specimens. Sample treatment buffer (4 to 6 drops) was added to a tube, followed by 1 drop of the uncentrifuged stool specimen. After the sample was mixed, it was immediately poured into the membrane flow test device. Using specific antibodies, *Giardia* antigens are isolated and immobilized on the substrate. Since the specimens were submitted in fixative, no mixing of the fecal sample was required prior to sampling; fluid above the stool was used, since the antigen was in solution. The assay results were read visually after 10 min. Positive results were seen as pink lines (regardless of intensity) on the membrane in the results window. A control was included in the device and visually appeared as a strong pink line.

In this study, the cartridge was tested against unconcentrated known positive and negative 10% formalin- or sodium acetate-acetic acid-formalin-preserved fecal specimens. The known positive and negative results were based on the reference methods, which were the O&P examination (concentration and permanent stained smear), the Meridian Bioscience, Inc., *Giardia/Cryptosporidium* Merifluor FA reagent, and the Medical-Chemical Corp. Para-Tect *Giardia/Cryptosporidium* FA reagent. For discrepant results obtained using the cartridge, if the specimen was positive by any two reference methods, the specimen was considered a true positive. Three specimens that tested negative using the cartridge were confirmed as true positives with very low parasite numbers by both of the more sensitive FA reference methods. Four specimens determined to be positive using the cartridge were originally considered negative based on the O&P examination; however, when retested, the four specimens were confirmed as true positives using the two FA reference methods.

Of the 210 specimens examined, 106 were positive for *Giardia* based on the results of the reference methods. Different parasites (10 protozoa, including trophozoites, cysts, and oocysts; seven helminths, including eggs and larvae; and four specimens containing human cells [polymorphonuclear leukocytes and macrophages]) were included in the negative specimens (n = 104). Both positive and negative specimens were tested by the two sites, 158 at LSG & Associates and 52 at the VA Greater Los Angeles facility. The known positive and negative specimens were randomly assigned to the two testing sites. All specimens were patient specimens, not seeded specimens, and some contained multiple organisms. Specific organisms included * Blastocystis hominis* (n = 16), *Cryptosporidium* spp. (n = 34), *Cyclospora cayetanensis* (n = 1), *Chilomastix mesnili* (n = 1), *Dientamoeba fragilis* (n = 6), *Endolimax nana* (n = 23), *Entamoeba coli* (n = 12), *Entamoeba hartmanni* (n = 1), *Entamoeba histolytica/Entamoeba dispar* (n = 7), *Iodamoeba bütschlii* (n = 3), *Ascaris lumbricoides* (n = 7), *Diphyllobothrium latum* (n = 1), *Enterobius vermicularis* (n = 5), *Hymenolepis nana* (n = 8), *Strongyloides stercoralis* (n = 4), *Taenia* spp. (n = 3), and *Trichuris trichiura* (n = 3). Although the results of the reference methods were known, the specimens were coded and tested blind when the SIMPLE-READ cartridge was used.

After resolution, the total number of positive specimens was 106, the sensitivity was 97.2%, the specificity was 100%, the positive predictive value (predictive value of a positive test result) was 100%, and the negative predictive value (predictive value of a negative test result) was 97.2% (Table 1). False-negative *G. lamblia* results were obtained from three speci-
The sensitivity was 97.2%, the specificity was 100%, and the negative predictive value (predictive value of a negative test result) was 97.2%.

The reference methods: O&P examination, Giardia/Cryptosporidium Merifluor FA reagent (Meridian Bioscience, Inc.), and Para-Tect Giardia/Cryptosporidium FA reagent (Medical-Chemical Corp.).

We thank Medical Chemical Corporation for providing the SIMPLE-READ diagnostic kits and the confirmative diagnostic kits. We thank Medical Chemical Corporation for financial support.


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<thead>
<tr>
<th>TABLE 1. Comparison of results with the reference methods and the SIMPLE-READ cartridgea</th>
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<tr>
<td>SIMPLE-READ cartridge result</td>
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<tr>
<td>Positive</td>
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<td>Negative</td>
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a The sensitivity was 97.2%, the specificity was 100%, the positive predictive value (predictive value of a positive test result) was 100%, and the negative predictive value (predictive value of a negative test result) was 97.2%.

b Reference methods: O&P examination, Giardia/Cryptosporidium Merifluor FA reagent (Meridian Bioscience, Inc.), and Para-Tect Giardia/Cryptosporidium FA reagent (Medical-Chemical Corp.).

c False-negative G. lamblia results were obtained from three specimens with very low parasite numbers. Both FA reference methods confirmed these specimens as true positives.

mens with low parasite numbers (one or two cysts/well using immunofluorescence). No cross-reactions were seen with other parasites.

In patients with giardiasis, the use of routine diagnostic methods such as concentration and permanent stain may be insufficient to demonstrate the presence of these organisms (5, 15). Although the fecal immunoassays are more sensitive than the routine O&P method, false-negative results may still occur with very light infections. It has been recommended that a second specimen be tested if the first fecal immunoassay for suspected giardiasis is negative (11).

Although the reagent costs may be more than those for the routine O&P methods, the labor costs will be considerably less when using the rapid cartridge format. When comparing diagnostic test costs, it is important to consider all aspects of testing including, but not limited to, reagents, labor, repeat tests, quality control, reagent storage, number of tests requested, staff expertise in microscopy, and training requirements.

The ability to detect Giardia antigens in formalin-fixed or unfixed fecal specimens with a 10-min nonenzymatic immunoassay provides the user with another useful diagnostic kit, the SIMPLE-READ Giardia rapid assay (Medical Chemical Corp.), which can also be used at the point of service like other rapid immunoassays currently available. The rapid immunoassays do not take the place of routine O&P examinations, but they are more sensitive and clinically relevant when trying to diagnose specific infections such as giardiasis (5).