Immunochromatography versus microscopy for the identification of *Giardia lamblia* and *Cryptosporidium parvum* in human feces

Imunocromatografie versus microscopie în identificarea *Giardia lamblia* si *Cryptosporidium parvum* din fecalele umane

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ABSTRACT

The purpose of this study has been to evaluate the benefits of rapid immunochromatographic qualitative assays, compared to classic microscopy, in the diagnosis of giardiasis. From a total number of 960 specimens received for ova & parasites examination during one year, 61.98% (95% CI 58.91-65.05%) arrived at our laboratory with a presumptive diagnosis of lambliasis/giardiasis. Only for 0.94% (95% CI 0.33-1.55%) of the total 960 specimens this diagnosis has been confirmed by microscopy and RIDA Quick *Cryptosporidium/Giardia* Combi - immune chromatography qualitative assay. There was a 100% concordance both in sensitivity and specificity between assays, when a total number of 3-4 samples/patient were analyzed by microscopy. The benefits of using a rapid assay were its higher sensitivity when a single sample (rapidity) was analyzed, due to antigen detection even when difficult to identify cysts, with modified morphology, were present. The limits of rapid assays were both objective, due to antigen persistence several days after treatment, and subjective, due to the low concordance between presumptive clinical diagnosis and certified parasitological diagnosis. In turn, the limits of microscopy were the need for an experienced microscopy technician and the fact that microscopy is a time consuming procedure.

Keywords: *Giardia lamblia*, *Cryptosporidium parvum*, antigen, microscopy, immunochromatography

Introduction

The diagnosis of human intestinal protozoa depends on microscopic detection of the various parasite stages in feces or duodenal fluid. Microscopy is considered to be the golden standard for diagnosis of *Giardia lamblia* (*G.lamblia*) infection [2] but, since fecal examination is very labor-intensive and requires a skilled microscopist, antigen detection tests have been developed as alternatives, using direct fluorescent antibody (DFA), enzyme immunoassay (EIA), and rapid, dipstick-like tests [6, 7]. Antigen detection methods can be performed quickly and do not require an experienced and skilled morphologist. Much work has been accomplished on the development of antigen detection tests, resulting in commercially available reagents for various intestinal parasites, such as the rapid immunochromatographic assay for the combined antigen detection of *Cryptosporidium parvum* and *G. lamblia* [7]. The objectives of our study were to identify the benefits and limits of rapid detection techniques versus microscopy, in the laboratory diagnosis of intestinal parasitic diseases.
Material and Methods

Stool samples from 960 patients clinically diagnosed with intestinal parasitoses were analyzed by optical microscopy and antigen detection method using a RIDA Quick Cryptosporidium / Giardia Combi - immunochromatographic qualitative assay.

Investigated patients had been hospitalized in different clinics of the Emergency Clinical County Hospital in Cluj-Napoca, Romania, between May 2007 and May 2008.

Optical microscopy was performed on wet smears, prepared from fresh feces samples, using iodine. The smears were analyzed by a well trained microscopist, using magnifications 10X and 40X (when needed). For 100 consecutive samples, antigen detection based on immunochromatography (RIDA Quick Cryptosporidium / Giardia Combi) was performed along with each microscopic examination.

Results and Discussions

From a total number of 960 ova & parasites examinations performed annually, 61.98% (95% CI 58.91-65.05%) were received by the laboratory with a presumptive diagnosis of lambliasi / giardiosis, 8.02% (95% CI 6.3-9.74%) suspected other types of parasitic infection and 30% (95% CI 27.1-32.9%) did not specify a particular type of suspected etiology, being sent to the laboratory with a clinical suspicion of “intestinal parasites” (Figure 1).

![Figure 1. Clinical diagnosis of the samples sent for analysis](image)

Only 9 specimens, representing 0.94% (95% CI 0.33-1.55%) of all samples were identified as G.lamblia by microscopy and antigen detection. Three specimens, representing 0.31% (95% CI 0-0.66%) were considered negative after initial microscopic examination but tested positive using the immunochromatographic method; two of these specimens were indeed found to be positive, after extensive microscopic reexamination (3 repeated samples); one specimen was confirmed positive by microscopy only after collecting a fourth sample from the patient.

Other four specimens that initially tested positive using the immunochromatographic method and negative in microscopy showed negative results with both diagnostic methods when a second and third sample were analyzed.

None of the samples tested positive for Cryptosporidium.
The sensitivity of microscopy was 66.66% when one sample was analyzed, 88.88% when two samples were analyzed, with multiple wet smears performed from the same sample, and reached 100% when four samples collected at 2-3 days interval were analyzed (Figure 2).

![Figure 2. Sensitivity of microscopy based on the number of samples analyzed from the same patient](image)

The antigen detection kit tested positive for four samples / 0.42% (95% CI 0.01-0.83%), coming from patients with no particular symptoms suggesting giardiasis and it became negative when a second sample was collected at five days interval. Microscopy was negative for these specimens.

Microscopy also identified other intestinal parasites: 1 case of *Hymenolepis nana* / 0.1% (95% CI 0-0.3%), 2 cases of *Trichuris trichiura* / 0.21% (95% CI 0-0.5%) and 3 cases of *Ascaris lumbricoides* / 0.31% (95% CI 0-0.66%). All cases were identified from patients with no specified diagnosis except “intestinal parasites” (Figure 3).
Of the 9 specimens in which *G. intestinalis* has been identified, only 5, representing 55.56% (95% CI 23.1-88.02%) of these specimens, had arrived at the laboratory with a specific clinical diagnosis of giardiasis (Figure 4).

We found a good concordance between microscopy and rapid diagnostic kit when multiple samples were analyzed. A similar result has been previously found by Katanik [7].

The sensitivity of microscopy increased, along with the number of analyzed samples, up to 100% when using four samples collected in a weeks interval. Similar results have been found by Hanson [5].

The main benefits of using a rapid assay were its high testing speed along with a higher sensitivity when single samples were analyzed, due to antigen detection even when difficult to identify cysts, with modified morphology, were present.
However, the importance of rapid detection for the patient has been reduced by the persistence of antigens for several days, either after treatment, an abortive infection or a cross-over reaction with other antigens. This, most probably explains the negative microscopy in the four samples obtained from patients with no particular symptoms of giardiasis [1, 3, 4].

Subjective limits of rapid detection also rose from the low concordance between presumptive clinical diagnosis and certified parasitological diagnosis.

The limits of microscopy consisted mainly in the necessity for an experienced microscopist and in the fact that microscopy is a highly time-consuming procedure.

An important issue of our study has been to highlight the lack of a specific clinical diagnosis in a large number of samples sent to our laboratory for analysis, thus making microscopy a compulsory examination technique in order to obtain the needed data for confirming and specifying irrelevant diagnoses, such as “intestinal parasites”.

Conclusions
1. Antigen detection methods may facilitate diagnosis of Giardia lamblia in stool specimens when a single sample is analyzed.

2. Use of a rapid test does not eliminate the need to analyze stool specimens by microscopy, for specific detection of parasites.

3. Microscopy and rapid immunochromatographic tests are comparable when at least three or more samples/patient are analyzed.

References


