Development of an External Control Panel to Monitor the Performance of a Multiplexed Gastrointestinal Pathogen Test

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Introduction

Gastrointestinal (GI) infections are a significant global health issue and are caused by a variety of parasites, bacteria, and viruses. Rapid and accurate identification of these causative agents improves time to diagnosis and treatment decisions. Instruments that perform qualitative nucleic acid testing, including the QIAGEN QIAstat-Dx® Analyzer 1.0, improve rapid detection; however, CLIA and good manufacturing practice dictates that all clinical tests must be monitored to identify shifts, trends, and random errors due to variations in the test system. A multiplexed control panel has been developed to monitor detection of all 23 pathogenic targets which include 5 viral, 14 bacterial, and 4 parasitic pathogens on the QIAstat-Dx GI Panel 2 assay.

Materials and Methods

Non-infectious, multi-target constructs, containing genome segments of all GI pathogens detected by the QIAstat-Dx GI Panel 2 were designed *in silico*, and ligated into engineered vectors to create stable frozen clones. All plasmids were then purified from frozen clones. *In vitro* RNA transcripts were generated for all viral targets. Plasmids and transcripts were quantified and formulated in a proprietary matrix to stabilize and carry the genetic material throughout the entire process. Testing was performed using the QIAstat-Dx GI Panel 2 assay on the QIAstat-Dx Analyzer® 1.0.

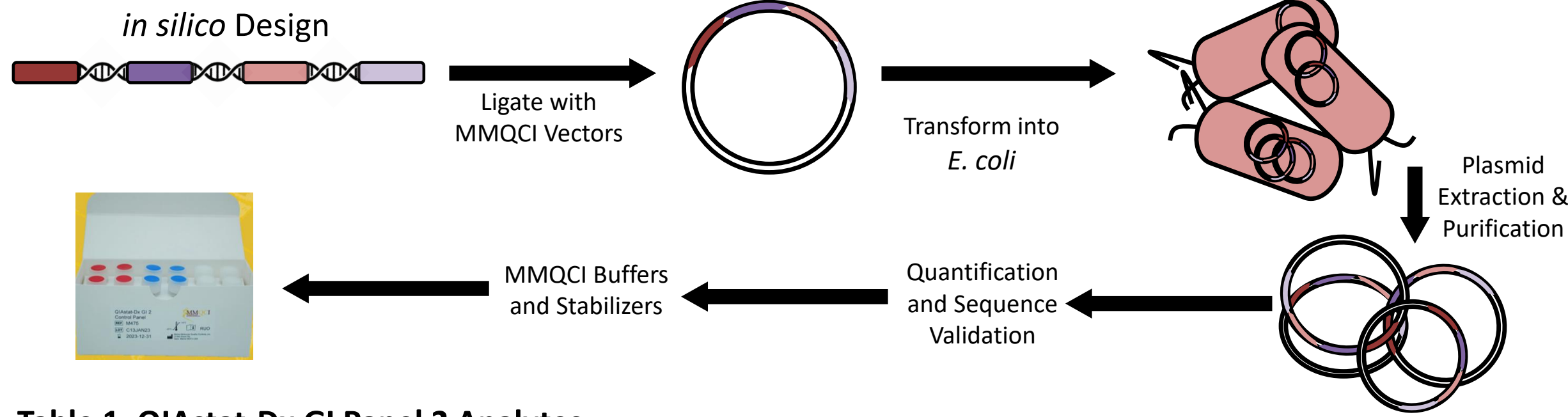**Figure 1. Design Strategy for QIAstat-Dx GI 2 Control Panel**

Table 1. QIAstat-Dx GI Panel 2 Analytes

QIAstat-Dx GI Panel 2 Analytes	
Viruses	
• Adenovirus F40/F41	• Norovirus GI/GII
• Astrovirus	• Rotavirus A
• Sapovirus	
Bacteria	
• *Campylobacter*	• *Salmonella*
• *E. coli* O157	• Shiga-like toxin *E. coli* (STEC)
• Enteropathogenic *E. coli* (EPEC)	• *Shigella/Enteroinvasive E. coli* (EIEC)
• Enterotoxigenic *E. coli* (ETEC)	• *Yersinia enterocolitica*
• *Plesiomonas shigelloides*	• *Vibrio cholerae*
• *Vibrio vulnificus*	• *Vibrio parahaemolyticus*
• *Clostridium dificile* toxin A/B	• Enteroaggregative *E. coli* (EAEC)
Parasites	
• *Cryptosporidium*	• *Entamoeba histolytica*
• *Cyclospora cayetanensis*	• *Giardia lamblia*

Table 2. Summary Table of Validation Data

A total of 92 tests of QIAstat-Dx GI 2 Positive Control Panel (n=92 positive controls) were performed across 2 sites utilizing 3 manufactured lots of QIAstat-Dx GI 2 Positive A and B controls and 4 different QIAstat-Dx GI 2 cartridge lots. Concordant calls were reported in all 92 tests across two sites, for an overall successful testing rate of 100%. An additional 10 tests of QIAstat-Dx GI 2 Negative Control Panel (n=10) were performed with 100% concordant negative results.

Sites	Total Tests	Invalid Tests	Concordant Positive Control Results	Incorrect Positive Control Results	Percent Correct Positive Control	Concordant Negative Control Results	Incorrect Negative Control Results	Percent Correct Negative Control	Total Percent Correct
MMQCI	90	0	80	0	100%	10	0	100%	100%
QIAGEN Spain	12	0	12	0	100%	-	-	-	-

Validation of QIAstat-Dx GI 2 Control Panel

Table 3. Internal Site Testing of the QIAstat-Dx GI 2 Control Panel

Three manufactured lots of QIAstat-Dx GI 2 Positive A and Positive B controls were tested at MMQCI (n=80) using 3 different QIAstat-Dx GI 2 cartridge lots. All controls showed 100% correct detection and all targets resulted in Cycle Thresholds (Cts) with %CV's of less than 5%.

QIAstat-Dx GI 2 Control Panel Internal Reproducibility Testing		Average Ct	SD	%CV	No. Samples Tested	No. Valid Tests	No. Correct Results	Percent Correct Results
Positive A	Astrovirus	25.1	1.12	4.47	80	80	80	100%
	Rotavirus A	26.1	1.19	4.58	80	80	80	100%
	Norovirus GI/GII	26.1	0.97	3.72	80	80	80	100%
	Sapovirus	25.7	0.80	3.12	80	80	80	100%
	Vibrio vulnificus	25.4	0.99	3.91	80	80	80	100%
	Cryptosporidium	25.0	0.62	2.46	80	80	80	100%
	Shiga-like toxin *E. coli*	25.2	0.64	2.53	80	80	80	100%
	Campylobacter	25.7	0.71	2.76	80	80	80	100%
	Clostridium difficile toxin A/B	24.1	0.89	3.69	80	80	80	100%
	E. coli O157	24.9	0.81	3.27	80	80	80	100%
	Enterotoxigenic *E. coli* lt/st	24.6	0.65	2.66	80	80	80	100%
	Salmonella	24.6	0.59	2.41	80	80	80	100%
	Entamoeba histolytica	25.0	0.65	2.59	80	80	80	100%
Positive B	*Plesiomonas shigelloides*	29.2	0.54	1.85	80	80	80	100%
	Vibrio cholerae	26.4	0.87	3.31	80	80	80	100%
	Vibrio parahaemolyticus	26.5	0.68	2.56	80	80	80	100%
	Giardia lamblia	25.9	0.51	1.98	80	80	80	100%
	Adenovirus F40/F41	26.5	0.82	3.09	80	80	80	100%
	Enteroaggregative *E. coli*	26.6	0.67	2.53	80	80	80	100%
	Enteropathogenic *E. coli*	26.5	0.58	2.17	80	80	80	100%
	Shigella/Enteroinvasive E. Coli	26.8	0.79	2.94	80	80	80	100%
	Yersinia enterocolitica	26.9	0.53	1.96	80	80	80	100%
	Cyclospora cayetanensis	26.4	0.66	2.49	80	80	80	100%

Table 5. Intra Lot Precision (Repeatability) of the QIAstat-Dx GI 2 Control Panel

One manufactured lot of QIAstat-Dx GI 2 Positive A and Positive B controls were tested across two QIAstat-Dx GI 2 Panel cartridge lots, Lot A (n=5) and Lot B (n=5), to assess intra lot precision of the QIAstat-Dx GI 2 Control Panel.

QIAstat-Dx GI 2 Control Panel Intra Lot Precision		Cartridge Lot A			Cartridge Lot B			Average Ct (n=10)	%CV
		Average Ct (n=5)	Standard Deviation	%CV	Average Ct (n=5)	Standard Deviation	%CV		
Positive A	Astrovirus	26.6	0.502	0.019	25.7	0.192	0.75	26.1	2.28
	Rotavirus A	25.9	0.472	0.018	25.2	0.141	0.56	25.5	1.87
	Norovirus GI/GII	25.9	0.508	0.020	25.2	0.277	1.10	25.6	2.18
	Sapovirus	26.4	0.385	0.015	25.9	0.249	0.96	26.2	1.57
	Vibrio vulnificus	24.6	0.602	0.024	23.7	0.230	0.97	24.1	2.69
	Cryptosporidium	25.0	0.540	0.022	24.2	0.335	1.38	24.6	2.49
	Shiga-like toxin *E. coli*	25.1	0.466	0.019	24.3	0.255	1.05	24.7	2.20
	Campylobacter	25.3	0.456	0.018	24.4	0.265	1.08	24.8	2.31
	Clostridium difficile toxin A/B	25.4	0.508	0.020	24.7	0.130	0.53	25.1	2.12
	E. coli O157	25.9	0.602	0.023	26.0	0.502	1.93	25.9	2.02
	Enterotoxigenic *E. coli* lt/st	24.9	0.288	0.012	25.0	0.458	1.83	25.0	1.45
	Salmonella	24.6	0.532	0.022	24.4	0.572	2.34	24.5	2.15
	Entamoeba histolytica	25.6	0.391	0.015	25.7	0.442	1.72	25.6	1.56
Positive B	*Plesiomonas shigelloides*	29.4	0.342	0.012	29.6	0.164	0.55	29.5	0.96
	Vibrio cholerae	27.4	0.332	0.012	26.2	0.383	1.46	26.8	2.64
	Vibrio parahaemolyticus	27.2	0.089	0.003	26.8	0.471	1.76	27.0	1.49
	Giardia lamblia	26.3	0.251	0.010	26.1	0.259	0.99	26.2	1.02
	Adenovirus F40/F41	27.4	0.230	0.008	26.5	0.308	1.16	27.0	2.07
	Enteroaggregative *E. coli*	27.2	0.084	0.003	26.8	0.396	1.48	27.0	1.27
	Enteropathogenic *E. coli*	27.1	0.277	0.010	26.6	0.152	0.57	26.8	1.35
	Shigella/Enteroinvasive E. coli	27.5	0.308	0.011	26.8	0.455	1.70	27.1	1.94
	Yersinia enterocolitica	27.4	0.114	0.004	27.0	0.265	0.98	27.2	1.11
	Cyclospora cayetanensis	27.1	0.192	0.007	26.4	0.148	0.56	26.7	1.51

Table 4. External Site Testing of the QIAstat-Dx GI 2 Control Panel

Three manufactured lots of QIAstat-Dx GI 2 Positive A and Positive B controls were tested at an external site (n=12) using 2 different QIAstat-Dx GI 2 cartridge lots across different instruments. All controls showed 100% correct detection and all targets resulted in Cycle Thresholds (Cts) with %CV's of less than 7%.

QIAstat-Dx GI 2 Control Panel Internal Validation		Average Ct	SD	%CV	No. Samples Tested	No. Valid Tests	No. Correct Results	Percent Correct Results
Positive A	Astrovirus	23.3	1.56	6.70	12	12	12	100%
	Rotavirus A	24.5	1.13	4.62	12	12	12	100%
	Norovirus GI/GII	25.3	1.58	6.26	12	12	12	100%
	Sapovirus	24.5	1.24	5.06	12	12	12	100%
	Vibrio vulnificus	24.8	1.10	4.44	12	12	12	100%
	Cryptosporidium	24.2	0.94	3.87	12	12	12	100%
	Shiga-like toxin *E. coli*	24.8	0.90	3.62	12	12	12	100%
	Campylobacter	25.2	0.95	3.77	12	12	12	100%
	Clostridium difficile toxin A/B	22.6	1.42	6.28	12	12	12	100%
	E. coli O157	23.4	1.26	5.38	12	12	12	100%
	Enterotoxigenic *E. coli* lt/st	23.5	1.03	4.37	12	12	12	100%
	Salmonella	23.6	0.94	3.98	12	12	12	100%
	Entamoeba histolytica	23.8	0.96	4.02	12	12	12	100%
Positive B	*Plesiomonas shigelloides*	28.3	0.67	2.35	12	12	12	100%
	Vibrio cholerae	24.4	0.53	2.16	12	12	12	100%
	Vibrio parahaemolyticus	25.0	0.64	2.57	12	12	12	100%
	Giardia lamblia	24.6	0.65	2.65	12	12	12	100%
	Adenovirus F40/F41	24.8	0.48	1.93	12	12	12	100%
	Enteroaggregative *E. coli*	25.1	0.52	2.09	12	12	12	100%
	Enteropathogenic *E. coli*	25.2	0.45	1.77	12	12	12	100%
	Shigella/Enteroinvasive E. coli	24.9	0.52	2.10	12	12	12	100%
	Yersinia enterocolitica	25.6	0.35	1.36	12	12	12	100%
	Cyclospora cayetanensis	25.0	0.42	1.69	12	12	12	100%

Table 6. Inter Lot Precision (Reproducibility) of the QIAstat-Dx GI 2 Control Panel

Three manufactured lots of QIAstat-Dx GI 2 Positive A and Positive B controls were tested across two sites (n=92) using 4 different QIAstat-Dx GI 2 cartridge lots across different instruments. All controls showed 100% correct detection and all targets resulted in Cycle Thresholds (Cts) with %CV's of less than 6%.

QIAstat-Dx GI 2 Control Panel Internal and External Site Testing Data		Average Ct	SD	%CV	No. Samples Tested	No. Valid Tests	No. Correct Results	Percent Correct Results
Positive A	Astrovirus	24.8	1.32	5.30	92	92	92	100%
	Rotavirus A	25.9	1.29	4.99	92	92	92	100%
	Norovirus GI/GII	26.0	1.08	4.16	92	92	92	100%
	Sapovirus	25.5	0.95	3.71	92	92	92	100%
	Vibrio vulnificus	25.3	1.02	4.01	92	92	92	100%
	Cryptosporidium	24.9	0.71	2.84	92	92	92	100%
	Shiga-like toxin *E. coli*	25.2	0.64	2.53	92	92	92	100%
	Campylobacter	25.6	0.75	2.92	92	92	92	100%
	Clostridium difficile toxin A/B	23.9	1.09	4.56	92	92	92	100%
	E. coli O157	24.7	1.00	4.04	92	92	92	100%
	Enterotoxigenic *E. coli* lt/st	24.4	0.79	3.24	92	92	92	100%
	Salmonella	24.5	0.72	2.94	92	92	92	100%
	Entamoeba histolytica	24.8	0.79	3.16	92	92	92	100%
Positive B	*Plesiomonas shigelloides*	29.0	0.63	2.16	92	92	92	100%
	Vibrio cholerae	26.1	1.07	4.08	92	92	92	100%
	Vibrio parahaemolyticus	26.3	0.84	3.20	92	92	92	100%
	Giardia lamblia	25.8	0.69	2.67	92	92	92	100%
	Adenovirus F40/F41	26.3	0.97	3.70	92	92	92	100%
	Enteroaggregative *E. coli*	26.4	0.81	3.07	92	92	92	100%
	Enteropathogenic *E. coli*	26.3	0.72	2.72	92	92	92	100%
	Shigella/Enteroinvasive E. coli	26.5	0.98	3.70	92	92	92	100%
	Yersinia enterocolitica	26.7	0.68	2.53	92	92	92	100%
	Cyclospora cayetanensis	26.2	0.77	2.95	92	92	92	100%

Results

The QIAstat-Dx GI 2 Control Panel, which includes two positive controls and a negative control, has been validated across multiple cartridge lots at 2 site locations which utilized different QIAstat-Dx® Analyzers. The QIAstat-Dx GI 2 Control Panel had a 100% successful testing rate across all samples (n=102), showing both positive controls with 100% concordant calls across all runs (n=92) with zero failures (Table 2). Similarly, all negative controls (n=10) resulted in negative results for all analytes detected within the QIAstat-Dx GI 2 Panel with zero failing tests. All positive control tests showed Cts within the expected range for each analyte on the QIAstat-Dx GI 2 Panel, and had coefficient of variation values ≤ 5.30% (Table 6).

Conclusion

- QIAstat-Dx GI 2 Control Panel provides the ability to monitor the accurate detection of all viral, bacterial, and parasitic, pathogens detected on the QIAstat-Dx GI 2 Panel using a total of 2 positive samples.

- QIAstat-Dx GI 2 Control Panel demonstrates a robust performance across multiple control lots, cartridge lots, instruments and operators.

- MMQCI's multiplex external control covering all targets within the QIAstat-Dx GI 2 Panel offers a simple, ready to use, non infectious, robust solution to monitor a comprehensive, integrated molecular assay that can be used in any laboratory.

Acknowledgements