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

Interior Health
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FACULTY OF MEDICINE

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Abstract

Background: Multiplexed molecular panels allow rapid detection of multiple stool pathogens, reducing workload and turn around time compared to conventional methods. This study evaluated the performance of Seegene Allplex™ GI-Bacteria (I) and (II) panels and Roche LightMix® gastrointestinal bacterial assays.

Methods: A total of 233 frozen, pre-characterized clinical or simulated Copan fecal swab™ specimens were tested on Allplex™ panels, while 219 were tested on LightMix® panels. Fecal swab fluid (190uL) was combined with 10uL of each Allplex™ GI-BP internal control and LightMix® phocine herpesvirus extraction control and extracted on the Roche MagNA Pure Compact. PCR was performed in parallel on the Bio-Rad CFX96 and LightCycler 480II, using reaction parameters specified by the manufacturers. *Campylobacter spp, Salmonella spp, Shigella spp, Y. enterocolitica, Aeromonas spp* and *Shiga toxin* 1/2 genes were compared between both assays, with *C. difficile, Vibrio spp* and *E. coli* O157 only evaluated on Allplex™, and *P. shigelloides* only evaluated on LightMix®. Sensitivity and specificity were calculated using a modified gold standard, composed of at least 2 positive between routine stool culture, Allplex™ and LightMix® PCR.

Results: Allplex™ panels provided accurate results in 192/198 (97%) clinical specimens while LightMix® was accurate in 183/189 (96.8%). When both clinical and simulated specimens were considered, Allplex™ had excellent performance for all targets, apart from *Campylobacter spp* (87.5% sensitivity) and *Vibrio spp* (66.7% sensitivity), since more unusual species (eg: *C. upsaliensis, V. fluvialis*) were not detected (Table 1). LightMix® performed well with >90% sensitivity and >99% specificity for all targets.

Conclusions: Both assays performed well in detecting clinically relevant infections, compared to conventional methods.

Introduction

- Infectious diarrhea is a leading cause of morbidity and mortality worldwide (1).
- This study compared the performance of the Seegene Allplex™ GI-Bacteria (I) and (II) panels to the Roche LightMix® Modular Gastrointestinal Bacterial Assays

Acknowledgements: Thanks to the Colin & Lois Pritchard Foundation for donating the PCR equipment and funding April's work term. Thanks to the KGH microbiology laboratory staff for performing stool culture testing on specimens used in the study. Thanks to St. Paul's hospital, BCCDC and LifeLabs laboratories for performing additional PCR testing on discordant specimens.

Methods

- A total of 233 samples were tested on Allplex™ panels and 219 samples were tested on LightMix® panels comprising 45 bacterial, viral and parasitic organisms.

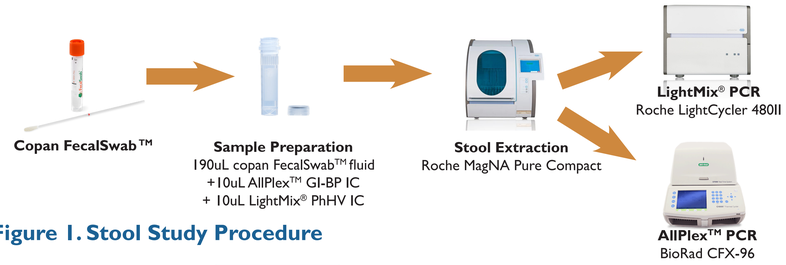

Figure 1. Stool Study Procedure

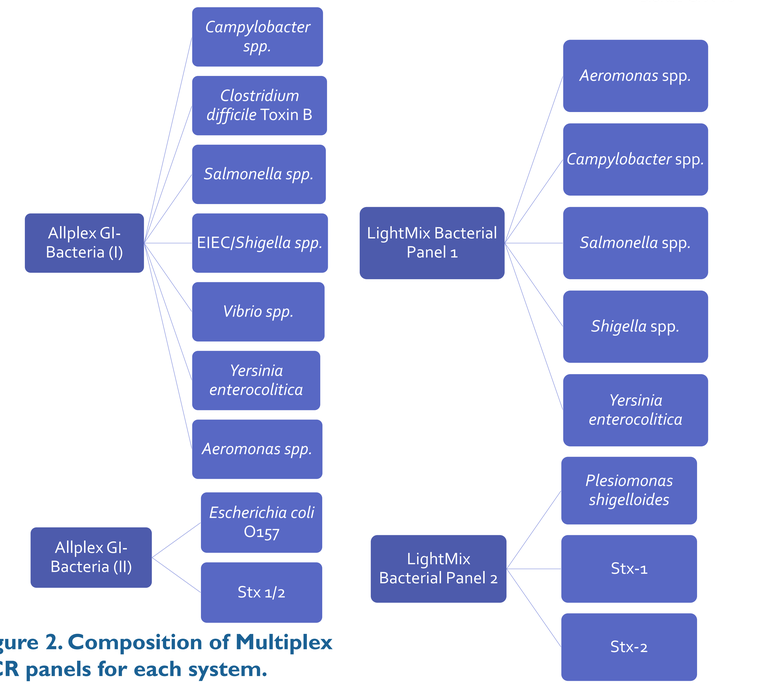

Figure 2. Composition of Multiplex PCR panels for each system.

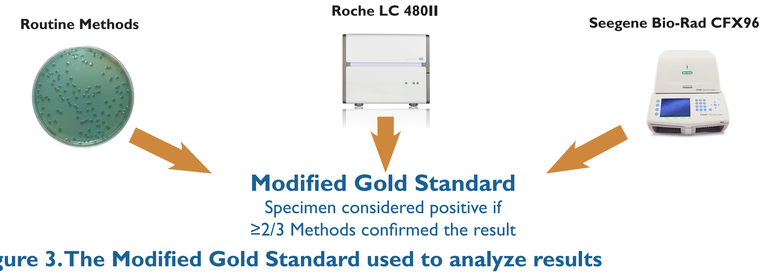

Figure 3. The Modified Gold Standard used to analyze results

Results

- Allplex™ panels provided accurate results in 192/198 (97%) clinical specimens while LightMix® was accurate in 183/189 (96.8%).
- Allplex™ demonstrated lower sensitivity for *Campylobacter spp* (87.5%) and *Vibrio spp* (66.7%), since more unusual species (eg: *C. upsaliensis, C. fetus, V. fluvialis, and V. alginolyticus*) were not detected, as primers were designed to detect more common species
- LightMix® performed well with >90% sensitivity and >99% specificity for all targets.
- For Allplex™ assays, inhibition was observed in 6.4% of samples run on Panel (I) and 4.3% run on Panel (II).
- For LightMix® assays, inhibition was observed in 12.3% of samples run on Panel 1 and 14.2% run on Panel 2

Table 1. Sensitivity and specificty results for all targets

Assay		Allplex™		LightMix®	
Target	n	Sens	Spec	Sens	Spec
Aeromonas spp	30	96.7%	99.5%	93.3%	100%
All Campylobacter spp	40	87.5%	99.5%	100%	100%
C. coli/C. jejuni only	33	100%	100%	100%	100%
Clostridium difficile	19	100%	99.5%		
Plesiomonas shigelloides	5			100%	99.5%
All Salmonella spp	28	100%	100%	100%	100%
All Shigella spp	10	100%	100%	100%	100%
All Vibrio spp	9	66.7%	100%		
Vibrio alginolyticus	1	0.0%	100%		
Vibrio cholerae	2	100%	100%		
Vibrio fluvialis	1	0.0%	100%		
Vibrio mimicus	1	0.0%	100%		
Vibrio parahaemolyticus	3	100%	100%		
Vibrio vulnificus	1	100%	100%		
Yersinia enterocolitica	16	100%	100%	93.8%	100%
E. coli O157	7	100%	100%		
Stx-1	9			100%	100%
Stx-2	15			100%	100%
Stx-1/2	17	100%	100%		

Conclusion

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

References

(1) Humphries RM, Linscott AJ. Laboratory diagnosis of bacterial gastroenteritis. Clin Microbiol Rev 2015;28(1):3-31.