SHORT RESEARCH ARTICLE

Detection of *Giardia lamblia* stool samples: a comparison of two enzyme-linked immunosorbent assays [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

Two commercially produced enzyme-immunosorbent assays (EIAs) to detect antigens of *Giardia lamblia* in stool specimens, Ridascreen Giardia and Serazym Giardia, were evaluated. A total of 88 stool specimens were collected from patients who presented for various medical complaints in our outpatient clinic. Every specimen was examined by a conventional microscopic examination (CME), PCR and tested by both EIA kits. When microscopy was used as the reference standard, the Ridascreen Giardia showed a sensitivity of 72.9% and a specificity of 100%. Serazym Giardia had a sensitivity of 93.8% and a specificity of 100%. Antigen detection by EIA has been established as a valuable tool to make parasite stool diagnostics more effective.

Keywords

Giardia lamblia diagnosis, antigen detection

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Introduction

Intestinal parasites have a world-wide distribution. One of the most common intestinal protozoan parasites is *Giardia lamblia*. Since it has a fecal-oral transmission cycle and is contracted by ingestion of contaminated water or food or by person-to-person contact, the highest disease burden is found in areas where sanitary conditions are poor. The highest rates of infection are therefore encountered in developing countries (10–30% in young children), 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 (2–5% of symptomatic patients).

The protozoan flagellate *Giardia lamblia* has reported a global prevalence of approximately 30% in cases of acute or persistent diarrhea. Acute giardiasis has been repeatedly described in travellers returning from highly endemic areas. The infection may be asymptomatic or present with various symptoms. The main symptom is watery, foul smelling diarrhoea, often accompanied by nausea, abdominal cramps or gurgling, bloating and weight loss. Symptoms of variable severity may persist for weeks. *Giardia lamblia* does not invade the tissue. Its life cycle consists of two different stages: the trophozoite and the infectious form (the cyst).

The diagnosis of giardiasis is frequently based on microscopic detection of the organisms in stool samples. Yet the method is time and labour intensive and depends on the skill of an experienced microscopist. Diagnosis via microscopical examination of a single stool specimen has a low sensitivity and may therefore miss up to 50% of *Giardia* infections. Because of the intermittent shedding of the parasites, microscopic examination of three consecutive stool specimens is required to reach a sensitivity of over 90%. Given these difficulties the development of sensitive, cost-effective and rapid diagnostic methods is of utmost importance. Enzyme immuno-assays (EIAs) for detection of the specific antigens in stools have developed into sensitive, cost-effective and rapid diagnostic methods with the Serazym Giardia test, sensitivity was 72.9%, whilst the specificity was 100%. With the Ridascreen Giardia test, sensitivity was calculated at 93.8% (45 samples positive), with a specificity of 100%. PCR produced several false negative results against CME and IFM, which are considered gold standard tests. The sensitivity of PCR was 85.4% (41 samples correctly positive), whilst the specificity was 100% (Table 2). With PCR used as a background for the EIAs, results became more divergent (Table 3): Ridascreen Giardia performed with a sensitivity of 73.2% and a specificity of 83.4%, while Serazym Giardia showed a sensitivity of 95.1%, and a specificity of 87.2%.

Discussion

In order to find simple, inexpensive and reliable diagnostic techniques for detecting intestinal infections with *Giardia lamblia*, several EIA test kits have been developed and tested in various studies. In this study, we evaluated the performance of two commercially available EIA kits for detecting *Giardia lamblia* antigens. We tested 88 specimens and compared the results of EIA to the results of stool microscopy (CME and IFM) of the same sample. When microscopy was used as the reference standard, the EIA kits both showed a specificity of 100%, while...
sensitivity varied considerably (Table 1). Ridascreen Giardia showed 72.9%, whilst Serazym Giardia performed considerably better with a sensitivity of 93.8%. PCR detection of Giardia DNA was also compared to microscopy (Table 2). Interestingly, PCR showed a lower sensitivity than one of the EIAs (85.4% vs. 93.8% Serazym Giardia). This may be explained by the high concentration of DNase in stool samples that traditionally hamper PCR diagnostics from this material\(^\text{18}\). It is important to notice though that the use of PCR as gold standard for the evaluation of EIAs in this setting would have produced false low specificity results for both tests (Table 3: 83.4% for Ridascreen, and 87.2% for Serazym, as opposed to the actual 100% specificities in both tests against CME and IFM).

As mentioned earlier, microscopic examination of one single stool specimen has a low sensitivity\(^\text{7,9}\). It has been demonstrated that antigens of *Giardia lamblia* 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\(^\text{9}\) and therefore provide low specificity results when only one CME is used as a reference standard. In a study conducted by Aldeen *et al.*\(^\text{16}\), nine different immunoassay kits for the detection of *Giardia lamblia* were evaluated: the sensitivity ranged from 93% to 100% and the specificity in all EIAs was above 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. In conclusion, we found that the EIAs evaluated are highly sensitive and specific for detection of *Giardia lamblia*. In our setting, one of the two EIAs proved to be more reliable than PCR. Antigen detection by EIA methods certainly has developed into a powerful additional method for increasing the effectiveness of stool diagnostics. Results of this study also demonstrate that considerable differences in sensitivity might occur between commercial tests. Thus, careful selection of appropriate test kits seems mandatory. For screening purposes, the antigen tests remain additional tools: there is currently no replacement for microscopical examination of stool specimens, since other potential pathogens could otherwise escape detection.

**Author contributions**

TJ designed the study, collected part of the samples, did part of the testing, and wrote part of the paper. SN collected part of the samples, did part of the testing and wrote part of the paper. Both authors approved the final manuscript for publication.

**Competing interests**

No competing interests have been disclosed.

**Grant information**

The authors declare that no grants were involved in supporting this work.

**Acknowledgements**

Sekisui Virotech GmbH provided the Serazym Giardia assays as well as financial support for purchase of the Ridascreen Giardia assays and for PCR.

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**Table 1 Results of Ridascreen Giardia and and Serazym Giardia tests against conventional and immunofluorescence stool microscopy.**

<table>
<thead>
<tr>
<th>Antigen test</th>
<th>Microscopy</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Ridascreen Giardia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>35</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>13</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Serazym Giardia</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>45</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity Ridascreen Giardia 72.9%.
Specificity Ridascreen Giardia 100%.
Positive predictive value Ridascreen Giardia 100%.
Negative predictive value Ridascreen Giardia 75.5%.
Sensitivity Serazym Giardia 93.8%.
Specificity Serazym Giardia 100%.
Positive predictive value Serazym Giardia 100%.
Negative predictive value Serazym Giardia 93%.

**Table 2 Results of PCR against conventional and immunofluorescence stool microscopy.**

<table>
<thead>
<tr>
<th>PCR</th>
<th>Microscopy</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>41</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>7</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity PCR 85.4%.
Specificity PCR 100%.
Positive predictive value PCR 100%.
Negative predictive value PCR 85.1%.

**Table 3 Results of Ridascreen Giardia and Serazym Giardia tests against PCR.**

<table>
<thead>
<tr>
<th>Antigen test</th>
<th>PCR</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Ridascreen Giardia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
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<td>5</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>11</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Serazym Giardia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>39</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>41</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity Ridascreen Giardia 73.2%.
Specificity Ridascreen Giardia 83.4%.
Positive predictive value Ridascreen Giardia 85.7%.
Negative predictive value Ridascreen Giardia 79.2%.
Sensitivity Serazym Giardia 95.1%.
Specificity Serazym Giardia 87.2%.
Positive predictive value Serazym Giardia 86.7%.
Negative predictive value Serazym Giardia 95.4%.
References

Open Peer Review

Current Peer Review Status: ✓ ❓

Version 1

Reviewer Report 12 June 2013

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Selwyn Lowe
Maastricht University Medical Center, Maastricht, The Netherlands

Abstract: The PCR is mentioned, but not the results compared to the standard.

Article content:

- **Materials and methods:** "Every slide was read for at least 10 min by two experienced microscopists ..." Was this done in parallel for 2 different slides or sequentially for 1 slide? If sequentially, microscopy was done for 20 min and this could influence the results.
- If different results between the EIA, PCR or microscopy (CME) were obtained, was immunofluorescence microscopy used for additional confirmation of true positive results? This is not in line with the Results: The 88 specimens were examined by CME, IFM, PCR and two EIA's.
- Please clarify the difference between what is stated in the Materials and Methods section and in the Results section. Where all 5 assays performed on all 88 stool samples?
- **Results:** It is not quite clear how the different assays compare to each other. Maybe a 5 by 5 table with only the positive results would give a better insight?

**Competing Interests:** No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 14 February 2013

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**Competing Interests:** No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.