Eur J Clin Microbiol Infect Dis (2001) 20:389–391 

*Article*

# Detection of *Giardia lamblia* and *Entamoeba histolytica* in Stool Samples by Two Enzyme Immunoassays

M. Schunk, T. Jelinek, K. Wetzel, H.D. Nothdurft

**Abstract** Two commercially produced enzyme immunoassays (EIAs) to detect antigens of *Giardia lamblia* and *Entamoeba histolytica* in stool specimens were evaluated. A total of 276 stool specimens were collected from patients who presented with various medical complaints in the outpatient clinic of the Department of Infectious Diseases and Tropical Medicine, University of Munich. Every specimen was examined by conventional microscopy and tested by both EIA kits. When microscopy was used as the reference standard, the EIA kit detecting *Giardia lamblia* showed a sensitivity of 100% and a specificity of 99.6%. The EIA kit detecting *Entamoeba histolytica* had a sensitivity of 81.8% and a specificity of 99.2%. Both tests showed no cross-reactivity with other intestinal protozoa. Antigen detection by EIA has the potential to become a valuable tool capable of making stool diagnostics more effective, although it should not be considered as a replacement for microscopic examination, since other potential pathogens could otherwise escape detection.

## Introduction

Intestinal parasites are distributed worldwide. The two most common intestinal protozoan parasites are *Giardia lamblia* and *Entamoeba histolytica* [1]. Since both of them have a faecal-oral transmission cycle and are contracted by ingestion of contaminated water or food or by person-to-person contact, they are most prevalent in areas where sanitary conditions are poor. Therefore, the highest rates of infection are found in developing countries, while in developed countries infections occur mostly in persons living in closed communities, homosexual men, immigrants and, of increasing importance, travellers returning from highly endemic countries.

The protozoan flagellate *Giardia lamblia* has a reported global prevalence of approximately 30% [2, 3]. Acute giardiasis has been well described in travellers returning from highly endemic areas [4]. The infection may be asymptomatic or it may present with various symptoms. The main symptom is watery, foul-smelling diarrhoea, often accompanied by nausea, abdominal cramps or gurgling, bloating and weight loss [5]. Symptoms may persist for weeks in variable severity. *Giardia lamblia* does not invade tissue. It's life cycle consists of two different stages: the initial trophozoite and, the infectious form, the cyst [6].

*Entamoeba histolytica/dispar* is found worldwide, with infection rates reaching up to 80% in some developing countries. Estimates suggest that approximately 10% of the global population is infected with this parasite and up to 110,000 deaths per year can be attributed to complications it has caused [7, 8]. Recently, it has been demonstrated that distinct species of *Entamoeba* are morphologically identical [9, 10], i.e., *Entamoeba dispar*, which solely appears with an asymptomatic carrier state, and the pathogenic species *Entamoeba histolytica* sensu strictu, which has the ability to invade tissue and cause symptomatic disease. Clinical manifestations of *Entamoeba histolytica* infection vary wildly and include diarrhoea with mucus or blood, accompanied by nausea, fever, colic and tenesmus. Infection of the liver with subsequent amoebic liver abscess might develop as a sequelae due to invasion into the portal circulation.

M. Schunk, T. Jelinek (✉), K. Wetzel, H.D. Nothdurft
Department of Infectious Diseases and Tropical Medicine, University of Munich, Leopoldstrasse 5, 80802 Munich, Germany
e-mail: jelinek@lrz.uni-muenchen.de
Tel.: +49-89-21803517
Fax: +49-89-336112

The diagnosis of giardiasis and intestinal amoebiasis is still based primarily on microscopic detection of the organisms in stool, but the method is time- and labour-intensive and depends on the skill of an experienced microscope technician [11, 12]. Diagnosis via the microscopic examination of a single stool specimen has a low sensitivity and may therefore miss up to 50% of infections caused by *Giardia* or *Entamoeba* spp. [11, 13]. Because of intermittent shedding of the parasites, the microscopic examination of three consecutive stool specimens is required to reach a sensitivity exceeding 90%. Another difficulty encountered with the microscopic diagnosis of intestinal amoebiasis is that of distinguishing the morphologically identical, nonpathogenic *Entamoeba dispar* from the pathogenic *Entamoeba histolytica* [14]. Given these difficulties, the development of sensitive, cost-effective and rapid diagnostic methods is of utmost importance. The recently developed enzyme immunoassays (EIAs) for the detection of specific antigens in stools hold the potential to become an efficient diagnostic technique for the detection of *Giardia lamblia* and *Entamoeba histolytica* [10, 15–17]

In the study presented here we tested two commercially available EIA kits, one that detect antigens of *Giardia lamblia* and another that detects *Entamoeba histolytica/dispar* in stool (Ridascreen Giardia and Ridascreen Entamoeba; R-Biopharm, Germany). The results were compared with those of conventional microscopic examination (CME).

## Materials and Methods

After informed consent was obtained, stool specimens were collected from patients who presented with diarrhoea plus various other complaints at our outpatient clinic from September 1999 to March 2000. All of the patients included in the study were German nationals returning from vacations abroad. All stool samples were investigated for ova and parasites by direct microscopy (iron-hematoxylin stain) and the sodium-acetate-acetic acid-formaline/ethyl acetate-concentration technique. Every slide was read for at least 10 min by two experienced microscope technicians before being considered negative. One part of every fresh stool sample was stored immediately at –20 °C and tested later by EIA, according to the manufacturer's instructions, by one lab technician who was not aware of the microscopy results. EIA results were obtained using a microplate reader (SLT-Labinstruments, Germany) with a wavelight setting of 450 nm. The cut-off in both tests was determined by adding 0.15 absorbance units to the measured absorption of the negative control. Samples were considered positive if the absorbance value was higher than 10% above the determined cut-off. If different EIA and microscopy results were obtained, both tests were repeated. EIA results were compared with those obtained by CME. The samples that had a positive result in CME were considered true positive. The sensitivity, specificity and the positive predictive value of both EIAs were calculated.

**Table 1** Results achieved using commercial enzyme immunoassays (EIAs) to detect *Giardia* and *Entamoeba* compared with conventional microscopy

| EIA | Conventional microscopy | |
|---|---|---|
| | Positive | Negative |
| *Giardia* | | |
| Positive | 21 | 1 |
| Negative | 0 | 254 |
| *Entamoeba* | | |
| Positive | 9 | 2 |
| Negative | 2 | 263 |

## Results

All 276 specimens were examined by CME and two EIAs (G-EIA to detect *Giardia* and E-EIA to detect *Entamoeba*). *Giardia lamblia* was detected in 21 (7.6%) stool samples by both CME and G-EIA. A total of 254 stools were negative for *Giardia lamblia* by both methods. One specimen was positive by G-EIA and negative by microscopy. This sample was considered to be false positive. There was no specimen that was false negative, i.e., negative by G-EIA and positive by microscopy (Table 1). The sensitivity of G-EIA versus microscopy was therefore calculated at 100% and the specificity at 99.6%.

*Entamoeba histolytica/dispar* was detected in nine stool samples by both CME and E-EIA. A total of 263 stools were negative for *Entamoeba histolytica* by both methods. Two specimens were positive by E-EIA and negative by microscopy. These samples were considered to be false positive. There were two specimens that were positive by microscopy and negative by E-EIA, which were considered to be false negative (Table 1). The sensitivity of E-EIA versus microscopy was calculated at 81.8% and the specificity at 99.2%.

Cross-reactions of the EIA with other antigens were not observed. Of the 276 patients included in the study, 48 (17.4%) carried at least one parasite other than the two investigated in their stool: 24 patients were infected with *Blastocystis hominis*, 12 with *Entamoeba coli*, 10 with *Endolimax nana* and 16 with a variety of other intestinal parasites, including *Iodamoeba bütschlii*, *Trichomonas hominis*, *Chilomastix mesnili*, *Isospora belli*, *Ancylostoma duodenale/Necator americanus*, *Strongyloides stercoralis*, *Trichura trichuris*, *Ascaris lumbricoides*, and others. No stool sample from any of these patients reacted positive in either of the EIAs.

## Discussion

In order to find simple, inexpensive and reliable diagnostic techniques for detecting intestinal infections with *Giardia lamblia* and *Entamoeba histolytica/dispar*, several EIA test kits have been developed recently and tested in various studies [10, 13, 15–21]. In this study,

we evaluated the performance of two commercially available EIA kits for the detection of *Entamoeba histolytica*/*dispar* or *Giardia lamblia*. We tested 276 specimens and compared the results of EIA to the results of stool microscopy of the same sample. When microscopy was used as the reference standard, the EIA kit detecting *Giardia lamblia* showed a sensitivity of 100% and a specificity of 99.6%. The EIA kit detecting *Entamoeba histolytica*/*dispar* had a sensitivity of 81.8% and a specificity of 99.2%. Both tests showed no cross-reactivity with other intestinal protozoa.

As mentioned earlier, microscopic examination of one single stool specimen has a low sensitivity [11, 13]. It is likely that antigens of *Giardia lamblia* or *Entamoeba histolytica*/*dispar* can be detected by EIA even in the absence of intact organisms (cysts or trophozoites). This may result in a greater sensitivity of EIA tests compared with microscopy [16], thereby providing low specificity results when only one CME is used as the reference standard. In a study conducted by Aldeen et al. [20], nine different immunoassay kits for the detection of *Giardia lamblia* were evaluated, with the resulting sensitivity values ranging from 93 to 100% and the specificity values for all EIAs exceeding 99%. The authors suggest that the high specificity results are due to the examination of up to seven individual slide preparations on an initially negative microscopic finding. Haque et al. [10] examined the results of EIA-based stool antigen kits for detecting *Entamoeba histolytica*/*dispar* in comparison with stool microscopy and culture. When culture was used as the reference standard, microscopy had a sensitivity of 60% and a specificity of 79% while the E-EIA showed a higher sensitivity (80%) and specificity (99%). In conclusion, we found that the EIAs evaluated are highly sensitive and specific for the detection of *Giardia lamblia* or *Entamoeba histolytica*/*dispar*. Antigen detection by EIA certainly has the potential to become a valuable additional method for increasing the effectiveness of stool diagnostics. However, there is currently no replacement for microscopic examination of stool specimens, since other potential pathogens could otherwise escape detection.

## References


1. Cook GC: *Entamoeba histolytica* and *Giardia lamblia* infections: current diagnostic strategies. Parasite (1995) 2:107–112
2. Petersen H: Giardiasis (lambliasis). Scandinavian Journal of Gastroenterology (1972) 14:1–44
3. Jelinek T, Peyerl G, Löscher TH, Nothdurft HD: Giardiasis in travellers: evaluation of an antigen-capture EIA for the detection of *Giardia lamblia*-antigen in stool. Zeitschrift für Gastroenterologie (1996) 34:237–240
4. Farthing M: Giardiasis as a disease. In: Reynoldson J, Thompson R, Lymbery A (eds): *Giardia*: from molecules to disease and beyond. CAB International, London (1993) pp15–37
5. Ortega YR, Adam RD: *Giardia*: overview and update. Clinical Infectious Diseases (1997) 25:545–549
6. Wolfe MS: Giardiasis. Clinical Microbiology Reviews (1992) 5:93–100
7. Ravdin JI, Jackson TFHG, Petri WA, Murphy CF, Ungar BLP: Association of serum antibodies to adherence lecitin with invasive amebiasis and asymptomatic infection with pathogenic *Entamoeba histolytica*. Journal of Infectious Diseases (1990) 162:768–772
8. Anonymous: Amoebiasis and its control. Bulletin of the World Health Organization (1985) 63:417–426
9. Tannich E, Horstmann R, Knobloch J, Henning A: Genomic DNA differences between pathogenic and nonpathogenic *Entamoeba histolytica*. Proceedings of the National Academy of Sciences of the USA (1989) 86:5118–5122
10. Haque R, Neville LM, Hahn P, Petri WA: Rapid diagnosis of *Entamoeba* infection by using *Entamoeba* and *Entamoeba histolytica* stool antigen detection kits. Journal of Clinical Microbiology (1995) 33:2558–2561
11. Ravdin J: Amebiasis. Clinical Infectious Diseases (1995) 20:1453–1466
12. Thompson RCA, Reynoldson JA, Mendis AHW: *Giardia* and giardiasis. Advances in Parasitology (1993) 32:71–160
13. Addis DG, Mathews HM, Stewart JM, Wahlquist SP, Williams RM: Evaluation of a commercially available enzyme-linked immunosorbent assay for *Giardia lamblia* antigen in stool. Journal of Clinical Microbiology (1991) 29:1137–1142
14. Ellen L, Stanley SL: Parasitic infections of the gastrointestinal tract. Gastroenterology (1996) 12:82–87
15. Elkadi IA, Smith DH, Hommel M: Early diagnosis of giardiasis by faecal antigen detection using capture EIA in a cohort of children in the United Arab Emirates. Transactions of the Royal Society of Tropical Medicine and Hygiene (1992) 86:520–521
16. Rosenblatt JE, Sloan LM, Schneider SK: Evaluation of an enzyme-linked immunosorbent assay for the detection of *Giardia lamblia* in stool specimens. Diagnostic Microbiology and Infectious Diseases (1993) 16:337–341
17. Pillai DR, Keystone JS, Sheppard DC, MacLean JD, MacPherson DW: *Entamoea histolytica* and the diagnostic methods in a setting of nonendemicity. Clinical Infectious Diseases (1999) 29:1315–1318
18. Rosoff JD, Stibbs HH: Physical and chemical characterisation of a *Giardia lamblia*-specific antigen useful in the coprodiagnosis of giardiasis. Journal of Clinical Microbiology (1986) 23:905–910
19. Goldin AJ, Hall A, Sarker RA, Warhurst DC, Miles MA: Diagnosis of *Giardia lamblia* infection in Bangladeshi infants: faecal antigen capture EIA. Transactions of the Royal Society of Tropical Medicine and Hygiene (1993) 87:428–432
20. Aldeen WE, Carroll K, Robison A, Morrison M, Hale D: Comparison of nine commercially available enzyme-linked immunosorbent assays for detection of *Giardia lamblia* in fecal specimens. Journal of Clinical Microbiology (1998) 36:1338–1340
21. Benzeguir AK, Aust KA: Evaluation of an enzyme-immunoassay test kit for diagnosing infections with *Entamoeba histolytica*. Archives of Medical Research (1997) 28:276–278