Rapid Molecular Diagnostics of Multiple Pathogens in Feces in a Single Automated Workflow

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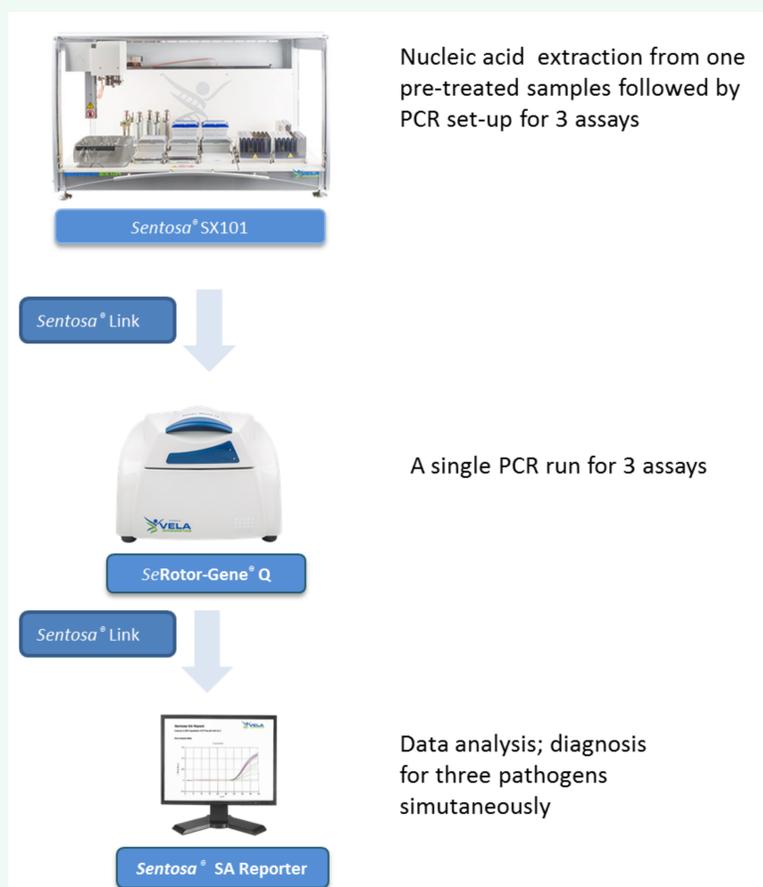
Background: Infectious gastroenteritis causes substantial morbidity and mortality worldwide. Various bacterial and viral species have been involved in gastroenteritis, among which *Clostridium difficile*, Norovirus and Rotavirus are considered as major causes of hospital-associated infectious diarrhea, especially in acute care settings and intensive care unit. Accurate diagnosis of pathogens for gastroenteritis will aid in patient management and proper therapy.

Results: We developed three real-time PCR based assays, namely, *Sentosa*® SA C. diff PCR Test, *Sentosa*® SA Norovirus RT-PCR Test and *Sentosa*® SA Rotavirus RT-PCR Test, with analytical sensitivities of 1.1, 5.4 and 1.7 copies/μl, and clinical sensitivities of 98.8%, 98.7% and 98.7%, respectively. All three assays demonstrated 100% clinical specificity. These assays share the same instrumentation and data reduction software as well as sample preparation method. An automated robotic workflow developed for the *Sentosa*® SX101 instrument from Vela Diagnostics was used to isolate nucleic acid (including both DNA and RNA) from the same stool sample followed by PCR setup of the three individual assays simultaneously. The result from a single PCR run provides diagnosis for all three pathogens. In a validation study with 83 stool samples from diarrhea patients, we achieved 98.8% agreement with the test results performed with individual assays separately.

Methods

- Stool sample was pretreated with S.T.A.R buffer manually
- Nucleic acid from individual sample was extracted using *Sentosa*® SX Virus Total Nucleic Acid Kit
- PCR was set up with *Sentosa*® SA C. diff PCR Test, *Sentosa*® SA Norovirus RT-PCR Test and *Sentosa*® SA Rotavirus RT-PCR Test
- The Nucleic acid extraction and PCR setup was conducted on *Sentosa*® SX101 instrument, an automated robotic workflow from Vela Diagnostics
- PCR reaction was conducted with Rotor-Gene Q MDx 5plex HRM Platform
- Data analysis was performed by *Sentosa*® SA reporter

Sentosa®SX101 — Nucleic acid extraction from one pre-treated samples followed by PCR set-up for 3 assays

Sentosa® Link

SeRotor-Gene® Q — A single PCR run for 3 assays

Sentosa® Link

Sentosa® SA Reporter — Data analysis; diagnosis for three pathogens simutaneously

Workflow of combination assay for Gastro panel

Results

Analytical performance of individual assay

Assay	Channel Target	Analytical Sensitivity (LoD)	Analytical Specificity	Reproducibility
Sentosa® SA C. diff PCR Test	Green: *C. difficile* Red: EC	1.1 copy/μL	All 22 *C. difficile* strains tested are detectable	98.3%
Sentosa® SA Norovirus RT-PCR Test	Green: Norovirus GII Orange: Norovirus GI	Norovirus GI: 5.4 copies/μL Norovirus GII: 1 copy/μL	Genotype GI. 3, GI. 7, GI.8, GII, 4, and GII.b tested are detectable	100%
Sentosa® SA Rotavirus RT-PCR Test	Green: Rotavirus Red: EC	1.67copies/μL	Six Rotavirus A tested are detectable	100%

EC, Extraction control

Clinical performance for each individual assay

Sentosa® SA C. diff PCR Test

Reference Method (Tissue Culture Cytotoxin)					95%CI		
		POS	NEG	Total	Sensitivity	98.8%	93.4%	99.8%
Sentosa® SA C. diff PCR Test	POS	81	0	81	Specificity	100%	95.1%	100%
	NEG	1	75	76				
	Total	82	75	157				

Sentosa® SA Norovirus RT-PCR Test

Reference Method (Nucleic Acid Test)					95%CI		
		POS	NEG	Total	Sensitivity	98.7%	92.9%	99.8%
Sentosa® SA Norovirus RT-PCR Test	POS	74	0	74	Specificity	100%	95.1%	100%
	NEG	1	75	76				
	Total	75	75	150				

Sentosa® SA Rotavirus RT-PCR Test

Reference Method (Real Time PCR assay)					95%CI		
		POS	NEG	Total	Sensitivity	98.7%	92.7%	99.8%
Sentosa® SA Rotavirus RT-PCR Test	POS	73	0	73	Specificity	100%	95.2%	100%
	NEG	1	76	77				
	Total	74	76	150				

Equivalent test between individual assay and combo assay

Equivalent test for analytical performance with 3× LoD simulated samples
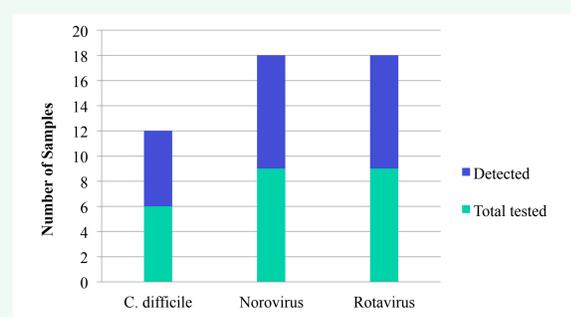
Equivalent test for clinical performance with 83 clinical diarrhea samples

		Individual Assay					
		POS	NEG	Total	Agreement	95% CI	
Combo Assay	POS	44	0	44	98.8%	92.9%	100%
	NEG	1	38	39			
	Total	45	38	83			

Conclusions

We have developed three real time PCR-based assays to detect three major pathogens for gastroenteritis. The assays demonstrated robust performance in our test. The combination of these three assay in an automated workflow enable us to provides diagnosis for all three pathogens in a single run. The combo assay has a similar performance as the individual assays.