Validation of a Multiplexed, External Control Panel to Monitor the Performance of a Multiplexed Gastrointestinal Pathogen Nucleic Acid Test

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Introduction

Gastrointestinal infections are a significant global health issue caused by a variety of parasites, bacteria, and viruses. Rapid and accurate identification of these agents improves time to diagnosis and treatment decisions. Instruments that perform qualitative nucleic acid testing, including the QIAGEN QIAstat-Dx® Analyzer and Rise, improve rapid detection; however, CLIA guidelines dictate that all clinical tests must be monitored to identify shifts, trends, and random errors due to variations in the test system. To that extent, MMQCI developed and validated a multiplexed control panel to monitor the detection of all viral, bacterial, and parasitic pathogens on the QIAstat-Dx GI Panel 2 assay.

Materials and Methods

The QIAstat-Dx GI 2 Control Panel is a multiplex molecular quality control containing in silico designed genome segments of all GI pathogens detected by the QIAstat-Dx GI Panel 2 assay. All segments were ligated into engineered vectors to create stable frozen clones. *In vitro* DNA plasmids and RNA transcripts were generated, quantified, and formulated into a proprietary matrix to stabilize and carry the nucleic acids throughout the entire testing process. Precision studies were conducted to demonstrate reproducibility across multiple reagents and operators. Shipping, Matrix, and stability studies were conducted to confirm robustness and reliability.

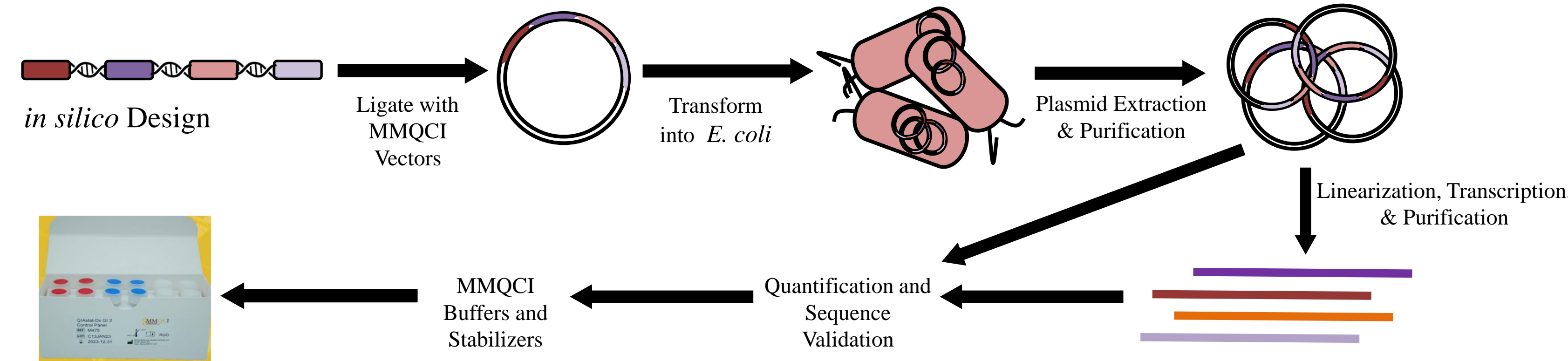Figure 1. Design Strategy for QIAstat-Dx GI 2 Control Panel

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 Reproducibility Study
A total of 181 tests of QIAstat-Dx GI 2 Control Panel were performed at MMQCI, utilizing 3 manufactured lots of QIAstat-Dx GI 2 Positive A and B controls, and 3 manufactured lots of QIAstat-Dx GI 2 Negative Controls, across 5 different QIAstat-Dx GI Panel 2 cartridge lots. Concordant calls were reported in all but one test, for an overall correct call rate of 99.4%.

Total Tests	Total Invalid Tests	Correct Positive Control Results	Incorrect Positive Control Results	% Correct Positive Control Results	Correct Negative Control Results	Incorrect Negative Control Results	% Correct Negative Control Results	Total % Correct
181	0	120	1	99.2%	60	0	100%	99.4%

Validation of QIAstat-Dx GI 2 Control Panel

Table 3. Inter Lot Precision (Reproducibility) of the QIAstat-Dx GI 2 Control Panel
3 manufactured lots of QIAstat-Dx GI 2 Positive A, Positive B, and Negative controls were tested at MMQCI (n=181) using 5 QIAstat-Dx GI 2 cartridge lots. The QIAstat-Dx GI 2 Positive A and Negative controls showed 100% correct calls. One sample of QIAstat-Dx GI 2 Positive B gave false negative calls (Not Detected) for 4 targets resulting in a 95.2% correct call rate for that lot and an overall 98.4% correct call rate across the three lots. This run was repeated and resulted in correct calls for all targets. All targets across all tests resulted in Cycle Thresholds (Cts) with %CV's of 2.8% or less.

Control	Control Lot #	No. of Tests	Invalid	Correct Results	Incorrect Results	Percent Correct
QIAstat-Dx GI 2 Positive A Control	B26OCT23A	20	0	20	0	100%
	F08NOV23A	20	0	20	0	100%
	E14NOV23A	20	0	20	0	100%
QIAstat-Dx GI 2 Positive B Control	M24JAN23A	20	0	20	0	100%
	C14FEB23A	21	0	20	1	95.2%
	B15NOV23A	20	0	20	0	100%
QIAstat-Dx GI 2 Negative Control	K10AUG22E	20	0	20	0	100%
	F16AUG22C	20	0	20	0	100%
	D10NOV23A	20	0	20	0	100%

Table 4. Summary of Within-Run Precision of the QIAstat-Dx GI 2 Control Panel (M527) at MMQCI
Within-run precision (repeatability) was demonstrated by 1 operator testing 1 lot of each component of the QIAstat-Dx GI 2 Control Panel (M475), using 1 lot of QIAstat-Dx GI Panel 2 cartridges on the QIAstat-Dx Analyzer 1.0 within one day at MMQCI. Each component resulted in 100% correct calls for all samples tested.

Control	Control Lot #	No. of Tests	Correct Results
QIAstat-Dx GI 2 Positive A Control	E14NOV23A	6	6/6
QIAstat-Dx GI 2 Positive B Control	B15NOV23A	6	6/6
QIAstat-Dx GI 2 Negative Control	D10NOV23A	6	6/6

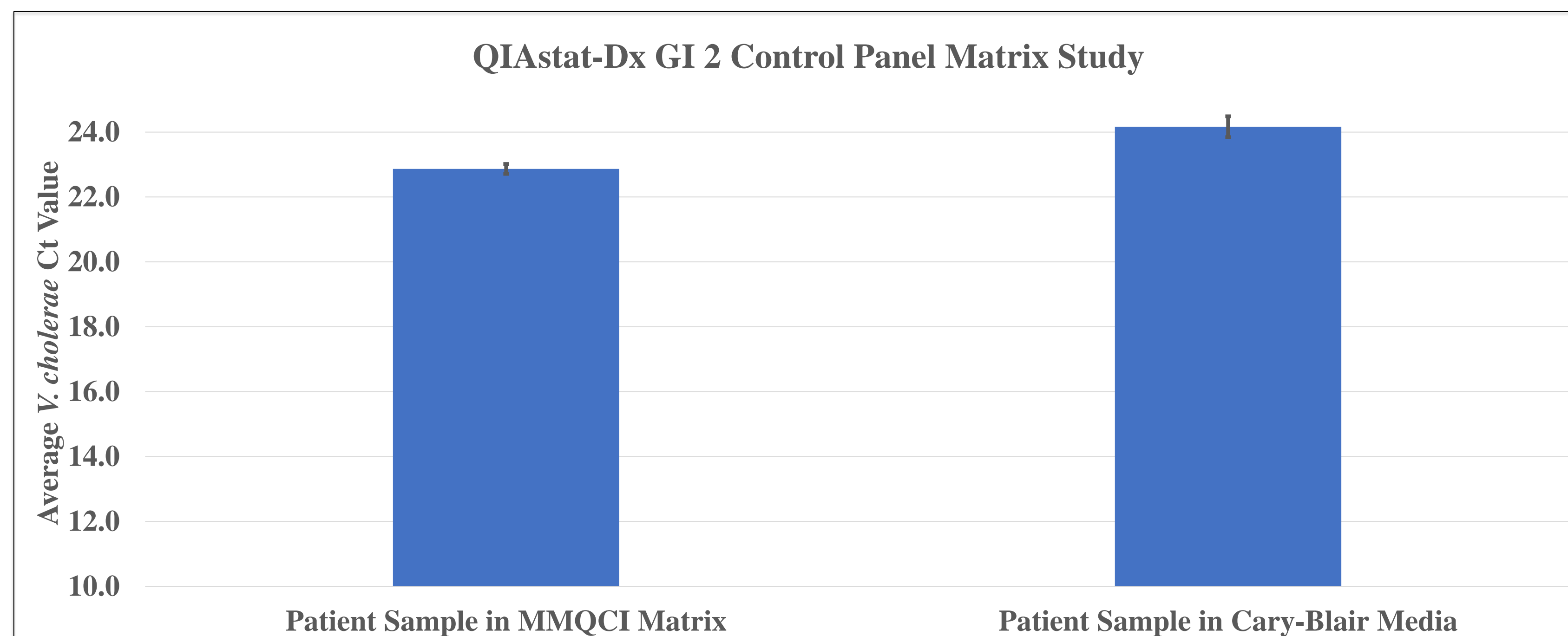QIAstat-Dx GI 2 Control Panel Matrix Study

Figure 1. MMQCI's Negative Matrix spiked with *V. cholerae* M045 gDNA (Cat#: ATCC 51394D-5) mimicked a simulated patient sample composed of Cary-Blair Media spiked with *V. cholerae* M045 gDNA (Cat#: ATCC 51394D-5). The simulated patient samples composed of *V. cholerae* M045 gDNA (Cat#: ATCC 51394D-5) diluted in Cary-Blair Media or Negative MMQCI Matrix underwent identical dilutions and were tested (n=3) using QIAstat-Dx GI Panel 2 cartridges on the QIAstat-Dx 1.0 Analyzer in order to determine any potential matrix effects.

Results

The QIAstat-Dx GI 2 Control Panel, consisting of two positive controls and one negative control, has undergone extensive precision, robustness, and performance studies using the QIAstat-Dx GI Panel 2 on the QIAstat-Dx 1.0 Analyzer as the testing method. Inter-lot precision studies demonstrated a greater than 99% accuracy when multiple lots of QIAstat-Dx GI 2 Control Panel were tested incorporating variability of QIAstat-Dx GI Panel 2 reagent lots, operators and testing days (Table 2 and 3). Intra-lot precision studies demonstrated that the QIAstat-Dx GI 2 Control Panel (M475) has 100% accuracy when testing variables are removed (Table 4). Matrix performance studies indicated that MMQCI's control matrix does not affect the performance of the assay compared to a patient sample in Cary-Blair transport media (Figure 1). Additionally, stability studies have indicated that the QIAstat-Dx GI 2 Control Panel (M475) is stable when stored frozen for 12+ months (Figures 2 and 3).
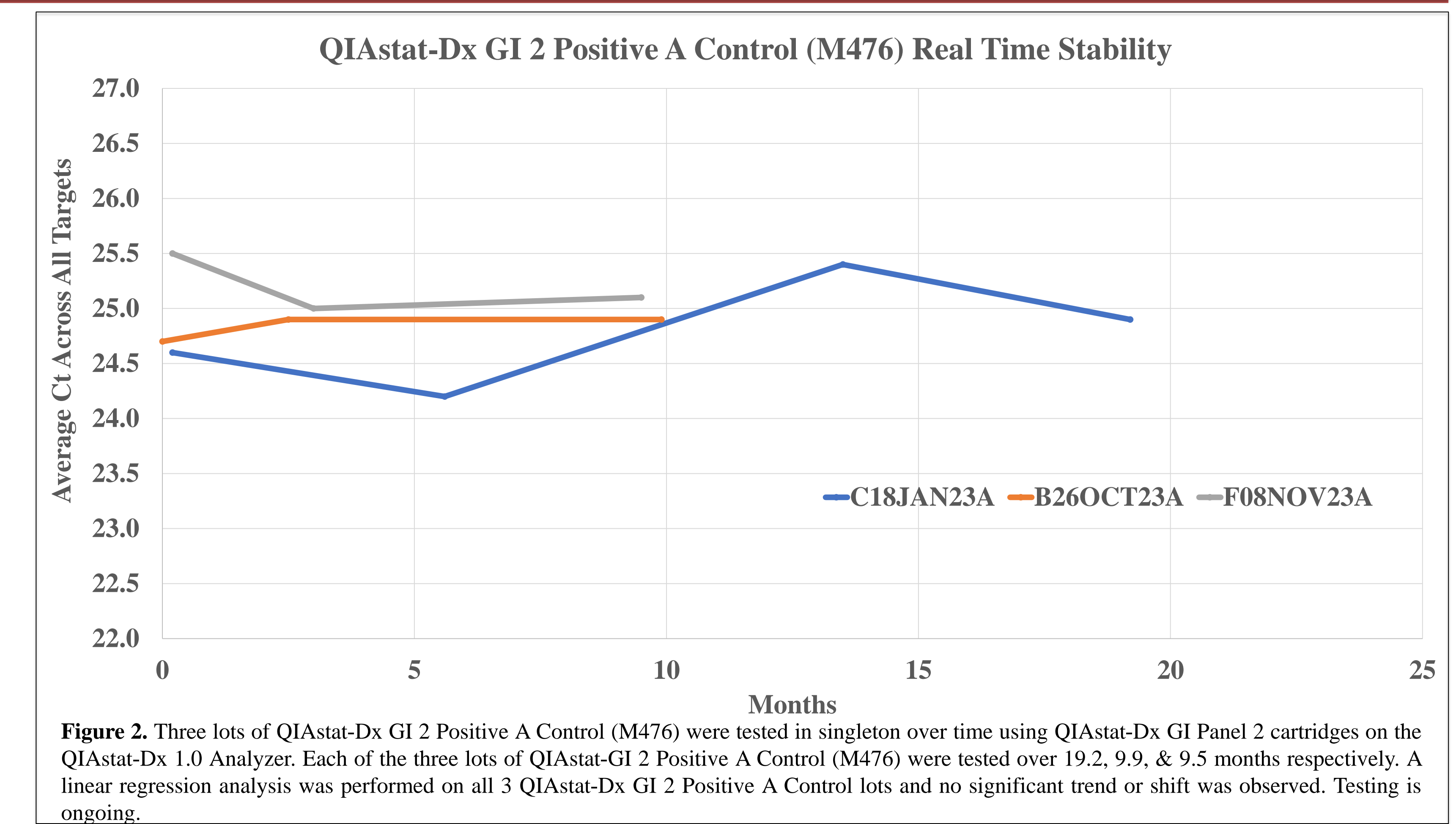

Figure 2. Three lots of QIAstat-Dx GI 2 Positive A Control (M476) were tested in singleton over time using QIAstat-Dx GI Panel 2 cartridges on the QIAstat-Dx 1.0 Analyzer. Each of the three lots of QIAstat-Dx GI 2 Positive A Control (M476) were tested over 19.2, 9.9, & 9.5 months respectively. A linear regression analysis was performed on all 3 QIAstat-Dx GI 2 Positive A Control lots and no significant trend or shift was observed. Testing is ongoing.
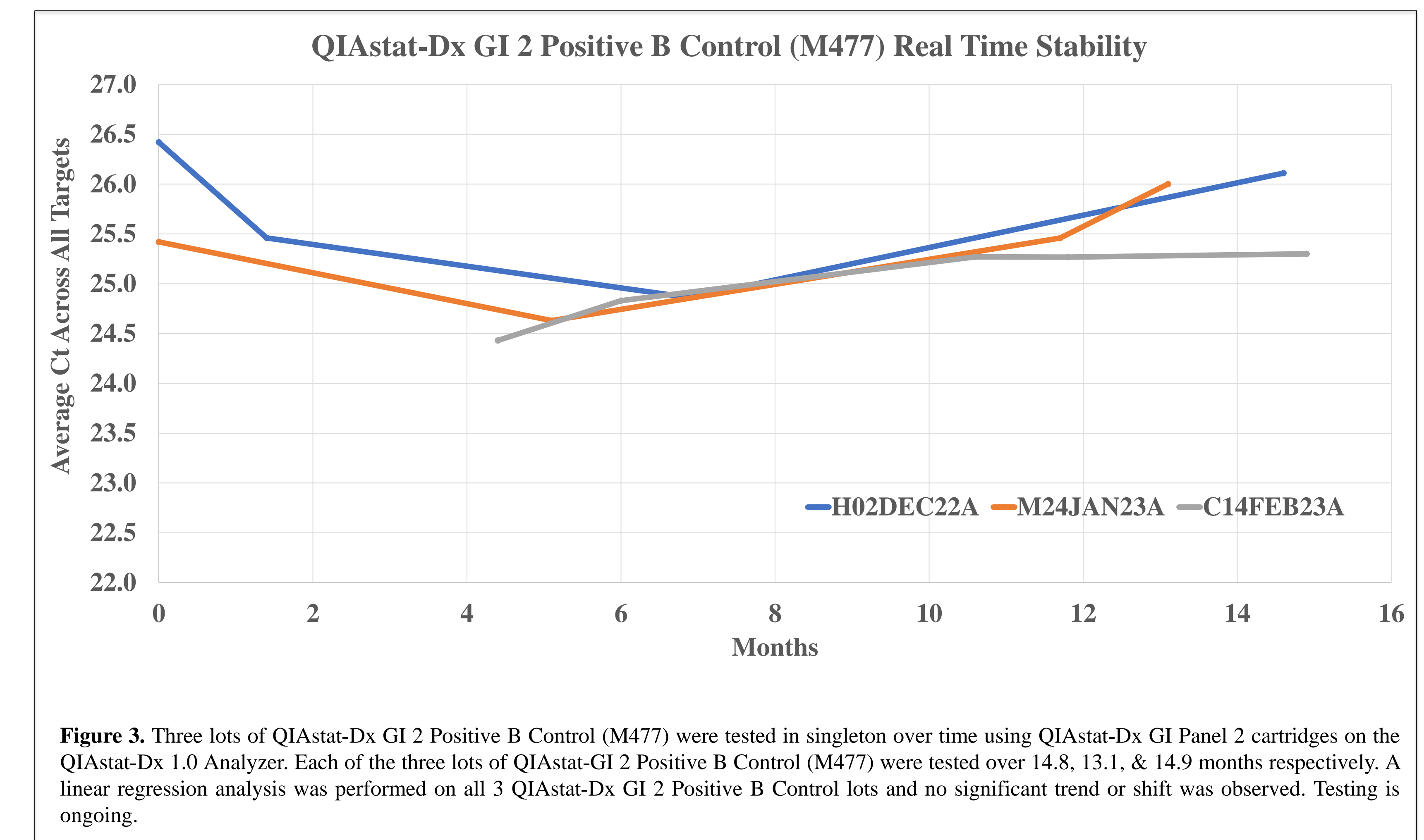

Figure 3. Three lots of QIAstat-Dx GI 2 Positive B Control (M477) were tested in singleton over time using QIAstat-Dx GI Panel 2 cartridges on the QIAstat-Dx 1.0 Analyzer. Each of the three lots of QIAstat-GI 2 Positive B Control (M477) were tested over 14.8, 13.1, & 14.9 months respectively. A linear regression analysis was performed on all 3 QIAstat-Dx GI 2 Positive B Control lots and no significant trend or shift was observed. Testing is ongoing.

Conclusion

• QIAstat-Dx GI 2 Control Panel provides the ability to accurately monitor the 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 and precise performance across multiple control lots, cartridge lots, instruments and operators.

• MMQCI's multiplex external control designed for use with the QIAstat-Dx GI 2 Panel offers a simple, ready to use, non infectious, robust solution to monitor a comprehensive, integrated molecular assay which will support patient gastrointestinal health worldwide.

Acknowledgments and References