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Multiplex Real-Time PCR for the Detection of Gastrointestinal Parasites: A Comparison of Two Commercial Assays

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Abstract

Background: Kelowna General Hospital (KGH) laboratory uses traditional and time consuming microscopic methods to detect ova & parasites (O&P), including both permanent slides using a modified iron haemotoxylin stain, and fecal concentrate slides. Multiplexed molecular panels provide rapid detection of multiple pathogens, reducing workload and turn around time. We compared the performance of the Seegene Allplex™ GI-Parasite Assay and the Roche LightMix® Modular Gastrointestinal Parasite Assay for detection of stool parasites.

Methods: 185 frozen, pre-characterized stool specimens were tested on Allplex™ and LightMix® panels. Specimens included 46 *Blastocystis hominis*, 2 *Cryptosporidium spp*, 2 *Cyclospora spp*, 18 *Dientamoeba fragilis*, 4 *Entamoeba histolytica*, 10 *Giardia lamblia* and 91 negatives. Copan fecal swab fluid (190uL) was combined with 10uL of each Allplex™ GI-BP exogenous internal control and LightMix® phocine herpesvirus extraction control, then extracted on the Roche MagNA Pure compact. PCR was performed in parallel on the LightCycler 480II and Bio-Rad CFX96 systems, using reaction parameters specified by the manufacturers. The LightMix® multiplex panel included *Blastocystis hominis*, *Cryptosporidium spp*, *Dientamoeba fragilis*, *Entamoeba histolytica* and *Giardia lamblia*, while Allplex™ included *Cyclospora spp* in addition to previously mentioned targets. Sensitivity and specificity of each assay were calculated using a modified gold standard, composed of at least 2 positive between O&P examination, Allplex™ and LightMix® PCR.

Results: The Allplex™ panel provided accurate results in 182 of 185 (98.4%) specimens while LightMix® was accurate in 175 of 185 (94.6%). For Allplex™ panels, sensitivity and specificity were >99% for all targets, while LightMix® had a sensitivity and specificity of 82.0% and 100.0% for *B. hominis*, 90.0% and 100% for *G. lamblia*, and 100% for all other targets (Table 1). Most *B. hominis* cases missed by LightMix® had low microscopic quantities of organism and were likely of limited clinical significance.

Conclusions: Both assays performed well in detecting clinically relevant parasitic infections, compared to conventional O&P microscopy.

Introduction

- This study evaluated the performance of the Seegene Allplex™ GI-Parasite Assay and the Roche LightMix® Modular Gastrointestinal Parasite Assay for detection of stool parasites.

Methods

- A total of 185 samples were tested on Allplex™ panels and LightMix® panels comprising 26 bacterial, viral and parasitic organisms.

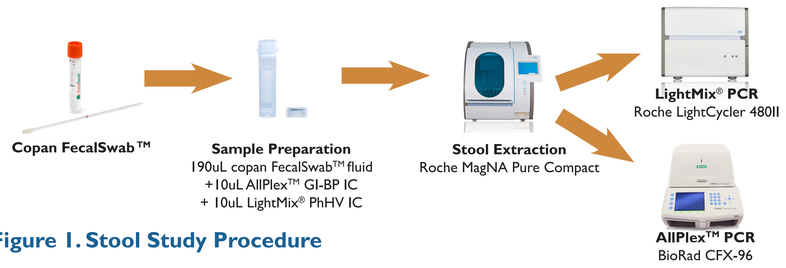

Figure 1. Stool Study Procedure

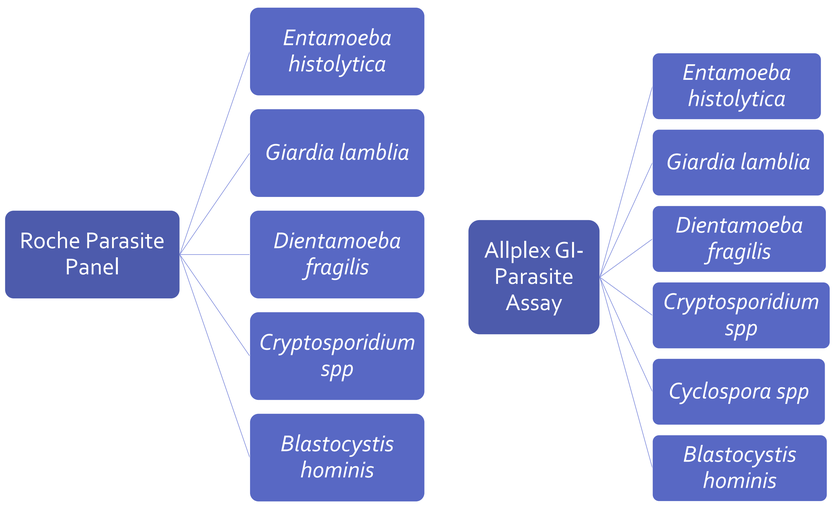

Figure 2. Composition of Multiplex PCR panels for each system.

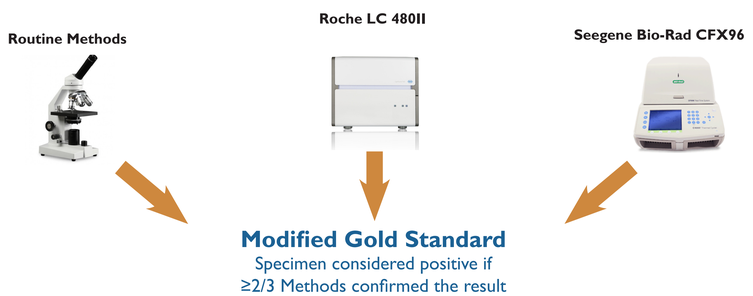

Figure 3. The Modified Gold Standard used to analyze results

Results

- The Allplex™ panel provided accurate results in 182 of 185 (98.4%) specimens while LightMix® was accurate in 175 of 185 (94.6%).
- The LightMix® assay showed decreased sensitivity for *B. hominis*. However, most false negative specimens had few organisms seen on microscopic examination, so they were likely of limited clinical relevance.
- The inhibition rate was 4.9% for the Allplex™ assay, and 5.4% for the LightMix® assay.

Table 1. Sensitivity and specificty results for all targets

Assay		Allplex™		LightMix®	
Target	**n**	**Sens**	**Spec**	**Sens**	**Spec**
Blastocystis hominis	50	100%	99.2%	82.0%	100%
Cryptosporidium spp	2	100%	100%	100%	100%
Cyclospora spp	2	100%	100%		
Dientamoeba fragilis	18	100%	100%	100%	100%
Entamoeba histolytica	4	100%	100%	100%	100%
Giardia lamblia	10	100%	100%	90.0%	100%

Conclusion

- Both assays performed well in detecting clinically relevant infections, compared to traditional methods

Acknowledgements

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