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ORIGINAL ARTICLE

Molecular detection of common intestinal parasites: a performance evaluation of the BD Max™ Enteric Parasite Panel

R. Batra[1] · E. Judd[2] · J. Eling[3] · W. Newsholme[1] · S. D. Goldenberg[1]

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Abstract The purpose of this study was to evaluate the level of agreement of the BD Max™ Enteric Parasite Panel (EPP) with microscopy for the detection of *Giardia duodenalis*, *Cryptosporidium* spp. and *Entamoeba histolytica* in stool samples. A total of 372 stool samples (partly collected on the basis of positive microscopy and partly unselected, consecutive sample submitted for parasite investigation) were tested with EPP according to manufacturer's instructions and also using microscopy according to standard techniques. Discrepant samples were further tested using PCR by the National Parasitology reference laboratory. Levels of agreement and laboratory turnaround times were measured and compared. Overall, positive and negative percent agreement was high between the two methods. However, microscopy resulted in four false positives and one false negative for *G. duodenalis* and two false positives for *Cryptosporidium*. Additionally, microscopy could not differentiate between *E. histolytica* and *Entamoeba dispar*. Median laboratory turnaround time was 65 hours for microscopy; results from EPP could be available after four hours. *Blastocycstis hominis* was detected by microscopy in one sample and would have been missed if only EPP was performed. The EPP was a good alternative to microscopy, detecting a small number of additional positives that were missed by microscopy. The assay is significantly faster than microscopy and allows laboratory workflows to be streamlined. The risk of missing parasites that are not included in the EPP appears to be minimal in the studied population; however, there may be certain patient groups who would benefit from microscopic examination of stools.

✉ S. D. Goldenberg
Simon.goldenberg@gstt.nhs.uk

[1] Centre for Clinical Infection and Diagnostics Research, King's College, London and Guy's & St Thomas' NHS Foundation Trust, 5th Floor North Wing, St Thomas' Hospital, Westminster Bridge Road, London SE 17EH, UK

[2] ViaPath LLP, London, UK

[3] Healthcare Inclusion Team, Guy's & St Thomas' NHS Foundation Trust, London, UK

Introduction

In industrialised countries the most commonly encountered intestinal parasites are *Giardia duodenalis*, *Cryptosporidium* spp. and *Entamoeba histolytica*. Morphological determination of these and other intestinal parasites using light microscopy has long been the method routinely employed by the majority of diagnostic laboratories [1]. However, this method is operator dependent and can be subjective. It requires a skilled workforce and is labour intensive, leading to delays in reporting [2]. Even with an experienced operator, microscopy may result in a significant number of false negatives. Additionally, it cannot differentiate between *E. histolytica* and *Entamoeba dispar*, which is non-pathogenic.

Recently, several commercial Nucleic Acid Amplification Tests (NAATs) have become available for the detection of a range of gastrointestinal pathogens, including parasites [2–7]. In particular, several of these panels demonstrate increased sensitivity over microscopy for detection of the more common parasites [2, 4, 7, 8].

The BD Max™ Enteric Parasite Panel (EPP) (Becton, Dickinson and Company, Oxford, UK) is a fully automated real-time PCR system designed to detect *C. parvum* and/or *hominis*, *G. duodenalis* and *E. histolytica* from stool samples. Use of this system has previously been described for detection of bacterial pathogens (*Campylobacter* spp., Salmonella,

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Shigella and *E. coli*) [5, 9, 10], but description of its use to detect parasites is more limited [8].

We performed a parallel diagnostic accuracy study comparing the performance of the EPP to microscopy in both collected stool samples known to be positive and in unselected stool samples with clinician requests for parasite investigation.

Material and methods

Setting

The study was conducted at Guy's & St Thomas' NHS Foundation Trust, a 1200 bed academic hospital in Central London serving a local population of approximately 800,000.

Samples and patient population

Samples were initially selected on the basis of a positive microscopy result for one or more of the targets of interest. A total of 29, 15 and 22 samples positive by microscopy for *C. parvum* and/or *hominis, G. duodenalis* and *E. histolytica/dispar*, respectively, were selected and stored at –20 °C for up to 9 months. A further set of 305 unselected, consecutive samples submitted to the laboratory for parasite investigation were also tested. Following microscopical examination (described below) these samples were stored at 4 °C for up to one week before testing with EPP.

All samples were unpreserved and were derived from both hospital inpatients, those attending for outpatient appointments, and from patients in community settings including those attending general practitioner appointments and refugee's clinic appointments.

Samples consisted only of anonymised, residual material for the purposes of a performance evaluation only, and thus considered exempt from requiring research ethics approval.

Microscopy

An aliquot of stool of approximately 1 g was added to a Midi Parasep® Faecal Parasite Concentrator (Apacor, Wokingham, UK) and processed according to manufacturer's instructions. The resulting deposit was re-suspended and stained with double strength Lugol's iodine. An unstained wet mount was also prepared and both preparations were examined under low power.

For *Cryptosporidium* oocysts, a medium smear of faeces was prepared on a microscope slide and allowed to air dry, then fixed in 70 % methanol for 2–3 minutes and allowed to air dry further. Auramine-phenol stain (1:10 v/v) was applied according to standard methods [11] and examined using fluorescence microscopy.

BD Max™ Enteric Parasite Panel

Samples were processed in accordance with manufacturer's instructions. Briefly, a 10-uL loopful of sample was introduced to the BD Max™ sample buffer tube and vortexed for 30 seconds. Samples were then heated on the BD Max™ Pre-Warm station for 50 minutes to facilitate parasite lysis. Samples were then loaded onto the automated BD Max™ platform in batches of up to 24 samples.

Resolution of discrepant samples

Discrepant samples were referred to the Public Health England National Parasitology Reference Laboratory in London for confirmation using a multiplex PCR. This consisted of a real-time PCR assay based on previously published methods [12]. True results were taken as concordance in at least two of the three methods.

Data analysis

Turnaround time

Laboratory turnaround time was measured from the point of specimen arrival in the laboratory to the point of availability of final report. This was only measured for microscopy, since the EPP was not performed at the same time as microscopy, nor were results reported back to clinicians.

Results

Giardia duodenalis

A total of 275 samples underwent microscopic examination for *Giardia* and produced evaluable results with EPP. Two samples remained unresolved by EPP after retesting; these were not sent to the reference laboratory and were excluded from the analysis.

Thirty-one samples were positive by microscopy, 30 were also positive by EPP, and the remaining sample was negative. This sample was also negative by the reference laboratory multiplex PCR and was classified as a false positive by microscopy.

A total of 244 samples were negative by microscopy for *Giardia*, 240 of which were also negative by EPP. The remaining four samples were positive by EPP and confirmed by the reference laboratory PCR, therefore were classified as false negatives for microscopy. Overall agreement of EPP with microscopy for was 98.2 % (95 % confidence interval [CI] 95.8–99.4 %), positive percent agreement (PPA) was 96.8 % (95%CI 83.3–99.9 %) and negative percent agreement

(NPA) was 98.4 % (95%CI 95.9–99.6 %). Results for *Giardia* are shown in Table 1.

Cryptosporidium parvum / hominis

A total of 372 samples underwent microscopic examination for *Cryptosporidia* and produced evaluable results with EPP. Three samples remained unresolved by EPP after retesting; these were not sent to the reference laboratory and were excluded from the analysis. Twenty-four samples were positive by microscopy, 22 were also positive by EPP, and the remaining two samples were negative. These samples were also negative by the reference laboratory multiplex PCR and were classified as false positives for microscopy. A total of 348 samples were negative by microscopy and EPP. Overall agreement of EPP with microscopy was 99.5 % (95%CI 98.1–99.9 %), PPA was 91.7 % (95%CI 73.0–99.0 %) and NPA was 100 % (95%CI 98.9–100 %). Results for *Cryptosporidium* are shown in Table 2.

Entamoeba histolytica

A total of 275 samples underwent microscopic examination for Entamoeba and produced evaluable results with EPP. Two samples remained unresolved by EPP after re-testing; these were not sent to the reference laboratory and were excluded from the analysis.

Seventeen samples were positive by microscopy for *E. histolytica/dispar*, two of which were also positive by EPP and confirmed as *E. histolytica* by the reference laboratory test. The remaining 15 samples were negative by EPP and the reference laboratory test and are assumed to be *E. dispar*; however, this could not be confirmed since no further work such as genus specific PCR or sequencing was undertaken on these samples.

A total of 258 samples were negative by microscopy for Entamoeba, 257 of which were also negative by EPP. The remaining sample was positive by EPP and confirmed by the reference laboratory PCR, therefore was classified as a false negative for microscopy.

Table 1 Sample results for detection of *Giardia duodenalis*

Test method		Microscopy		
		Positive	Negative	Total
EPP	Positive	30	4[a]	34
	Negative	1[b]	240	241
	Total	31	244	275

EPP BD Max™ Enteric Parasite Panel

[a] All four samples were positive by the reference lab multiplex PCR

[b] This sample was negative by the reference lab multiplex PCR

Table 2 Sample results for detection of *Cryptosporidium*

Test method		Microscopy		
		Positive	Negative	Total
EPP	Positive	22	0	22
	Negative	2[a]	348	350
	Total	24	348	372

EPP BD Max™ Enteric Parasite Panel

[a] Both samples were negative by the reference lab multiplex PCR

Percent agreement was not calculated for this parasite, due to the inability of microscopy to differentiate between *E. histolytica* and *E. dispar*.

Unresolved samples

A total of 19 samples (5.1 %) gave unresolved or indeterminate results on initial testing and underwent repeat testing using a new sample buffer tube. Sixteen samples gave valid results and three remained unresolved and were excluded from further analysis.

Turnaround times

The median turnaround time for microscopy was 65 hours, which is in keeping with our previous published work [2]. The EPP requires approximately four hours from the start of sample preparation to results.

Additional parasites detected by microscopy

Of the 211 unselected, consecutive samples that were examined by microscopy, only two samples had other parasitic organisms detected. One sample had cysts of the non-pathogenic parasite *Iodamoeba bütschlii* and another sample had cysts of *Blastocystis hominis*, the relevance of which is uncertain [13].

Discussion

Overall there was a high degree of agreement between the EPP and microscopy results. In the case of discrepant results, the reference laboratory result resolved in favour of the EPP in all cases. This is perhaps unsurprising for the samples that were negative by microscopy since the enhanced sensitivity of molecular assays is well described and expected [2, 4–6, 14, 15]. However, there were three samples that were likely microscopy false positives (one for Giardia and two for *Cryptosporidium*). This highlights the need for laboratory staff to be adequately skilled and undergo continuous training.

A molecular test such as this can help to standardise laboratory procedures and minimise inter-operator variability. Alternatively it is possible that the nucleic acids within the sample had deteriorated during storage. In support of this is the fact that all three discrepant samples were from retrospectively stored samples and none were found in the unselected, consecutive sample cohort.

The difference in turnaround times of the two methods is stark, with microscopy taking a median of 65 hours. This is likely to be a result of a number of reasons. First, samples are batched and processed only once per day, often being one of the last tasks that laboratory staff undertake, as there is a perception that they are not urgent. Second, there is a tendency for laboratory staff to seek a 'second opinion' from other staff members, where there is a degree of uncertainty. Although actual turnaround times for the EPP were not measured, the assay is likely to take around four hours and results could easily be returned within a day, particularly if more than one assay run per day is undertaken. Perhaps equally important, use of this highly automated assay frees up laboratory staff time to perform other tasks.

The initial unresolved rate for EPP was higher than expected at 5.1 %, although the majority produced a valid result on retesting and could be related to over-inoculation of the sample buffer tube. Nevertheless, this wastage cost should be factored in when undertaking any cost effectiveness analysis.

The main advantage of microscopy is that it allows for the detection of a broad range of pathogens including those that are unexpected and which are not targeted by the molecular panels. Although in reality the number of parasite infections that are potentially missed if only a PCR test is performed is probably quite limited, especially if algorithms based on clinical information are implemented to identify risk factors for samples that might require alternative methods [16]. An example of this might be in the investigation of eosinophilia to detect *Strongyloides* or hookworm infection. In our study only one (0.47 %) additional potential pathogenic parasite was detected by microscopy. Furthermore, since microscopy is unable to discriminate between cysts of *E. histolytica* and *E. dispar*, its utility is somewhat limited in this situation.

Ultimately, decisions on the methods of diagnosis are dependent on a combination of test performance, cost, workflow, throughout and patient population to be tested [7]. We are all familiar with the urgent need to practice good antimicrobial stewardship, but less attention is placed on good laboratory stewardship. Clinicians must be encouraged to select the tests that are likely to give the highest yield [17].

In conclusion, multiplex molecular panels for diagnosis of gastrointestinal parasites will be increasingly adopted in clinical practice and are a good alternative to microscopy, particularly in low-risk patients (e.g. non-travellers living in industrialised countics), where the risk of missing parasites not included on the panel is limited [1].

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Compliance with ethical standards

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Conflict of interest Simon Goldenberg reports speakers fees from BD; there were no other conflicts of interest from other authors.

Ethical approval and informed consent All samples were residual and fully anonymised; research ethics approval and informed consent was not required.

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