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# Comparison of microscopy, ELISA, and real-time PCR for detection of *Giardia intestinalis* in human stool specimens

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**Background/aim:** This study included patients who had digestive system complaints between August 2015 and October 2015. The research was designed to compare conventional microscopy with an antigen detection ELISA kit and the TaqMan-based real-time PCR (RT-PCR) technique for detection of *Giardia intestinalis* in human stool specimens.

**Materials and methods:** Samples were concentrated by formalin-ether sedimentation technique and microscopic examinations were carried out on wet mount slides. A commercially available ELISA kit (Giardia CELISA, Cellabs, Brookvale, Australia) was used for immunoassay. DNA was extracted from fecal samples of about 200 mg using the QIAamp Fast DNA Stool Mini Kit (QIAGEN, Hilden, Germany) and the LightCycler Nano system (Roche Diagnostics, Mannheim, Germany) was used for the TaqMan-based RT-PCR assay.

**Results:** A total of 94 stool samples, 38 of them diagnosed positive (40.4%) and 56 of them diagnosed negative by microscopy, were selected for evaluation by antigen detection and molecular assays. The prevalence of *G. intestinalis* infection was found as 46.8% (n: 44) and 79.8% (n: 75) by ELISA and RT-PCR, respectively. RT-PCR revealed by far the highest positivity rate compared to the other two methods. The difference between these methods was found to be statistically significant ($P < 0.05$). In comparison to PCR, the sensitivity and specificity of microscopy and ELISA were 50.7% and 100% and 53.3% and 79%, respectively.

**Conclusion:** RT-PCR seems to be much more sensitive and beneficial for rapid and accurate diagnosis of *G. intestinalis* in human stools.

**Key words:** *Giardia*, detection, ELISA, real-time PCR, comparison

## 1. Introduction

*Giardia intestinalis* (*G. duodenalis*) is a flagellated intestinal protozoan that infects humans and several animal species. In certain parts of the world, *G. intestinalis* is more commonly known as *G. lamblia*. It is one of the most common pathogens responsible for diarrhea. *Giardia* has two stages, trophozoites and cysts. The second one is the infectious stage of the organism and ingesting as few as 10 cysts is sufficient to acquire the illness. Contaminated water and food and person-to-person contact are the main sources of the infection (1). The parasite has a worldwide distribution and it is particularly common in developing countries, where sanitation and personal hygiene are problematic. *Giardia* is responsible for 500,000 new cases every year in Asia, Africa, and Latin America (2) and an estimated 280 million cases annually in the world (3,4). The parasite is also one of the agents most responsible for waterborne outbreaks of diarrhea. The infection is mainly asymptomatic, but acute and chronic gastrointestinal clinical symptoms such as vomiting, anorexia, diarrhea, flatulence, abdominal pain, greasy stools, and nausea may occur. It also threatens human health, especially that of children, via growth retardation and nutritional damage (1,3,5).

The diagnosis of giardiasis in asymptomatic cases plays an important role for controlling the disease. Thus, obtaining reliable results in the diagnosis is quite significant. There are different methods used to detect *G. intestinalis* cysts or trophozoites. Currently, microscopic techniques on fresh and concentrated fecal samples are still commonly utilized. However, analysis of only one stool specimen and the skill of the microscopist can reduce the accuracy of detection (6,7). In addition, immunoenzymatic and molecular techniques are also available for routine diagnosis and research studies. Antigen detection methods are fast, easy to perform, and more sensitive compared to microscopy, but they have some disadvantages such as the need for certain reagents and high cost compared to microscopy. Recently, molecular approaches were developed and shown to be more efficient and sensitive

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for the detection of low numbers of parasites (8,9). In addition, the real-time PCR (RT-PCR) technique is an example of a recent advancement that allows monitoring of PCR products during the analysis. It is also beneficial with its short analysis time and reduced risk of contamination (10).

This study was designed to compare microscopy with a commercially available stool antigen detection enzyme linked immunosorbent assay (ELISA) kit and a TaqMan-based RT-PCR technique for the detection of *G. intestinalis* in human stool specimens. This study compared these three methods in the detection of *G. intestinalis* in human stool specimens.

## 2. Materials and methods

### 2.1. Specimen collection and microscopic examination

The study was conducted with patients referred to the parasitology laboratory from various clinics with suspicion of giardiasis between July 2015 and October 2015. Whereas PCR and light microscopy can detect the actual presence of the parasite, ELISA only detects the antigen of cysts. In diarrheic patients, cysts are not formed and antigens cannot be detected by ELISA. Therefore, to compare these three methods properly, nondiarrheic stool samples were selected.

Each specimen was dived into three parts for microscopy, ELISA, and PCR. The fresh first portions of stool samples were instantly concentrated by formalin-ether sedimentation technique and microscopic examinations were carried out with saline and iodine wet mounts slides (6). Slides were screened at 400× magnification (Olympus CH2) to determine cysts of *Giardia intestinalis* (Figure 1A). The unpreserved stool samples were stored at –20 °C for performing PCR later.

### 2.2. ELISA

A commercial ELISA test (Giardia CELISA, Cellabs, Brookvale, Australia) was performed on fresh stool samples as well. The kit was designed to detect *Giardia intestinalis* cyst antigens in fecal specimens and included negative and positive controls. Antigens from stool specimens were bound to microplates, coated with purified mouse monoclonal antibodies. The test was performed according to the manufacturer's instructions and the absorbance was measured within 10 min by spectrophotometer (Biotek ELx800, Winooski, VT, USA) at a wavelength of 450 nm. Values above 0.150 were considered as positive.

### 2.3. DNA extraction and RT-PCR

Fecal samples were subjected to three rapid freeze–thaw cycles before the application. DNA was extracted from samples of about 200 mg using the QIAamp Fast DNA Stool Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's recommendations. DNA samples were stored at –20 °C until PCR was performed.

The LightCycler Nano system (Roche Diagnostics, Mannheim, Germany) was used for the TaqMan-based RT-PCR assay. Amplification reactions were performed in a volume of 20 µL, including 10.0 µL of FastStart Essential DNA Probes Master (Roche Cat. No. 06402682001), 0.5 µL of Modular Giardia Kit solution (TIB Molbiol Cat. No. 53-0612-96, containing primers and probes), 4.5 µL of PCR-grade water, and 5 µL of control or sample DNA in each reaction. One thousand copies that included the DNA provided in the LightMix Modular Kit and sterile water were used for the positive and negative control, respectively. Amplification consisted of 5 min at 95

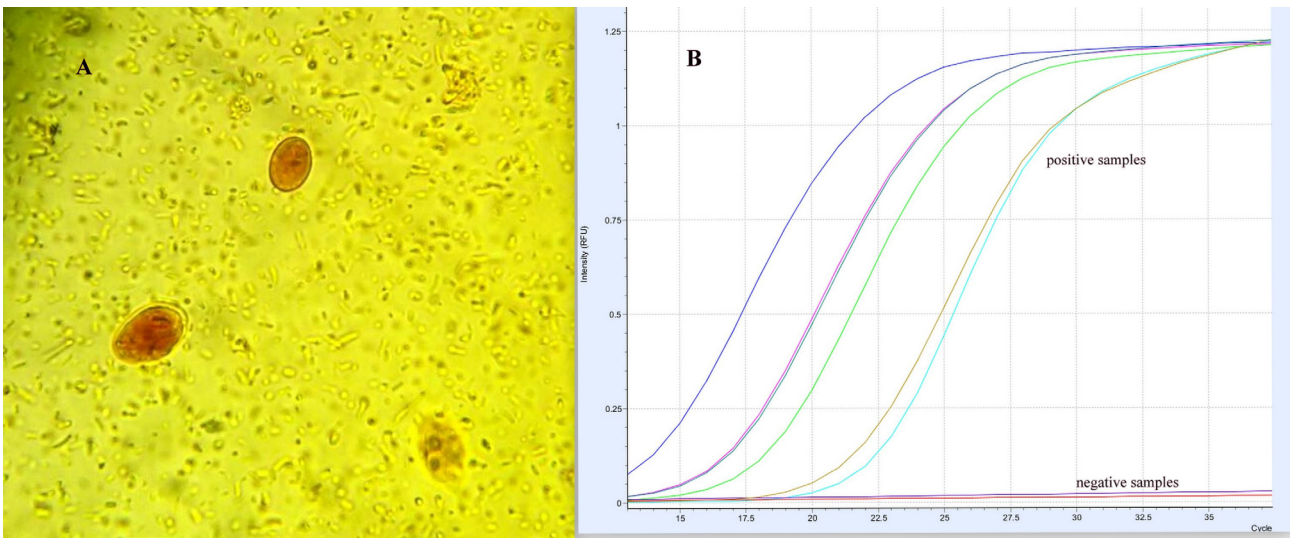


**Figure 1.** Microscopy (A) and RT-PCR results (B) of stool samples.

°C followed by 45 cycles of 15 s at 95 °C, 30 s at 60 °C, and 1 s at 72 °C followed by a cooling period of 30 s at 40 °C. A fragment of the *18S RNA* gene 62 bp long was amplified and the fluorescence was measured with a FAM label. The results were analyzed by absolute quantification (abs-quant 2nd derivative) and the samples that produced Ct values and amplifications were considered as positive (Figure 1B).

The statistical difference between the techniques was analyzed with the chi-square test. The differences were considered statistically significant at $P \leq 0.05$. To quantify the relationship between the techniques, RT-PCR was used as the reference test. Evaluation of the test results was based on the sensitivity and specificity. They were computed by universal formulas.

## 3. Results

A total of 94 stool samples, 38 of which were diagnosed as positive (40.4%) and 56 of which were diagnosed as negative by microscopy, were selected for evaluation by antigen detection and molecular assays. The prevalence of *G. intestinalis* infection was 46.8% (n: 44) and 79.8% (n: 75) by ELISA and RT-PCR, respectively. RT-PCR revealed by far the highest positivity rate compared to the other two methods.

Forty-four samples were found negative and 32 were found positive by both microscopy and ELISA. Additionally, 12 samples were detected negative by microscopy while they were positive by ELISA. False negative results were obtained in six samples by ELISA, which were positive by microscopy. All positive findings by microscopy (n: 38) were also confirmed with RT-PCR. Out of 94 samples, 37 were negative by microscopy while positive by RT-PCR (Table 1). In the comparison of ELISA and RT-PCR, 40 samples were positive and 15 negative by both techniques. However, 35 samples that the ELISA kit failed to detect were positive by RT-PCR. Furthermore, four samples in which the *Giardia* antigen was detected (one of them a weak positive) were negative by PCR (Table 2).

The statistical analyses demonstrated significant differences between microscopy and ELISA ($P = 0.001$), microscopy and PCR ($P = 0.001$), and ELISA and PCR ($P = 0.012$). In comparison to PCR, the sensitivity and specificity of microscopy were 50.7% and 100%, and the sensitivity and specificity of ELISA were 53.3% and 79%, respectively.

## 4. Discussion

*G. intestinalis* is one of the most common human parasites and the cause of giardiasis. The infection affects individuals worldwide, especially in areas where sanitation is poor. Due to outbreaks and its effects on growth in children, *G. intestinalis* is still a significant public health problem. The prevalence of infection is 2%–5% in developed countries and up to 20% in developing countries. Additionally, the highest number of cases of infection occurs in children younger than 10 years old (11–13). The incidence of giardiasis has been reported as 4.5%–22% in Turkey (14).

*G. intestinalis* infection has a wide clinical spectrum, which ranges from asymptomatic cases to acute or chronic diarrhea, abdominal pain, and weight loss (15). The laboratory diagnosis of giardiasis generally depends on detection of cysts/trophozoites of the parasite in stool samples. Examination of only one single specimen by microscopy decreases the sensitivity due to the intermittent excretion of the parasite. Therefore, at least three multiple samples should be examined on separate days for a definitive diagnosis (16,17). One other significant drawback of microscopic examination is that it requires an experienced microscopist (16). For the above-mentioned reasons, there might be many false negative results making the sensitivity of microscopy lower. Additionally, antigen detection immunoassays and molecular-based methods can only be performed in certain specialized laboratories.

The direct fluorescent antibody test and enzyme immunoassays (EIAs) have high sensitivity and specificity similar to the most widely used immunological techniques (18). EIA is practical when numerous samples should

**Table 1.** Comparison of microscopy results with ELISA and RT-PCR.

| | | Microscopy | | |
|---|---|---|---|---|
| | | Positive (+) | Negative (-) | |
| ELISA | + | 32 | 12 | 44 |
| | - | 6 | 44 | 50 |
| RT-PCR | + | 38 | 37 | 75 |
| | - | - | 19 | 19 |
| Total | | 38 | 56 | 94 |

**Table 2.** Comparison of ELISA results with RT-PCR.

| | | ELISA | | |
|---|---|---|---|---|
| | | Positive (+) | Negative (-) | |
| RT-PCR | + | 40 | 35 | 75 |
| | - | 4 | 15 | 19 |
| Total | | 44 | 50 | 94 |

be screened. Several commercially available ELISA kits that detect *G. intestinalis* antigens in stool samples are used in the diagnosis of giardiasis with varied sensitivity and specificity. They have significant advantages in terms of turnaround time and easy usefulness and they enable the quantitative reading of results. Generally, this technique provides over 90% sensitivity and specificity when compared to microscopy (19). Recently, a variety of PCR assays (nested PCR, PCR-RFLP, RT-PCR etc.) were developed for diagnosis of giardiasis. They have excellent sensitivity and specificity but require more specialized technical skills and high-cost equipment. RT-PCR is the most powerful method and it has the following advantages: targeting the small specific gene regions of the parasite, rapid cycling time (approximately 1 h), low contamination risk, and ability to measure the DNA amount during the assay without post-PCR analysis. The infection could be detected in patients with a low parasite count by ELISA or by PCR when only when two cysts are present (20). These methods capture the infection using the parasite antigen or DNA molecule, so even when the live parasite is absent, they produce accurate results (21,22). RT-PCR was accepted as the gold standard in this study; however, there is no true reference test for the diagnosis of the disease. The use of different diagnostic techniques together would increase the chances of obtaining true positives (11).

In this study, out of 94 patients, 44 were diagnosed as positive for *G. intestinalis* infection by the CELISA *Giardia* kit, which produced better results than wet mount microscopy, which detected only 38 positive cases of giardiasis. There were six false negatives by EIA, possibly associated with intermittent shedding of the parasite cysts. Various commercial EIA kits in the detection of *G. intestinalis* might detect different values. In a recent study, ELISA's sensitivity and specificity compared to microscopy was 72.9% and 100%, respectively (22). In several other studies conducted with different immunoassay kits, excellent specificity rates within the range of 91.5%–100% were reported and the specificity values for all EIAs exceeded 99% (23,24). In a study conducted with the same commercial EIA kit used in this study (Giardia CELISA, Cellabs), the sensitivity (63%) was found to be in concordance with our findings (13). Aldeen et al. (23) suggested that EIAs could replace microscopic examination when giardiasis is the most likely diagnosis. However, considerably lower results were detected in sensitivity with different EIAs with the sensitivity of 63% and 73.2% (22,25).

While *Giardia* was detected in 38 patients by microscopy, 37 additional individuals were also found to be *Giardia*-positive by RT-PCR. Compared to RT-PCR, microscopy exhibited false negative results in 39.4% of the patients. These findings confirm the limitation of microscopy in *G. intestinalis* detection. It was previously demonstrated that microscopy had lower sensitivity (50%) and PCR had higher sensitivity and specificity in detection of *Giardia* (26). In another study, RT-PCR showed 100% sensitivity, whereas conventional assays (microscopic examination including immunochromatographic and direct immunofluorescence assays) revealed 86.7% specificity (20). The lower sensitivity and specificity of ELISA compared to RT-PCR was reported by several other studies, as well (20,26). Verweij et al. (27) found that the sensitivity of PCR was the same as that of the antigen detection method (98%), although higher than that of microscopy (89%). However, PCR produced false negative results against enzyme EIAs, and lower sensitivity of PCR (85.4%) against immunofluorescence was detected (22). In another study, microscopy, RT-PCR, and rapid immunoassay were compared and all three techniques were highly sensitive within the range of 98%–100% (28). In this study, ELISA exhibited 35 false positive and four false negative results compared to RT-PCR. One of the false negative results exhibited by PCR was borderline positive by ELISA. Most of the low Ct values detected by PCR generated negative results by ELISA. This demonstrates that ELISA could not capture positives in the presence of small parasite counts, which were detected only by RT-PCR based on DNA levels.

Traditionally, wet mount microscopy and concentration techniques were used for routine analysis in the diagnosis of *Giardia* infection. They must be used as a first choice due to their economical and easy-to-use features. EIAs are generally utilized and are useful to analyze large numbers of samples. In summary, the RT-PCR assay seems to be beneficial for rapid and accurate diagnosis of *G. intestinalis* in human stool samples. Unfortunately, due to high costs related to PCR, molecular methods are still not widely available, especially in developing countries. The most important benefit of molecular assays is that they have contributed progress towards better public health while reducing the cost of unnecessary treatment due to misdiagnosis. At least one molecular technique must be utilized in routine diagnosis for evaluation and confirmation of the stool antigen assays or conventional microscopic results.

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## References


1. Adam RDA. Biology of *Giardia intestinalis*. Clin Microbiol Rev 2001; 14: 447-475.
2. WHO. The World Health Report. Fighting Disease, Fostering Development. Geneva, Switzerland: World Health Organization; 1996.
3. Hill DR. *Giardia intestinalis*. In: Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 8th ed. New York, NY, USA: Churchill Livingstone; 2010. pp. 3527-3534.
4. Lane S, Lloyd D. Current trends in research into the waterborne parasite *Giardia*. Crit Rev Microbiol 2002; 28: 123-147.
5. Ortega YR, Adam RD. Giardia: overview and update. Clin Infect Dis 1997; 25: 545-550.
6. Garcia LS. Diagnostic Medical Parasitology. 5th ed. Washington, DC, USA: ASM Press; 2007.
7. Johnston SP, Ballard MM, Beach MJ, Causer L, Wilkins PP. Evaluation of three commercial assays for the detection of *Giardia* and *Cryptosporidium* organisms in fecal specimens. J Clin Microbiol 2003; 41: 623-626.
8. Babaei Z, Oormazdi H, Rezaie S, Rezaeian M, Razmjou E. *Giardia intestinalis*: DNA extraction approaches to improve PCR results. Exp Parasitol 2011; 128: 159-162.
9. McGlade TR, Robertson ID, Elliot AD, Read C, Thompson RC. Gastrointestinal parasites of domestic cats in Perth, Western Australia. Vet Parasitol 2003; 117: 251-262.
10. Klein D. Quantification using real–time PCR technology: applications and limitations. Trends Mol Med 2002; 8: 257-260.
11. Caccio SM, Sprong H. Epidemiology of giardiasis in humans. In: Lujan HD, Svard S, editors. *Giardia*: A Model Organism. Vienna, Austria: Springer-Verlag; 2011. pp. 17-25.
12. CDC. Division of Parasitic Diseases Program Review. National Center for Infectious Diseases. Atlanta, GA, USA: Centers for Disease Control; 1995.
13. Marshall MM, Naumovitz D, Ortega Y, Sterling CR. Waterborne protozoan pathogens. Clin Microbiol Rev 1997; 10: 67-85.
14. Balcioglu C, Limoncu E, Ertan P, Yereli K, Ozbilgin A, Onag A. Incidence of giardiasis among siblings in Turkey. Pediatr Int 2003; 45: 311-313.
15. Dutt P, Mehta S, Vinayak VK. Enzyme linked immunosorbent assay for copro-diagnosis of giardiasis and characterization of a specific *Giardia intestinalis* antigen in stools. J Med Microbiol 1991; 34: 271-275.
16. Clinical and Laboratory Standards Institute. Procedures for the Recovery and Identification of Parasites from the Intestinal Tract; Approved Guideline. CLSI Document M28-A2. 2nd ed. Wayne, PA, USA: Clinical Laboratory Standards Institute; 2005.
17. Hanson KL, Cartwright CP. Use of an enzyme immunoassay does not eliminate the need to analyze multiple stool specimens for sensitive detection of *Giardia intestinalis*. J Clin Microbiol 2001; 39: 474-477.
18. El-Nahas HA, Salem DA, El-Henawy AA, El-Nimr HI, Abdel-Ghaffar HA, El-Meadawy AM. Giardia diagnostic methods in human fecal samples: a comparative study. Cytometry B Clin Cytom 2013; 84: 44-49.
19. Al-Saeed AT, Issa SH. Detection of *Giardia intestinalis* antigen in stool specimens using enzyme-linked immunosorbent assay. East Mediterr Health J 2010; 6: 362-364.
20. Calderaro A, Gorrini C, Montecchini S, Peruzzi S, Piccolo G, Rossi S, Gargiulo F, Manca N, Dettori G, Chezzi C. Evaluation of a real-time polymerase chain reaction assay for the laboratory diagnosis of giardiasis. Diagn Microbiol Infect Dis 2010; 66: 261-267.
21. Addiss DG, Mathews HM, Stewart JM, Wahlquist SP, Williams RM, Finton RJ, Spencer HC, Juranek DD. Evaluation of a commercially available Enzyme-linked immunosorbent assay for *Giardia intestinalis* antigen in stool. J Clin Microbiol 1991; 29: 1137-1142.
22. Jelinek T, Neifer S. Detection of *Giardia intestinalis* stool samples: a comparison of two enzyme-linked immunosorbent assays. F1000Research 2013; 2: 39-44.
23. Aldeen WE, Carroll K, Robison A, Morrison M, Hale D. Comparison of nine commercially available enzyme-linked immunosorbent assays for detection of *Giardia intestinalis* in fecal specimens. J Clin Microbiol 1998; 36: 1338-1340.
24. Schunk M, Jelinek T, Wetzel K, Nothdurft HD. Detection of *Giardia intestinalis* and *Entamoeba histolytica* in stool samples by two enzyme immunoassays. Eur J Clin Microbiol Infect Dis 2001; 20: 389-391.
25. Maraha B, Buiting AGM. Evaluation of four enzyme immunoassays for the detection of *Giardia intestinalis* antigen in stool specimens. Eur J Clin Microbiol Infect Dis 2000; 19: 485-487.
26. Stark D, Al-Qassab SE, Barratt JLN, Stanley K, Roberts T, Marriott D, Harkness J, Ellis JT. Evaluation of multiplex tandem real–time PCR for detection of *Cryptosporidium* spp., *Dientamoeba fragilis*, *Entamoeba histolytica*, and *Giardia intestinalis* in clinical stool samples. J Clin Microbiol 2011; 49: 257-262.
27. Verweij JJ, Schinkel J, Laeijendecker D, van Rooyen MA, van Lieshout L, Polderman AM. Real-time PCR for the detection of *Giardia intestinalis*. Mol Cell Probes 2003; 17: 223-225.
28. Schuurman T, Lankamp P, van Belkum A, Kooistra-Smid M, van Zwet A. Comparison of microscopy, real-time PCR and a rapid immunoassay for the detection of *Giardia intestinalis* in human stool specimens. Clin Microbiol Infect 2007; 13: 1186-1191.