Detection of Intestinal Parasites With Automated Sedimax2®. A New Step in the Parasitological Diagnosis in High Throughput Laboratories.

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Abstract

Despite the low prevalence of parasitic infections in Europe, the diagnosis of intestinal parasites is difficult and laborious for microbiology laboratories. Currently, the antigens detection kits and the molecular biology have allowed an easier diagnosis. But these techniques have also limitations due to the fact that they do not detect all possible parasites presents in the samples. The objective of the study was to evaluate the accuracy and the usefulness of SediMAX2® (77 Elektronika, Budapest, Hungary) automated microscopy in the detection of parasitic structures from feces. A total of 197 formol-fixed stool samples were processed in parallel with wet mount examination and by SediMAX2®. Sensitivities, specificities and predictive values were analyzed, reaching sensitivity of 89.51% and specificity of 98.15%. Predictive values were also calculated with a very good positive predictive value (99.22%). SediMAX2® is a good tool for a reliable diagnosis of intestinal parasitic infections. The easiness of use, processing and the flexibility in the images analyse allows its incorporation in the day to day laboratory work as an extra step for the parasitologists workload.

Introduction

Parasitic diagnose still relies on microscopic examination although the limitations of these methods. Improving the parasitic diagnose is mandatory and combined with clinical symptoms, clinical and travel history, and geographic location of patient may help, especially in non endemic countries but there is still a lack. Despite the low prevalence of parasitic infections in Europe, the diagnosis of intestinal parasites is difficult and laborious for microbiology laboratories. In recent years, there has been a tremendous effort to focus the research on the development of newer diagnostic methods focusing on serological, molecular, and proteomic approaches (Ricciardi and Ndao, 2015) but these techniques have also limitations due to the fact that they do not detect all possible parasites, they are expensive and no available for all microbiology laboratories. Molecular tools have emerged to be the solution in a lot of infectious diseases (Yang and Rothman, 2004) and several targets have been developed but, more of them are homemade and not standardized.

Especially remarkable is the development of the loop-mediated isothermal amplification (LAMP) (Notomi et al., 2015) that has attracted much attention in the field of parasitology. Several in house methods have been evaluated for different parasitic infectious (Fernández-Soto P et al., 2014; Mugambi et al., 2015), but is in the malaria diagnose in which this technology has represented the trigger for the development of new and available diagnostic tools showing best results than other point-of-care tests (De Koninck et al., 2017). In this case, there is the only commercial LAMP tool available in the market. The future of the malaria diagnosis has also changed with the incorporation of a new microscopic way to perform its detection. Parasight is an enhanced computer vision device for the diagnosis of malaria (Eshel et al., 2017) and it is able to novel malaria test system that is able to provide highly sensitive malaria evaluations faster and more accurate than current malaria tests. This is a big step in the evolution of the classical microscopic diagnose and leads to apply the technology to other pasitological areas.

In 2015, a group of Italian researchers published an interesting article which evaluate the accuracy of an autoanalyzer that it is used for the diagnosis of urinary tract infection, the sediMAX 1 (77 Elektronika, Budapest, Hungary) (Intra et al., 2016), and recently they have also improved the detection of protozoos with the new version of the SediMAX2® (77 Elektronika, Budapest, Hungary) (Intra et al., 2017).

Digitalized microscopy is already used in pathology departments with the advent of Whole-Slide Imaging. SediMAX2® could be the first step in the parasitological virtual microscopy.

We aimed to evaluate the accuracy and the usefulness of the SediMAX2® for the diagnosis of intestinal parasitic infections from formalin-fixed stools and the usefulness in a high throughput laboratory.

Material And Methods

2.1. Study design

This was a prospective cross-sectional observational study designed for the clinical evaluation of SediMAX2® compared with microscopy for the routine diagnosis of gastrointestinal parasitic infections at the Microbiology Department of the Germans Trias i Pujol University Hospital (HUGTiP, Badalona, Spain). The study was approved by the Ethics Committee at our institution. The need for informed consent was waived.

2.2. Study population and clinical samples

During the period January 2016– to December 2017 a total of 197 fecal samples from patients with suspected intestinal parasitic infection fixed with sodium acetate-acetic acid-formalin (SAF) (Universal System 50 ml con SAF, Durviz, Valencia, Spain) and stored in the Microbiology Department of the HUGTiP were studied. Stools received from Monday to Friday during the morning schedule and processed at our hospital's clinical microbiology laboratory were included in this study. These samples were from patients with suspected parasitic infections in two different populations: (i) patients at the emergency department or hospitalized at HUGTiP and (ii) patients at primary care centers.

2.3. Stool samples processing

The samples were first processed by microscopic ova and parasite examination. Firstly fixed stools were diluted with 3 milliliters of ethyl acetate and the filtered and centrifuged 500 g during 5 minutes.

Then, the samples were analyzed using the automatic microscopy sediment analyzer, SediMAX2®. This autoanalyzer homogenizes and transfers 20 microliters the samples into special disposable cuvettes, which are centrifuged for a few seconds. Afterwards, whole-field high definition images are obtained. All images are stored on the computer, to be reviewed for a second independent reader. For the process in SediMAX2®, once the fixed feces were concentrated, sediment was subsequently diluted with saline solution (1:20) for later analysis in SediMAX2®2 described by Intra *et al* (8). Subsequently, high definition

images of the different photographed fields will be obtained and evaluated. For each sample, a triple analysis will be carried out with SediMAX2®2, for which a total of 60 images will be reviewed.

2.4. Statistical analysis

The correlation of the results was evaluated using the Kappa coefficient (κ ; CI 95%). Sensitivity (Se), specificity (Sp), positive and negative predictive values (PPV and NPV) for SediMAX2® were also calculated using openepi software, www.openepi.com (Emory University. Atlanta. USA).

2.5 Ethic statement.

Ethical approval from the Ethics Committee Research of Germans Trias i Pujol University Hospital Ethics Committee was obtained (PI-17-232) and the need for informed consent was waived.

Results

Out of the 197 samples processed from 178 patients, 146 were positive; 124 were in single infection and 22 co-harbored 2 or 3 parasites and 54 were negative by wet mount examination. Regarding the positives samples in single infection: 78 were *G. lamblia*, 10 *B. hominis*, 8 *Entamoeba coli*, 2 *Entamoeba histolytica/dispar*, 3 *Endolimax nana*, 4 *Enterobius vermicularis*, 3 *Hymenolepis nana*, 2 *Iodamoeba bustchlii*, 1 *Strongyloides stercoralis*, 2 *Trichuris trichura*, 1 *Taenia* spp and 3 Hookworm. Of the mixed infection samples, 2 were positive for *H. nana* y *G. lamblia*; 2 for *E.nana* and *B.hominis*; 2 for *E.coli* and *E.nana* 1 for *G. lamblia*, *E.nana* and *B.hominis*; 1 for *G. lamblia*, *E.nana*, *E. coli* and *B.hominis*; 3 for *E.nana*, *E. coli* and *B.hominis*; 4 for *E. coli* and *B.hominis*; 1 for *E.hystolitica*, *Entamoeba hartmanii* and *E.nana* and 1 for *G. lamblia*, *E.hystolitica* and *B.hominis*; 1 for *G. lamblia* and *E.hystolitica* and 1 for *A. lumbricoides* and *E. nana*.

Values of SE, SP, PPV, NVP and *Kappa* index were (IC 95%): 89.51%; 98.15%, 99.22%, 77.94% and 0.81 respectively comparing with wet mount examination.

Out of 197 samples studied there were 16 discrepancies shown in Table 1.

Table 1
Discordant results between SediMAX2® and wet mount examination.

Patient number	Microscopic examination	SediMAX2®
1	<i>E.coli</i>	Negative
2	<i>G.lamblia</i>	Negative
3	<i>G.lamblia</i>	Negative + <i>B.hominis</i>
4	<i>G.lamblia</i>	Negative
5	<i>E.vermicularis</i>	Negative
6	<i>I.bustchlii</i>	Negative
7	<i>G.lamblia</i>	Negative
8	<i>E.vermicularis</i>	Negative
9	<i>S.stercolaris</i>	Negative
10	<i>A.lumbricoides</i>	Negative
11	<i>A.lumbricoides</i>	Negative
12	<i>E.vermicularis</i>	Negative
13	Negative	<i>B.hominis</i>
14	<i>G.lamblia</i>	Negative
15	<i>G.lamblia</i>	Negative
16	Negative	<i>G. lamblia</i>

In these discrepancies, there 2 samples in whom detected parasites have debatable clinical significance such as *E.coli* or *I.bustchlii* and 1 sample with a positive result for *B.hominis* in SediMAX2®. Considering these results as negative the data obtained for SE, SP and predictive values improve the accuracy of the SediMAX2® with SE, SP, PPV, NVP and *Kappa* index (95% IC) of 90.78%; 100%; 100%; 81.16% and 0.8484 respectively. Three samples with *E. vermicularis* were included although the diagnosis must be performed with the "Scotch test", cellulose-tape slide test on the perianal skin.

In all samples 60 images were processed and reviewed but in 101 of the 143 positive samples (Fig. 1), the detection of the parasite was performed only with 20 images, reducing the time consuming problems of the parasitic diagnosis. In 18 cases 40 images were needed and in 23; 60 images were reviewed for a correct diagnosis. Additionally, a squared 15x15 micrometers was installed to allow the measure of the structures for a correct parasitological evaluation. Despite these results the evaluation of all the images performed is mandatory for a good diagnosis.

Discussion

Intestinal parasitic infections are a reality, and it is of great importance to find the correct way to diagnose them to avoid further disease transmission and chronic illnesses. Although for many years, parasitic infections are still neglected in terms of laboratory development. Still nowadays, microscopic examination of stool samples for the detection of cysts, trophozoites and ova remains the diagnostic method of choice for many laboratories; however, the method requires technical expertise, and it is laborious; it can also have low sensitivity with low levels of infection and time consuming (McHardy et al., 2014). The limitations of microscopy and antigen detection tests have influenced parasitologists towards the use of genomic amplification methods made possible with the advent of the molecular diagnose but it is still remain underused (Won et al., 2016). Step by step antigen detection tests for *G. lamblia*, *Cryptosporidium* spp and *Entamoeba histolytica* have been widely introduced in the day-to-day laboratory workflow especially in those laboratories with lower capacities for parasitic diagnosis and they are associated with a significant improvement (McHardy et al., 2014) and even, there are several tests cleared by the FDA.

Although growth in international travel and migration from endemic areas, in our settings *G. lamblia* and *E. vermicularis* are still the most prevalent pathogens. In high throughput laboratories, tools for detection of these parasites are mandatory. The results obtained for the *G. lamblia* diagnosis suggest that SediMAX2® could be a good tool and it can be implemented for the detection of this protozoon (SE, SP, PPV and NPV for *G. lamblia*; 89,29%; 98,15%; 98,68%; 85,48%, respectively). Out of the 84 samples positives for *G. lamblia*, in 9 cases SediMAX2® was not able to detect them. In the other hand, it is important to remember that a negative result does not rule out parasitic infection because several parasites (particularly *G. lamblia*) have an intermittent shedding (CLSI, 2005) and the probability of parasite detection increases more than 95% when 3 stools are tested (Marti and Koella, 1993), so a serial parastilological studies are still needed to confirm a high suspicion of *G. lamblia* infection in case of a previous negative result. For the rest of protozoa SediMAX2® was able to detect the protozoa with a pathogenic role, but only 4 *E. histolytica/dispar* were included in the study.

In case of worm infections, all eggs were properly identified with the exception of *E. vermicularis* in all three cases, 2 *A. lumbricoides* and 1 case of *S. stercoralis* larvae. In the rest 15 worm infections from 13 patients, all eggs were detected. The discrepancies in the worm infections must be explained by the fact that in case of *E. vermicularis*, stool wet mount examination is not the recommended tool for the diagnosis and, even the presence in the microscopic mount, a tape slide-test must be sent to the laboratory to a correct diagnosis. For *S. stercoralis*, it is known that the visualization of rhabditiform larvae in stools is not always possible and complemented techniques (parasitological or serology-based test) is needed. Diagnosis of *S. stercoralis*, is often delayed due the presence of subclinical or poorly-symptomatic cases and the usually low parasite load and irregular larvae output. This fact makes that this worm is also known as there is but not seen (Montes et al., (2010).

The most remarkable fact in this automatic microscopic system is the reducing in hand and microscope time. With the possibility of a big storage of images, this system could reduce the time in microscope with a high positive predictive value.

Parasitic diagnosis is heavy workload and relies exclusively on the experience of the trained technicians. Thus, it is difficult to maintain enough people with expertise in diagnostic medical parasitology. A recognized image system based in the same principle that urines must be developed by the biomedical engineering to provide new tools in case of intestinal parasitic infections. Additionally, the improvements in the sample process to avoid detritus that can difficult the interpretation of the images would help to better parasitological diagnose.

This study is a first step to implement engineering with medical practice to help medical microbiologist diagnose their patients. There is still an opportunity for improvement, especially with high throughput laboratories and when diagnosis is almost exclusively manual. A two-step algorithm including antigen detection and digital microscopic could be useful to help parasitologists in their day-to-day workload.

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Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee at our institution (PI-17-232). The need for informed consent was waived.

Consent for publication

The authors consent this paper for its publication

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Study design: GFR, BR, LM

Sample Recruitment: GFR, BR, NR

Sample Analysis: GFR, NR, JHW, MA.

Statistical analysis: GFR, BR

Wrote the manuscript: GFR, LM

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Figures

Figure 1

Images from SediMax2® software. 1A. *Giardia lamblia* cysts (circled). 1B. Hookworm egg. 1C. *Blastocystis hominis* vacuolar stage. 1D. *Entamoeba histolytica/dispar* cyst. 1E. *Entamoeba coli* cysts. 1F. Double infection of *Endolimax nana* cysts (arrows) and *Ascaris lumbricoides* egg.