

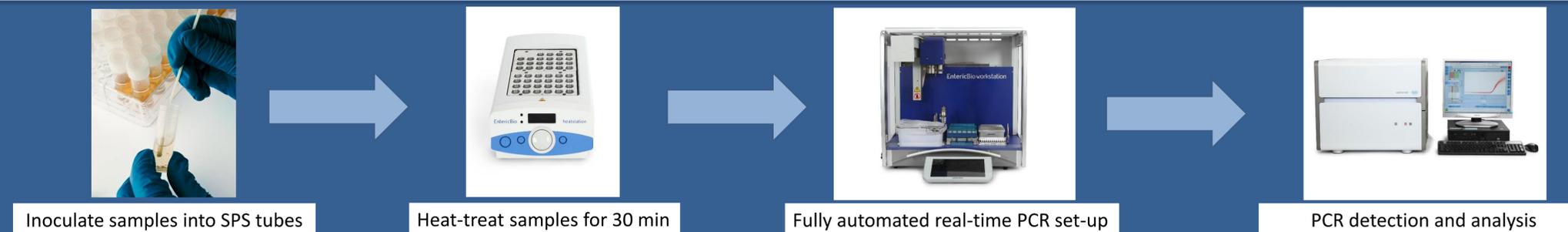
Introduction & Purpose

Cryptosporidium and Giardia are the leading causes of parasitic gastroenteritis. These organisms are usually transmitted through contaminated water, food or soil but person-to-person transmission, particularly in kindergarten, has also been described. There are many species of Cryptosporidium in nature, which can infect both humans and many types of animals. The common types responsible for infecting humans are *Cryptosporidium hominis* and *Cryptosporidium parvum*. In developing countries, Giardia and Cryptosporidium are rarely present as mono-infections and the resulting polyparasitic scenarios exacerbate the clinical impact of individual parasites, and complicate diagnosis, treatment and control. The clinical symptoms may include loss of appetite, constipation, bloody diarrhea, nausea, abdominal pain, fever, rash and, anemia. The infections can be severe and last for weeks. Often epidemics occur in daycare centers and kindergartens. Traditional laboratory methods used to identify these parasites, such as microscopy, tend to be labour-intensive, subjective and time-consuming and often lack sensitivity. Therefore molecular-based methods are exploited as an alternative in a clinical microbiology setting.

In this study, we evaluated the performance of EntericBio® Gastro Panel 2 assay (Serosep) for detection of *Cryptosporidium parvum/hominis* and *Giardia lamblia* in faecal samples.

Methods

Testing included frozen specimens, previously identified as positive for Giardia/ Cryptosporidium as well as fresh specimens submitted to the laboratory during the study. The routine investigation of *Giardia lamblia* was done by light microscopy. *Cryptosporidium* spp. is routinely identified by immunoassay – Crypto One-Step Test (Novamed). The test is routinely performed on samples with specific physician request only. These methods were compared to multiplex PCR - EntericBio® Gastro Panel 2 assay (GP2, Serosep), an automated system designed to detect bacteria and parasites using Real-Time PCR method directly from stool within 3 hours. For the GP2 test, the sample was inoculated into EntericBio® Stool Preparation Solution (SPS) using the EntericBio® FLOQSwab™. SPS tubes were heated for 30 min on the EntericBio® Heatstation. Lysed samples were then added to lyophilised PCR reagents using an EntericBio® Workstation. Amplification and detection was done using a LightCycler 480 II (Roche). Discordant samples were tested retrospectively with BD Max Enteric Parasite Panel.



Results

A total of 297 prospective samples were tested for Giardia in the study. Additionally, 19 Giardia and 20 Cryptosporidium retrospective positive samples were included. Breakdown of results is shown in the tables below.

Breakdown of retrospective *Cryptosporidium* spp. and *Giardia lamblia* positive samples

<i>Cryptosporidium</i> spp.		<i>Giardia lamblia</i>			
Retrospective n=20	EntericBio		Retrospective n=19	EntericBio	
	Pos	Neg		Pos	Neg
Routine Method	Pos	20	Routine Method	Pos	19
	Neg			Neg	

All *Cryptosporidium* and *Giardia* retrospective positive samples were detected by Multiplex PCR – EntericBio GP2. 100% sensitivity and PPV for *Cryptosporidium* spp. and *Giardia lamblia*.

Breakdown of prospective *Giardia lamblia* results

<i>Giardia lamblia</i>		EntericBio			
Prospective n=297	EntericBio		Prospective n=297	EntericBio	
	Pos	Neg		Pos	Neg
Routine Method	Pos	7	Routine Method	Pos	0
	Neg	7		Neg	283

100% sensitivity, 98% specificity and 100% NPV. Increased sensitivity comparing to routine methods - all discordant samples were confirmed as Giardia by alternative PCR.

Conclusions & Discussion

- The Serosep EntericBio® GP2 assay is a rapid, easy-to-use, reliable and labour-efficient system.
- The Gastro Panel 2 enables detection and identification of *Giardia lamblia* and *Cryptosporidium* spp. directly from stool within 3 hours.
- The multiplex PCR Gastro Panel 2 showed excellent specificity and greater sensitivity than routinely used methods for *Giardia lamblia*.
- Inhibition rate 0.68%

The EntericBio® system offers a great alternative method for routine diagnosis of gastroenteritis, especially for high throughput laboratories (up to 46 samples per run). The system enables simultaneous detection and identification of several parasites and bacteria, resulting in significant labour savings in manpower and decreased turnaround time for reporting of the results.