Journal of Microbiological Methods 110 (2015) 78–84

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Comparison of four rapid diagnostic tests, ELISA, microscopy and PCR for the detection of *Giardia lamblia*, *Cryptosporidium* spp. and *Entamoeba histolytica* in feces

Dorien Van den Bossche *, Lieselotte Cnops, Jacob Verschueren, Marjan Van Esbroeck

Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

ARTICLE INFO

Article history:
Received 7 December 2014
Received in revised form 19 January 2015
Accepted 20 January 2015
Available online 20 January 2015

Keywords:
Giardia
Cryptosporidium
Entamoeba
Rapid diagnostic tests

ABSTRACT

Purpose: Microscopy is the diagnostic reference standard for the detection of parasites, but it is labor-intensive and requires experience. Rapid diagnostic tests (RDTs) can provide an alternative to microscopy.
Methods: RDTs from four different manufacturers were compared to enzyme-linked immunosorbent assay (ELISA), microscopy and/or parasite-specific real-time PCR: Immuno*Card*STAT!®CGE (Meridian Bioscience Inc., Cincinnati, Ohio, USA) (A), *Crypto/Giardia* Duo-Strip (Coris Bioconcepts, Gembloux, Belgium) (B), RIDA®QUICK *Cryptosporidium/Giardia/Entamoeba* Combi (R-BioPharm, Darmstadt, Germany) (C) and *Giardia/Cryptosporidium* Quik Chek (Techlab Inc., Blacksburg, Virginia, USA) (D).
Results: Thirty frozen samples were analyzed retrospectively. For *Giardia lamblia* (n = 12) and *Cryptosporidium* (n = 12) sensitivities ranged from 58% (B), over 83% (A, C) to 100% (D) and from 92% (B) to 100% (A, C, D), respectively. Specificity for both *G. lamblia* and *Cryptosporidium* was 100% for all RDT brands. Sensitivity for *Entamoeba histolytica* (n = 5) was 100%, while specificity reached 80% (A) to 88% (C). In a prospective study, fresh samples were tested. For *G. lamblia* (n = 30), sensitivity ranged from 66% (B), over 79% (A) and 83% (C) to 100% (D) and specificity varied between 94% (D) and 100% (A, B, C). For *Cryptosporidium* (n = 3), sensitivity was 100% for all brands except (B) (67%) and specificities were 95% (A, B), 98% (C) and 100% (D). *E. histolytica* (n = 1) was detected by both (A) and (C), while specificity was 81% and 87% respectively.
Conclusion: RDTs can be a valuable tool when microscopic expertise is poor and in remote and outbreak settings where other techniques are often not available and rapid diagnosis is required.

1. Introduction

Giardia lamblia and *Cryptosporidium* spp. are both protozoan parasites which can be present without symptoms or cause diarrhea and abdominal discomfort with weight loss and malabsorption. *Entamoeba histolytica* is a unicellular parasite responsible for intestinal and hepatic amoebiasis and occasionally affects other organs. Clinical symptoms of intestinal amoebiasis range from colitis to dysentery or an ameboma, but can be asymptomatic as well. These three parasites can lead to human infection via fecal–oral transmission of the cysts through contaminated food and water and person-to-person contact. They are common in both developed and developing countries, but with an increased risk in the latter due to poor sanitation standards (Dillingham et al., 2002; Ali and Hill, 2003).

A variety of methods for diagnosis of all three parasites is available. *G. lamblia* cysts or trophozoites can be detected by microscopic, immunological and molecular methods in stool samples. The same techniques can be applied for *Cryptosporidium* spp. but oocysts cannot be positively identified in wet mounts and an additional staining, like acid fast Ziehl–Neelsen or carbol-fuchsin staining, is necessary. Microscopic examination of *E. histolytica* cysts does not allow one to make a distinction with *Entamoeba dispar*. Molecular investigation is indispensable as the latter is considered non-pathogenic as opposed to *E. histolytica*. Molecular techniques are sensitive and specific, but not easily accessible and expensive. Microscopy is time-consuming, labor-intensive, relies on the technician's experience and three stool samples may be required to increase sensitivity. Direct fluorescent antibody (DFA) is an immunological method which allows the visualization of the whole parasite through fluorescence. Enzymatic immunoassays (EIA) permit an objective result by the obtained optical densities (ODs). Still, these tests require more than an hour to generate a result and are optimally used in settings with a lot of samples allowing one to test the samples in batch. Rapid diagnostic tests (RDTs) are increasingly popular tools as they provide a solution to overcome these disadvantages. RDTs are immunochromatographic lateral-flow tests which allow the detection of antigens of one or more protozoan parasites in a single test format, are easy to perform and to interpret and can be used in settings with poor resources. In this study, the performance of four commercial

* Corresponding author at: Central Laboratory of Clinical Biology, Department of Clinical Sciences, Institute of Tropical Medicine, Kronenburgstraat 43/3, 2000 Antwerp, Belgium.
E-mail address: dorienvdb@hotmail.com (D. Van den Bossche).

http://dx.doi.org/10.1016/j.mimet.2015.01.016
0167-7012/

RDTs was compared to routine diagnostic methods for the detection of *G. lamblia*, *Cryptosporidium* spp. and *E. histolytica*.

2. Material and methods

2.1. Routine diagnostic methods

Analyses were performed by examining stool samples collected from patients presenting at the outpatient clinic of the Institute of Tropical Medicine (ITM), Antwerp, Belgium or stool samples that were submitted to the Central Laboratory of Clinical Biology (CLKB) of ITM for diagnosis of parasitic infections. Microscopic detection of ova and cysts was performed by the examination of direct smears with saline and wet mounts after formalin-ether concentration (Loughlin and Spitz, 1949). A carbol-fuchsin staining (Heine, 1982; Potters and Van Esbroeck, 2010) was performed on formalin-ether concentrates for the detection of *Cryptosporidium*. Freshly collected samples were fixed with a sodium acetate, acetic acid and formaldehyde (SAF) solution within 20 min and examined by microscopy after iron-hematoxylin Kinyoun staining. Copro-antigen enzyme-linked immunosorbent assays (ELISAs) were performed by the *G. lamblia* ProSpecT ELISA Microplate assay, *Cryptosporidium* ProSpecT ELISA Microplate assay and *E. histolytica* ProSpecT ELISA Microplate assay which detects both *E. histolytica* and *E. dispar* (Remel, Lenexa, Kansas, USA). A parasite-specific real-time polymerase chain reaction (PCR) to differentiate between *E. histolytica* and *E. dispar* (Cnops and Van Esbroeck, 2010), to detect *G. lamblia* (adapted from Verweij et al., 2003), or *Cryptosporidium hominis* and *Cryptosporidium parvum* (adapted from Hadfield et al., 2011) was performed on all samples positive for the corresponding parasite by microscopy and/or ELISA, real-time PCR. The primer and probe sequences were used as described before (Cnops, Verweij, Hadfield), while the extraction method and PCR conditions were slightly adapted. Briefly, primers and probes were purchased from Integrated DNA Technologies (IDT, Belgium, Leuven). The *lib13* target of *C. hominis* and *C. parvum* was detected in a duplex reaction. DNA extraction of samples of the retrospective study was performed with the QIAamp DNA stool kit (Qiagen Benelux, Venlo, The Netherlands) (Cnops and Van Esbroeck, 2010). Stool samples used in the prospective study were incubated in cobas® PCR media buffer (Roche Diagnostics, Vilvoorde, Belgium) and thoroughly mixed for 20 min with the Hulamixer (Invitrogen, Merelbeke, Belgium) before overnight storage at −20 °C (Cnops and Van Esbroeck, 2010). Prior to automated DNA extraction by the MagNA Pure LC 2.0 with the MagNA Pure LC Total Nucleic Acid High Performance kit (Roche), samples were heated for 10 min at 95 °C, centrifuged and 500 μL of fecal suspension incubated for 10 min at 56 °C after the addition of 4% polyvinylpolypyrrolidone (PVPP) and proteinase K (L. Cnops, K. Demeulemeester, E. Van Gintelenberg and M. Van Esbroeck, presented at the 8th European Meeting on Molecular Diagnostics, Scheveningen, The Netherlands, 2–4 Oct 2013). All PCRs were run on a SmartCycler II (Cepheid Benelux, Belgium) in a 25 μL reaction volume with 1× Hotstar Taq mastermix (Qiagen, Hilden, Germany) using 5 μL of DNA. Each sample was tested for efficient extraction and inhibition of the PCR by an exogenous extraction control (PhHV-1), and in each PCR a positive and negative control were tested to control the PCR process (Cnops and Van Esbroeck, 2010).

2.2. Rapid diagnostic tests

Four immunochromatographic RDT assays were evaluated and performed according to the manufacturer's instructions. Immuno*Card*STAT!®CGE (Meridian Bioscience Inc., Cincinnati, Ohio, USA), *Crypto/Giardia* Duo-Strip (Coris Bioconcepts, Gembloux, Belgium), RIDA®QUICK *Cryptosporidium/Giardia/Entamoeba* Combi (R-BioPharm, Darmstadt, Germany) and *Giardia/Cryptosporidium* Quik Chek (Techlab Inc., Blacksburg, Virginia, USA). All four brands are able to identify *G. lamblia* and *Cryptosporidium*, and Immuno*Card*STAT!®CGE and RIDA®QUICK Combi additionally detect *E. histolytica*. Specifications of the different RDTs with regard to specific identification of protozoa and sample storage are summarized in Table 1.

2.3. Retrospective study

The following 30 samples were selected for testing by all RDT brands: 6 *G. lamblia*, 8 *C. hominis*, 2 *C. parvum*, 3 *E. histolytica*, 3 *E. dispar*, 1 *Entamoeba hartmanni* (included because initially identified as *E. histolytica/dispar*), 1 *G. lamblia/C. hominis*, 1 *C. hominis/E. dispar*, 2 *E. histolytica/G. lamblia* and 3 *E. dispar/G. lamblia*.

The samples were kept frozen at −20 °C for a period ranging from 1 month to 5 years. Parasite detection was confirmed by PCR in 27/30 samples. *E. histolytica/dispar* PCR was negative in one sample containing *E. hartmanni* cysts. For 2 *Giardia* samples, of which one was positive by both microscopy and ELISA and one by microscopy (not tested by ELISA), insufficient material was available for confirmation by PCR. Each sample was tested with all RDT brands on the same day, except for Immuno*Card*STAT!®CGE, which was tested two months later due to the unavailability of this new test at study start. Two technicians independently interpreted the result of the test lines as negative, weaker than, equal to or stronger than the control line. In the case of differences in qualitative interpretation (positive versus negative) between both interpreters, the worst possible scenario (false positive or false negative) was recorded as the final result.

2.4. Prospective study

In a prospective study, fresh samples collected during routine work-up between April–August 2013 were included if enough material was available to perform all tests. Ten non-fixed negative samples and 60 non-fixed samples in which *G. lamblia*, *Cryptosporidium* and/or *E. histolytica/dispar*, were detected by either ELISA and/or microscopy, were selected for RDT testing. Parasite-specific PCR was performed on every sample positive by either microscopy or ELISA. In three cases (three *Giardia* positives) not enough sample was available. In case only *E. histolytica/dispar* was found by microscopy and/or ELISA, only Immuno*Card*STAT!®CGE and RIDA®QUICK were performed. Each sample was tested with all RDTs on the same day, except for Immuno*Card*STAT!®CGE for the reasons explained above.

Test results were interpreted independently by two lab technicians as described for the retrospective study. Specimens were considered true *G. lamblia* or *Cryptosporidium* positives if they were positive by PCR (or positive by microscopy and ELISA in case PCR analysis could not be performed). Specimens were considered true *E. histolytica* positives if a positive microscopic and/or ELISA result was confirmed by the *E. histolytica* specific PCR. Specimens were considered as true negative when both microscopy and ELISA were negative for *G. lamblia*, *Cryptosporidium* or *E. histolytica*.

2.5. Statistical analysis

Analyse-it Software (Leeds, England) was used to calculate 95% confidence intervals (95% CI) for sensitivity and specificity. Kruskal–Wallis test with Bonferroni correction allowed multiple pairwise comparison of the ELISA ODs classified according to test interpretation as true positive, true negative and false negative.

3. Results

3.1. Retrospective study

Sensitivities and specificities of all four RDTs in the retrospective study setting are summarized in Table 2. The specificity was 100.0% for *G. lamblia* and *Cryptosporidium*, and ranged from 80.0% to 88.0% for