Evaluation of the Verigene EP IUO Test for the Rapid Detection of Bacterial and Viral Causes of Gastrointestinal Infection

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Revised Abstract

Updated Abstract

Objectives: Laboratory fecal studies due to infectious diarrhea are dependent on the severity, duration and history of the patient. Bacterial work-ups are labor intensive and require time to process the culture or EIA. Viral studies are not as widely available and send outs can add to delayed results and cost. Molecular stool panels address a clinical need while also streamlining workflow in the clinical laboratory. We evaluated the performance of the sample-to-result Verigene® Enteric Pathogens Nucleic Acid (EP) IUO Test (Nanosphere, Northbrook, IL) for the detection of common bacterial (*Salmonella* spp., *Shigella* spp., *Campylobacter*, *Vibrio* spp., *Yersinia enterocolitica*, (*stx*1, *stx*2)) and viral (Norovirus, Rotavirus) causes of diarrheal illness directly from stool preserved in Cary-Blair transport media.

Methods: Prospectively collected and contrived stool specimens were analyzed using the Verigene EP Test. Results of the Verigene EP Test were compared to gold standard methods. Prospective clinical specimens were preserved in Cary-Blair media within 4 hours of collection and were tested on the Verigene EP Test within 48 hours of collection and with bacterial culture within 60 hours of collection. Reference testing for viral identification was performed at a third party service laboratory.

Results: A total of 839 stool specimens were analyzed using the Verigene EP Test. This included 611 prospectively collected clinical specimens and 228 contrived specimens. The overall sensitivity and specificity of the bacterial and toxin targets on the Verigene EP Test was 97.5% and 99.9%, respectively. The sensitivity and specificity of the Verigene EP Test norovirus target was 90.9% and 98.9%, respectively and the Rotavirus target was 66.7% and 99.9%, respectively. Viral discrepant testing is pending. For the sensitivity and specificity for each Verigene EP target see table. Results of the Verigene EP Test were available within 2.0 hours of test initiation.

Conclusions: The Verigene EP Test provides the necessary sensitivity and turnaround time to be considered a viable diagnostic option for the detection of common acute diarrheal agents. Rapid stool panels have the potential to improve patient management decisions, facilitate prompt infection control measures, and outbreak investigations.

Background

Acute Gastroenteritis is caused by both bacterial and viral etiologies. According to the CDC website each year roughly 48 million people get sick, 128,000 are hospitalized and 3,000 die of foodborne illnesses. The bacterial work-up of stools specimens for acute gastroenteritis is one of the more labor intense benches in the clinical microbiology laboratory, with the majority of cultures negative. Viral stool studies for rotavirus comprise of an EIA. For Norovirus diagnosis PCR is recommended, which is often times not widely available. The objective of this study was to evaluate the performance of the Verigene Enteric Pathogens Nucleic Acid (EP) IUO Test (Nanosphere, Northbrook, IL) for the detection of common bacterial and viral causes of diarrheal illness directly from stool preserved in Cary-Blair media.

Methods

Prospective specimens: Prospective specimens meeting institutional criteria for stool pathogen testing were analyzed using the Verigene® EP Test. A total of 611 specimens were submitted for routine stool culture to SCPMG Regional Reference Laboratories between July 2013 and December 2014. Fresh stool was preserved in Cary-Blair transport media within 4 hrs of collection. Residual unformed stools ≥ 5 mL were de-identified and enrolled in the study within 48 hrs of collection.
Contrived specimens: A total of 228 frozen contrived samples containing known enteric pathogens were sent blinded to the laboratory between November-December 2013.
Specimen preparation: Prospective: Cary-Blair specimens were screened for eligibility enrollment, de-identified and aliquoted into four tubes for study purposes.

Procedure:
Test specimens using Verigene EP Test for Investigation Use Only using manufacturer established protocol and package insert:

- Specimens were processed by dipping a swab into the stool specimen and inoculating Stool Preparation Buffer by inserting the swab into the Stool Preparation Buffer tube and breaking off the swab at pre-scored break point.
- Consumables were loaded on the instrument (See User's Manual for step by step directions).
- Specimen order was created using the Verigene Reader interface (See User's Manual for step by step directions).
- 200uL of Stool Preparation Buffer was pipetted into the Extraction Tray for processing (See Package Insert for step by step directions).
- Close consumable tray which prompts processing to start automatically.
- After Processing is complete, test cartridge is removed and placed into Verigene Reader for analysis (See User's Manual for step by step directions).

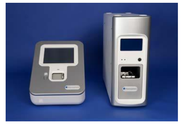

Figure 1. Verigene System: Reader and Processor *SP*

Figure 2. Extraction Tray

Figure 3. Utility Tray

Figure 4. Test cartridge and reagent pack

Table 1: Prospective Specimens (n=611)

Prospective (N=611)									
Organism	n	TP	TN	FP	FN	% Sensitivity	% Specificity	PPV	NPV
Campylobacter	611	8	593	8	2	80.0%	98.7%	50.0%	99.7%
Salmonella	611	7	598	4	2	77.8%	99.5%	63.6%	99.5%
Shigella	611	0	601	10	0	N/A	98.4%	0.0%	100.0%
Vibrio	611	2	609	0	0	100.0%	100.0%	100.0%	100.0%
Y. enterocolitica	611	0	611	0	0	N/A	100.0%	N/A	100.0%
STX1	611	3	607	1	0	100.0%	99.8%	75.0%	100.0%
STX2	611	3	607	1	0	100.0%	99.8%	75.0%	100.0%
Norovirus	611	20	580	9	2	90.9%	98.5%	69.0%	99.7%
Rotavirus	611	2	607	1	1	66.7%	99.8%	66.7%	99.8%

- Bacterial: FP = 24, FN = 4 (NOTE: No *Y. enterolitica* were detected in the prospective specimens tested)
- Viral: FP = 10, FN = 3

Table 2: Resolved Prospective Specimens (n=611)

Prospective (N= 611): BDS Resolved									
Organism	n	TP	TN	FP	FN	% Sensitivity	% Specificity	PPV	NPV
Campylobacter	611	16	593	0	2	88.9%	100.0%	100.0%	99.7%
Salmonella	611	10	598	1	2	83.3%	99.8%	90.9%	99.7%
Shigella	611	10	601	0	0	100.0%	100.0%	100.0%	100.0%
Vibrio	611	2	609	0	0	100.0%	100.0%	100.0%	100.0%
Y. enterocolitica	611	0	611	0	0	N/A	100.0%	N/A	100.0%
STX1	611	4	607	0	0	100.0%	100.0%	100.0%	100.0%
STX2	611	4	607	0	0	100.0%	100.0%	100.0%	100.0%
Norovirus*	611	20	580	9	2	90.9%	98.5%	69.0%	99.7%
Rotavirus*	611	2	607	1	1	66.7%	99.8%	66.7%	99.8%

*Viral discrepant analysis not complete at time of presentation

- Bacterial FP: 23/24 resolved by BDS
- NoroV – 4/9 FP PCR + by at least one Alt PCR
- RotaV - FP & FN Suspected Specimen Mix-up
- Dual Pos (N=1): TP STX 1 + FP NoroV

Table 3: Contrived (n=228)

Contrived (N=228)									
Organism	n	TP	TN	FP	FN	% Sensitivity	% Specificity	PPV	NPV
Campylobacter	228	24	203	0	1	96.0%	100.0%	100.0%	99.5%
Salmonella	228	45	183	0	0	100.0%	100.0%	100.0%	100.0%
Shigella	228	36	192	0	0	100.0%	100.0%	100.0%	100.0%
Vibrio	228	30	197	0	1	96.8%	100.0%	100.0%	99.5%
Y. enterocolitica	228	25	203	0	0	100.0%	100.0%	100.0%	100.0%
STX1	228	31	196	1	0	100.0%	99.5%	96.9%	100.0%
STX2	228	34	192	1	1	97.1%	99.5%	97.1%	99.5%
Norovirus	228	0	228	0	0	N/A	100.0%	N/A	100.0%
Rotavirus	228	0	228	0	0	N/A	100.0%	N/A	100.0%

- BDS did not resolve discrepant results
- FN (N=3): 3/3 Spiked at 2X LOD, Low Titer specimens
- FP (N=2): STX1 in Salmonella contrived specimen; STX2 in Yersinia contrived specimen

Table 4: Overall Combined Results Prospective & Contrived (n=839)

Combined (N=839): BDS Resolved									
Organism	n	TP	TN	FP	FN	% Sensitivity	% Specificity	PPV	NPV
Campylobacter	839	40	796	0	3	93.0%	99.7%	95.0%	99.6%
Salmonella	839	55	781	1	2	96.5%	99.7%	98.2%	99.8%
Shigella	839	46	793	0	0	100.0%	100.0%	100.0%	100.0%
Vibrio	839	32	806	0	1	97.0%	100.0%	100.0%	99.9%
Y. enterocolitica	839	25	814	0	0	100.0%	100.0%	100.0%	100.0%
STX1	839	35	803	1	0	100.0%	99.9%	97.2%	100.0%
STX2	839	38	799	1	1	97.4%	99.9%	97.4%	99.9%
Norovirus**	839	20	808	9	2	90.9%	98.9%	69.0%	99.8%
Rotavirus**	839	2	835	1	1	66.7%	99.9%	66.7%	99.9%
Total	**839**	**293**	**523**	**13**	**10**	**96.7%**	**97.6%**	**95.8%**	**98.1%**

*Viral discrepant analysis not complete at time of presentation

- Overall Sensitivity 96.7% and Specificity 97.6%
  - Initial No Call Rate = 4.2%; Final No Call Rate = 1.5%
- Prospective Specimens:
  - Bacterial: 100% increase in positivity
  - Viral: 32.3% overall positives [29.9% NorV]

DATA ANALYSIS

Bacterial
Reference Testing: Culture with biochemical ID for *Salmonella, Shigella, Campylobacter, Vibrio,* and *Y. enterocolitica;* MacConkey broth enrichment followed by EHEC EIA and PCR amplification/bi-directional sequencing for conformation and typing of *STX1* and *STX2*
Discrepant Testing from fresh nucleic acid: Bi-directional Sequencing

Viral
Reference Testing from residual Nucleic Acid: Real-Time PCR, End Point PCR with Bidirectional Sequencing [2/2 pos = TP]
Discrepant Testing* = Repeat of reference testing, plus repeat from a new nucleic acid extract

*Discrepant testing for vial targets pending at time of presentation

Results

A total of 611 prospective specimens and 228 contrived specimens were tested as part of an 8 site clinical trial for the Verigene® Enteric Pathogens Nucleic Acid (EP) IUO Test. Both prospective and contrived specimens had false negative and false positive results compared to culture or other testing (EIA for virus) but many were resolved when discrepant testing was performed (see data above). There was an overall increase of 45% in positivity for bacteria when comparing the Verigene to culture. In addition there was an overall increase of 34% in positivity for viruses (31% for Norovirus) when comparing the Verigene assay to EIA (some discrepant testing still pending at time of poster).

Conclusions

Overall the Verigene® Enteric Pathogens Nucleic Acid (EP) IUO Test performed well. Combining data from the prospective and contrived data set the overall sensitivity and specificity was 96.7% and 97.3% respectively for all pathogens. Some viral discrepant testing was still pending at the time of this poster. The discrepant testing data could enhance the overall performance for Norovirus and Rotavirus. The assay is moderately complex and takes approximately 2 hours to complete with minimal hands on time. PCR for enteric pathogens will not only increase the sensitivity of detection of commonly isolated bacterial but will also allow laboratories to easily test for pathogens such as Norovirus in which testing has historically only been available through public health laboratories or the reference laboratory setting. Time to detection is significantly improved over the traditional cultures methods which has the potential to impact clinical patient care. Ease of use allows laboratories with limited molecular experience the ability to perform testing in house.

References

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