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Evaluation of the BD Max Enteric Parasite Panel for Clinical Diagnostics

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We compared the performance of the BD Max enteric parasite panel to routine microscopy and an in-house PCR for the detection of *Giardia intestinalis*, *Entamoeba histolytica*, and *Cryptosporidium* spp. The enteric parasite panel showed good specificity for all targets and good sensitivity for *E. histolytica* and *Cryptosporidium* spp. Sensitivity for *G. intestinalis* with the BD Max enteric parasite panel was equivalent to that with microscopy.

The World Health Organization (WHO) ranks diarrheal disease the second most common cause of morbidity and mortality in children and in the developing world (1, 2). The major etiological agents of parasitic diarrhea are considered to be *Giardia intestinalis*, *Cryptosporidium* spp., and *Entamoeba histolytica* (3–5).

The detection of intestinal parasites can be improved, compared to microscopy, by the use of PCR-based methods (6–8). Recently, the BD Max enteric parasite panel (BD Diagnostics, Sparks, MD) was launched on the BD Max system (BD). The panel uses integrated DNA extraction and PCR to detect *G. intestinalis*, *E. histolytica*, and *Cryptosporidium* spp. (*C. hominis* and *C. parvum*). This study evaluated the enteric parasite panel on clinical samples and compared the performance to that of microscopy and an in-house PCR method.

A total of 132 clinical samples were used for the evaluation. Overall, 39% of the samples had been stored frozen before the analyses on the BD Max system. Sixty-six samples (27 positive and 39 negative) were previously analyzed with a modified multiplexed in-house PCR for the presence of *G. intestinalis*, *E. histolytica* (9), and *Cryptosporidium* spp. (10) at the Department of Clinical Microbiology, Halland County Hospital, Halmstad, Sweden. Briefly, the in-house method included a prepreparation step by adding feces to lysis buffer, kept at −20°C overnight, followed by DNA extraction with a QIAsymphony DSP virus/pathogen kit (Qiagen GmbH, Germany) and a multiplex PCR run on Rotor-Gene Q (Qiagen).

The remaining samples ($n = 66$) had previously been examined with microscopy for ova and cysts on concentrated fecal samples (32 positive and 34 negative) at the Department of Clinical Bacteriology, Sahlgrenska University Hospital, Gothenburg, Sweden. The samples analyzed with microscopy were all preserved in SAF transport medium (12.6 g/liter sodium acetate, 2% acetic acid, 4% formaldehyde, and 0.1% Triton X-100).

A loop of 10 μl of the fecal sample was added to the sample buffer tube (BD). The tube was vortexed and pretreated for 50 minutes on the BD prewarm heater prior to loading into the BD Max instrument along with the BD Max enteric parasite panel reagent strip. DNA extraction and real-time PCR were automatically performed by the instrument. Time from the start of sample preparation to the result, including automated data analysis, was ~3.5 h. The results were reported as negative or positive by the instrument. In the case of discordant results, the original sample was analyzed at a third laboratory (Ryhov County Hospital, Jönköping, Sweden) using an in-house PCR modified from the study by Verweij et al. (9), including specific primers for *Entamoeba dispar* (11).

The performance of the BD Max enteric parasite panel is presented in Table 1. The results were in agreement with the in-house PCR protocol with the exception of *G. intestinalis*, where 4 out of 12 samples were not detected (66.7% sensitivity; 95% confidence interval [CI], 40.0% to 93.4%). The presence of *G. intestinalis* DNA in these samples was confirmed in 3 out of 4 BD Max negative samples (no material left in 1 sample) using in-house PCR. All samples positive for *G. intestinalis or Cryptosporidium* spp. by microscopy were positive in the BD Max enteric parasite panel. In addition, the enteric parasite panel was positive for *G. intestinalis* in 1 specimen that was negative by microscopy (47/48, 97.9% specificity; 95% CI, 93.8% to 100%). All samples ($n = 12$) were reported to be negative for *E. histolytica*/*dispar* by the BD Max enteric parasite panel. All were verified as positive for *E. dispar* using in-house PCR.

In conclusion, this evaluation of the BD Max enteric parasite panel showed that the assay had good specificity for all targets and good sensitivity for the detection of *E. histolytica* and *Cryptosporidium* spp. Thirty-three percent of the samples positive for *G. intestinalis* by the in-house PCR were missed by the BD Max enteric parasite panel. However, it is important to note that two of the four *G. intestinalis* samples that were missed by the BD Max enteric parasite panel had been stored frozen. This may have affected the result, according to the kit insert. However, these were again verified by in-house PCR, indicating a possible lower sensitivity in the enteric parasite panel. Compared to the standard

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TABLE 1 Comparison between BD Max enteric parasite panel and multiplex in-house PCR on untreated feces or microscopy on SAF-treated feces

Test result	No. of positive results for:			
	In-house PCR	BD Max	Microscopy	BD Max
Giardia intestinalis	12	8	18	19[a]
Entamoeba spp/*E. histolytica*	5/5	5/5	12/0[b]	0/0[b]
Cryptosporidium spp	10	10	2	2
Negative	39	43	34	45

[a] The additional *G. intestinalis* positive sample was one of the 12 samples positive for *E. dispar* (verified with in-house PCR).
[b] Reported as *E. histolytica/dispar* with microscopy but verified as positive for *E. dispar* and negative for *E. histolytica* using in-house PCR in this study.

method for detection of intestinal protozoa, i.e., microscopy, the BD Max enteric parasite panel performed well. Although microscopy allows a very broad diagnostic approach, the method is hampered by its relatively low sensitivity (12) and its inability to exclude the presence of *E. histolytica* in samples where only cysts are present (13). In addition, some protozoa (e.g., *Cryptosporidium* spp.) are very difficult to detect unless a specific stain is used (14). Furthermore, the method is dependent on highly skilled technicians and is time consuming. Molecular techniques provide improved workflow and increased sensitivity, although the clinical relevance should always be evaluated (15, 16). With the BD Max enteric parasite panel, the workflow is further improved by the integrated DNA extraction and PCR. Considering the local epidemiology of the three pathogens in the enteric parasite panel and the results of this evaluation, we will primarily use the test in cases of travelers' diarrhea in our setting. However, as *Cryptosporidium* infections tend to be underdiagnosed in domestic cases of gastroenteritis in Sweden, the test can also be considered for use as a primary diagnostic tool, at least when waterborne outbreaks are suspected (17). Whether the test is useful in settings where enteric parasites are endemic remains to be elucidated, as the high sensitivity of molecular tests may decrease the predictive value of a positive result in these settings. Although comparable to microscopy, the detection of *G. intestinalis* with the BD Max enteric parasite panel was not optimal compared to that with in-house PCR and may thus be an area for improvement.

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