Comparison of in-house PCR and Commercial qPCR for Detecting Gastrointestinal Parasites

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Introduction

Gastrointestinal parasite (GIP) infection remains an important global health problem, disproportionally affecting the world's poorest communities. With increased travel and migration there is a drive to improve GIP diagnostics. Microscopic diagnosis requires significant expertise[1], and there is a need for improved data on the validity of molecular diagnostics, particularly for helminths[2]. This study compared faecal microscopy combined with an in-house multiplex parasite qPCR[3] to commercially available helminth/protozoa qPCR panels.

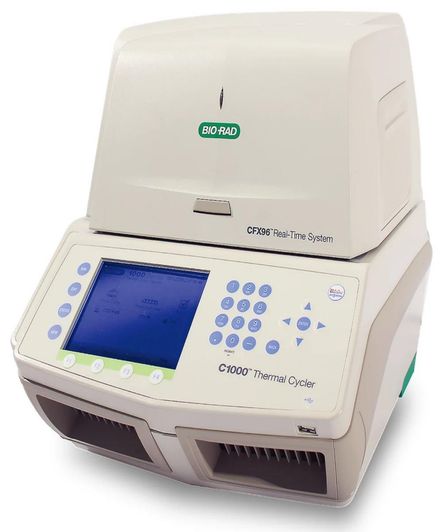

Figure 1. Biorad CFX96 PCR Machine

Methods

Concentration microscopy and in-house multiplex qPCR were performed on faecal samples sent to the Liverpool School of Tropical Medicine's Clinical Diagnostic Parasitology Laboratory (LSTM CDPL) as a reference standard. A selection of faecal samples, both positive and negative for a range of GIPs were then selected to be tested using the commercial Seegene Allplex™ GI-Parasite Assay and/or the commercial Seegene Allplex™ GI-Helminth(I) Assay with the Biorad CFX96 PCR machine. Samples were extracted using the Qiagen DNA extraction kits and stored at -20℃ freezers .The in-house multiplex reference standard qPCR panel included *Trichuris, Giardia*, *Hookworm spp*, *Strongyloides*, *Schistosoma*, *Ascaris*, *Entamoeba histolytica*, *Entamoeba dispar*, and *Enterobius*.

Results

A total of 125 samples were included. Results from the Seegene™ assays were compared to a reference standard (Microscopy plus in-house qPCR, or microscopy alone in the cases where the GIP target is not included the in-house qPCR panel). For all studied GIP, the Seegene Allplex™ assays showed substantial or near perfect agreement with the reference standard, with Cohen's Kappa ranging from 0.66 for *Ascaris* to 1.00 for *Trichuris*, *Taenia*, *Hymenolepis*, and *Cyclospora*. Sensitivity ranging from 100% for *Trichuris, Taenia, Hymenolepis, and Cyclospora spp* to as low as 50% for *Ascaris*. Specificity was 100% for most of the parasites except *Giardia, Strongyloides, and Cryptosporidia. Hymenolepis, Cyclospora* and *Taenia spp* were detected via microscopy alone. The Seegene Allplex™ panels found increased numbers of organisms compared with the reference standard, including four further instances of *Cryptosporidium*, three of *Giardia*, and 24 of *Blastocystis*. There was also 8 instances of microsporidia and nine instances of *Dientamoeba fragilis* not detected by the reference microscopy and in-house qPCR methods. Amongst *Strongyloides* culture positive samples, the seegene panel showed same sensitivity and specificity with in-house qPCR.

Figure 2. Chart showing sensitivity & specificity of each G.I Parasite Spp.

Figure 3. Photograph of the Seegene Allplex™ Result Platform.

Figure 4. Table showing Seegene results against microscopy and in-house PCR reference.

Organism	Ref pos (n)	Seegene (n)	Sens	Spec	Kappa
Trichuris	6	6	100.00	100.00	1.00
Giardia	11	12	90.91	96.43	0.84
Hookworm	8	7	87.50	100.00	0.93
Strongyloides	15	13	80.00	98.08	0.82
Enterobius	7	6	85.71	100.00	0.92
Ascaris	2	1	50.00	100.00	0.66
Cryptosporidia	8	12	87.50	91.53	0.65
Enttamoeba histolytica	11	7	63.64	100.00	0.75
Cycloposora	6	6	100.00	100.00	1.00
Hymenolepis	2	2	100.00	100.00	1.00
Taenia	7	7	100.00	100.00	1.00

Conclusion

The Seegene Allplex™ panels demonstrated good comparability to the reference standard and increased the overall numbers of organisms detected. There was good sensitivity for most helminths (except *Ascaris*, with a small sample size (n=2). The Seegene Allplex™ overall has a wider panel of organisms and had more identifications compared with the reference in-house methods, including identifying *Dientamoeba fragilis*, *Blastocystis hominis* and microsporidia which are not detected by the in-house qPCR panel. Despite the limitation of a small number of samples for comparison in some cases, the Seegene Allplex qPCR method gave very satisfactory results in this first phase of the comparative analysis. However, a limitation of the Seegene Allplex™ assays is the current absence of *Schistosoma* species, an important global pathogen, from the panels.

References.

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