Evaluation of the Copan Fecal Swab and Seegene Allplex Gastrointestinal multiplex panels for Accurate Molecular Detection of Stool Pathogens with Operational Efficiency for Implementation in a High Volume Laboratory Setting

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BACKGROUND

Traditional diagnostic methodologies for the detection of pathogens in stool have relied on microscopy for ova/parasites (O/P) or culture for pathogenic bacteria. Microscopy is highly subjective and reliant on technologist expertise. Culture based identification of bacterial pathogens requires plating for growth on various selective media followed by biochemical/phenotypic testing for confirmation. Dynacare Microbiology laboratories in Ontario, Canada receive approximately 200 specimens per day for independent diagnosis of O/P and bacterial pathogens. An accurate diagnostic method with efficient workflow was sought for high volume diagnostic testing for O/P and bacterial pathogens in stool specimens.

METHODS

The Seegene Allplex Gastrointestinal (GI) multiplex PCR panels for Parasites, and Bacteria (I, II) were evaluated against positive and negative specimens both prospectively with "fresh" stool specimens (n=88) and retrospectively with archived specimens (n=115). All specimens underwent diagnostic testing as per current standard operating protocols accredited by CAP and IQMH: (i) detection of O/P was performed on stool collected in sodium acetate formalin fixative via microscopic examination of wet mount and stained smears and (ii) detection of bacterial pathogens was performed on stool collected in Enteric Transport Media. For bacterial detection, stool was plated onto individual selective media for the isolation of Yersinia enterocolitica, E. coli O157 and *Campylobacter jejuni/coli*. Stool was also inoculated into Selenite broth for enrichment prior to plating for isolation of Salmonella sp and Shigella sp. Antibiotic susceptibility testing was performed for Salmonella and Shigella isolates. Serotyping was performed for presumptive identification of O157 strains amongst non-sorbitol fermenting *E. coli* isolates. In addition the limit of detection for bacterial targets was compared in triplicate using "spiked" specimens prepared in Enteric Transport Media and Copan Fecal Swabs. The complete value chain of activities was assessed starting with the preanalytical phase (specimen collection, transportation, set up for DNA extraction and PCR plates), analytical phase (PCR, reflex test setup for culture and antibiotic susceptibility testing) and post-analytical phase (reporting, referral for confirmatory testing).

RESULTS

The Seegene Allplex GI tests provided superior sensitivity and specificity compared to conventional methods. The limit of detection for bacterial pathogens (Salmonella sp, Shigella sp, Campylobacter jejuni/coli, Yersinia enterocolitica and E. coli O157) was 10-100 fold improved in Copan Fecal Swab specimens compared to Enteric Transport media. Copan Fecal Swab specimens can be loaded directly onto the Nimbus or Starlet platforms for greater operational efficiency and audit trail during automated DNA extraction and PCR plate setup.

Table 1. Analytical Sensitivity, Limit of Detection for Bacterial Targets a. Salmonella

Salmonella Detection - Allplex GI-Bacteria (I) Assay													
Sample # Dilutio		Without Selenite Enrichment					With Selenite Enrichment						
	Dilution	Enteric Transport Media			Copan Fecal Swab		Enteric Transport Media			Copan Fecal Swab			
	Dilution	Result	C(t) Value	Culture Detection	Result	C(t) Value	Culture Detection	Result	C(t) Value	Culture Detection	Result	C(t) Value	Culture Detectior
1	10^6 A	Sal	41.17	✓	Sal	34.84	✓	Sal	34.62	-	Sal	30.34	✓
2	10^6 B	Sal	42.11	✓	Sal	35.23	✓	Sal	35.55	✓	Sal	29.80	✓
3	10^6 C	Sal	41.41	-	Sal	35.03	✓	Sal	36.92	-	Sal	29.33	✓
4	10^5 A	-	N/A	-	Sal	38.25	✓	Sal	40.19	✓	Sal	32.64	✓
5	10^5 B	Sal	42.27	-	Sal	37.72	✓	Sal	38.08	-	Sal	33.14	✓
6	10^5 C	-	N/A	-	Sal	39.57	✓	Sal	40.31	-	Sal	33.58	✓
7	10^4 A	-	N/A	-	Sal	42.46	✓	-	N/A	-	Sal	36.36	✓
8	10^4 B	-	N/A	-	-	N/A	✓	-	N/A	-	Sal	37.93	✓
9	10^4 C	-	N/A	✓	-	N/A	-	-	N/A	-	Sal	36.61	-
10	10^3 A	-	N/A	-	-	N/A	-	-	N/A	-	Sal	42.60	-
11	10^3 B	-	N/A	-	-	N/A	-	-	N/A	-	Sal	40.98	-
12	10^3 C	-	N/A	-	-	N/A	-	-	N/A	-	Sal	41.41	-
13	10^2 A	-	N/A	-	-	N/A	-	-	N/A	-	-	N/A	-
14	10^2 B	-	N/A	-	-	N/A	-	-	N/A	-	-	N/A	-
15	10^2 C	-	N/A	-	-	N/A	-	-	N/A	-	-	N/A	-

b. Shigella

Shigella Detection - Allplex GI-Bacteria (I) Assay									
		Without Selenite Enrichment							
Sample #	Dilution	Ente	ric Transpo	rt Media	Copan Fecal Swab				
		Result	C(t) Value	Culture	Result	C(t) Value	Culture		
				Detection			Detection		
1	10^6 A	Sh/EI	39.84	✓	Sh/EI	33.20	✓		
2	10^6 B	-	N/A	✓	Sh/EI	33.23	✓		
3	10^6 C	Sh/EI	41.31	-	Sh/EI	33.75	✓		
4	10^5 A	-	N/A	-	Sh/EI	36.96	✓		
5	10^5 B	Sh/EI	44.59	-	Sh/EI	36.66	✓		
6	10^5 C	Sh/EI	44.10	-	Sh/EI	35.94	✓		
7	10^4 A	-	N/A	-	Sh/EI	41.96	✓		
8	10^4 B	-	N/A	-	Sh/EI	39.17	-		
9	10^4 C	-	N/A	-	Sh/EI	41.46	✓		
10	10^3 A	-	N/A	-	-	N/A	✓		
11	10^3 B	-	N/A	-	-	N/A	-		
12	10^3 C	-	N/A	-	Sh/EI	43.86	-		
13	10^2 A	-	N/A	-	-	N/A	-		
14	10^2 B	-	N/A	-	-	N/A	-		
15	10^2 C	-	N/A	_	-	N/A	_		

c. E.coli O157 eaeA

E. coli O157 Detection - Allplex GI-Bacteria (II) Assay									
	Dilution	Ente	ric Transport M	ledia	Copan Fecal Swab				
Sample #		Result	C(t) Value	Culture Detection	Result	C(t) Value	Culture Detection		
1	10^6 A	"eaeA,0157"	37.37, 42.42	✓	"eaeA,0157"	31.13, 33.69	✓		
2	10^6 B	"eaeA,0157"	36.53, 39.33	✓	"eaeA,0157"	31.16, 33.91	✓		
3	10^6 C	"eaeA,0157"	39.34, 40.04	✓	"eaeA,0157"	30.69, 32.87	√		
4	10^5 A	-	N/A	√	"eaeA,0157"	34.99, 37.45	√		
5	10^5 B	-	N/A	√	"eaeA,0157"	34.62, 37.17	✓		
6	10^5 C	eaeA	39.34	√	"eaeA,0157"	35.60, 38.75	✓		
7	10^4 A	-	N/A	- ✓	"eaeA,O157"	37.67, 39.75	✓		
8	10^4 B	-	N/A	-	0157	40.68	✓		
9	10^4 C	-	N/A	√	"eaeA,O157"	38.25, 40.15	-		
10	10^3 A	-	N/A	-	-	N/A	✓		
11	10^3 B	-	N/A	-	-	N/A	-		
12	10^3 C	-	N/A	-	0157	41.85	-		
13	10^2 A	-	N/A	-	-	N/A	-		
14	10^2 B	-	N/A	-	-	N/A	-		
15	10^2 C	-	N/A	-	-	N/A	-		

d. Yersinia enterocolitica

Yersinia Serial Dilutions - Allplex GI-Bacteria (I) Assay									
		Copan Fecal Swab							
Sample #	Dilution	Result	C(t) Value	Culture Detection (CFU/mL)					
1	Negative Stool	-	N/A						
2	Heavily Spiked	Yer	19.26						
3	10^6 A	Yer	32.26	✓					
4	10^6 B	Yer	32.40	✓					
5	10^6 C	Yer	33.01	✓					
6	10^5 A	Yer	36.77						
7	10^5 B	Yer	35.91						
8	10^5 C	Yer	36.08						
9	10^4 A	Yer	41.16						
10	10^4 B	Yer	39.75						
11	10^4 C	Yer	38.37						
12	10^3 A	-	N/A						
13	10^3 B	Yer	40.12						
14	10^3 C	-	N/A						
15	10^2 A	-	N/A						
16	10^2 B	-	N/A						
17	10^2 C	-	N/A						

Limit of Detection Summary

- Stool samples were spiked with pre-determined colony forming units of the five enteric pathogens of interest.
- Spiked stool samples were "inoculated" into an Enteric Transport Media vial (Bio-Media) and in parallel to a Copan Fecal Swab, as per standard stool sampling instructions.
- PCR detection consistently had greater LOD for all bacterial targets
- Detection of Salmonella was improved in samples processed from Copan Fecal Swab

Parasite detection from Copan Fecal Swab specimens was also verified. 100% Accuracy for all targets with lower Ct values than detection from ETM

e. Campylobacter jejuni/coli

Campylobacter Detection - Allplex GI-Bacteria (I) Assay								
		Copan Fecal Swab						
Sample #	Dilution	Result	C(t) Value	Culture Detection (CFU/mL)				
1	Heavy Inoculum	Cam	18.31					
2	10^6 A	Cam	32.23	✓				
3	10^6 B	Cam	31.69	✓				
4	10^6 C	Cam	31.25	✓				
5	10^5 A	Cam	35.89					
6	10^5 B	Cam	36.19					
7	10^5 C	Cam	34.92					
8	10^4 A	Cam	38.69					
9	10^4 B	Cam	39.51					
10	10^4 C	Cam	40.37					
11	10^3 A	Cam	40.35					
12	10^3 B	-	N/A					
13	10^3 C	-	N/A					
14	10^2 A	-	N/A					
15	10^2 B	-	N/A					
16	10^2 C	-	N/A					







Figure 3. Proposed Workflow for Molecular Detection

A. Pre-Analytical Phase: Load & Go DNA Extraction Instrument Order Entry, Audit Trail from point of specimen receipt with Copan Fecal Swab Fecal Swab → DNA+Master Mix aliquotted into 96-well Plates for 3 PCR panels





Nimbus IVD

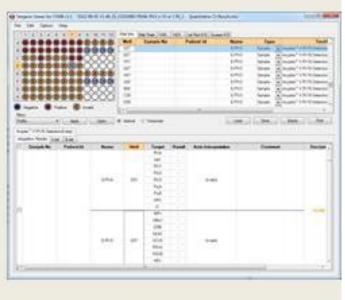
Starlet: 80 Samples/90 min

Nimbus: 48 Samples/90 min

B. Analytical: Load & Go Multiplex PCR and Results Analysis 3 reaction panels; Parasite, Bacteria I, Bacteria II (Ecoli O157 & toxin genes)







Seegene Viewer

MLT Reviews Positive Bacteria Samples Culture setup

• AST for Salmonella, Shigella • E coli O157 and Yersinia isolates **Reference Lab for surveillance**

Wet prep for Helminths

C. Post-Analytical: Interface of Seegene software to LIS Automated merging of PCR results and O/P microscopy and application of appropriate comment codes for reporting

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Faster Turn Around Time 2 days vs. 5 days Early Detection = Enhanced **Clinical Management Improved Patient Outcomes**

The combination of test performance characteristics, operational parameters (throughput, ease of use) highlight Seegene Allplex GI panels and the Startlet/Nimbus instruments as an attractive testing suite for high-volume diagnostic enteric testing of bacterial and

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