



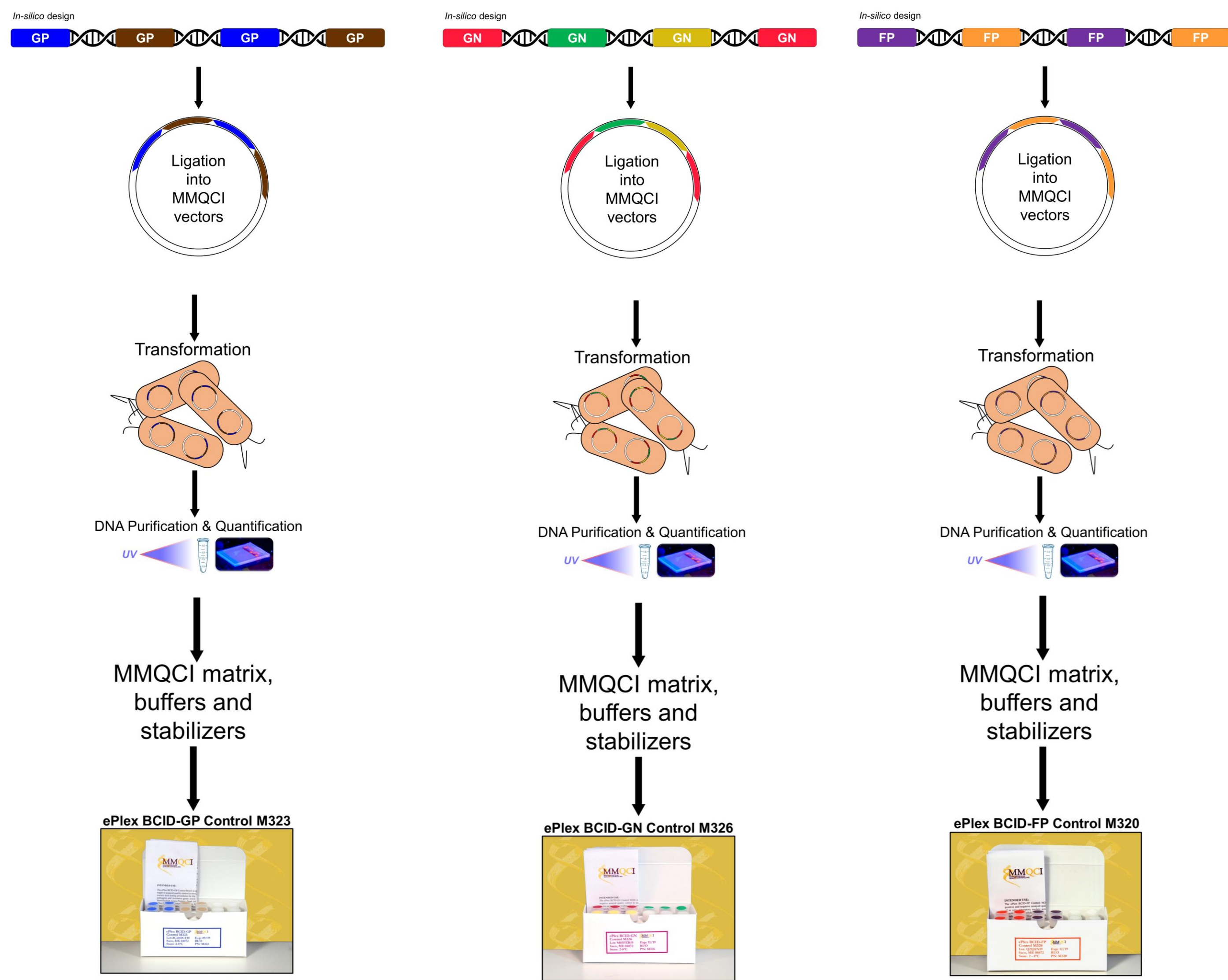
# Development of Synthetic Multiplexed External Controls for Monitoring the Performance of Qualitative Laboratory Nucleic Acid Testing Panels Used for Rapid Identification of Blood Culture Pathogens

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## Background

Bacterial and fungal blood infections that promote a hyperimmune response cause severe symptoms, including fever, pain, and tissue damage. If not treated quickly, this immune response can lead to a life-threatening condition called sepsis. Traditional blood culture methods including sub-culture isolations and antibiotic susceptibility testing take several days to obtain results. Rapid identification of bacterial pathogens and possible corresponding antibiotic resistance genes are critical to begin targeted treatment early. Reduced treatment time drastically diminishes the chance of lethality in septic patients.

Instruments that perform qualitative laboratory nucleic acid testing, such as GenMark's ePlex<sup>®</sup> system, aid in alleviating this issue; however, like any test in clinical use, their performance must be closely monitored to identify shifts, trends, and random errors caused by variations in the test system, such as failing reagents or operator errors. Maine Molecular Quality Controls Inc. (MMQCI) has developed a unique, extractable multiplex control panel designed to monitor all pathogenic organisms and antibiotic resistance genes detected by GenMark's ePlex<sup>®</sup> Blood Culture Identification (BCID) Gram-positive (GP), Gram-negative (GN), and Fungal Pathogen (FP) Panels.



**Table 1a: ePlex BCID-GP Control M323** **Table 1b: ePlex BCID-GN Control M326** **Table 1c: ePlex BCID-FP Control M320**

Gram-positive pathogens	Gram-negative pathogens <sup>†</sup>	Fungal pathogens
<ul style="list-style-type: none"> <li>Bacillus cereus group*</li> <li>Bacillus subtilis group*</li> <li>Enterococcus*</li> <li>Enterococcus faecalis*</li> <li>Enterococcus faecium*</li> <li>Streptococcus</li> <li>Streptococcus agalactiae*</li> <li>Streptococcus anginosus group*</li> <li>Streptococcus pneumoniae*</li> <li>Streptococcus pyogenes</li> <li>Staphylococcus*</li> <li>Staphylococcus aureus*</li> <li>Staphylococcus epidermidis*</li> <li>Staphylococcus lugdunensis*</li> <li>Cutibacterium acnes (P. acnes)</li> <li>Corynebacterium</li> <li>Lactobacillus</li> <li>Listeria</li> <li>Listeria monocytogenes</li> <li>Micrococcus</li> </ul>	<ul style="list-style-type: none"> <li>Acinetobacter baumannii</li> <li>Bacteroides fragilis</li> <li>Cronobacter sakazakii</li> <li>Enterobacter (Non-cloacae complex)</li> <li>Enterobacter cloacae complex</li> <li>Fusobacterium nucleatum</li> <li>Klebsiella pneumoniae</li> <li>Neisseria meningitidis</li> <li>Escherichia coli</li> <li>Fusobacterium necrophorum</li> <li>Proteus</li> <li>Pseudomonas aeruginosa</li> <li>Citrobacter</li> <li>Haemophilus influenzae</li> <li>Klebsiella oxytoca</li> <li>Salmonella</li> <li>Serratia</li> <li>Serratia marcescens</li> <li>Morganella morganii</li> <li>Proteus mirabilis</li> <li>Stenotrophomonas maltophilia</li> </ul>	<ul style="list-style-type: none"> <li>Candida albicans</li> <li>Candida glabrata</li> <li>Candida krusei</li> <li>Candida parapsilosis</li> <li>Candida dubliniensis</li> <li>Candida famata</li> <li>Candida kefyr</li> <li>Candida auris</li> <li>Candida guilliermondii</li> <li>Candida lusitanae</li> <li>Candida tropicalis</li> <li>Cryptococcus gattii</li> <li>Cryptococcus neoformans</li> <li>Fusarium</li> <li>Rhodotorula</li> </ul>
Resistance gene targets	Resistance gene targets	
<ul style="list-style-type: none"> <li>mecA</li> <li>mecC</li> <li>vanA</li> <li>vanB</li> </ul>	<ul style="list-style-type: none"> <li>OXA</li> <li>VIM</li> <li>CTX-M</li> <li>IMP</li> <li>KPC</li> <li>NDM</li> </ul>	
Pan-targets		
<ul style="list-style-type: none"> <li>Pan Gram-negative*</li> <li>Pan-Candida</li> </ul>		
Pan targets		
<ul style="list-style-type: none"> <li>Pan Gram-positive*</li> <li>Pan-Candida</li> </ul>		

## Materials and Methods

**Figure 1:** The synthetic, multiplex molecular controls contain genome segments of all Gram-positive, Gram-negative, and fungal pathogens, antimicrobial resistance markers, and Pan-targets that detected by the GenMark ePlex<sup>®</sup> BCID-GP, BCID-GN, and BCID-FP Panels. Genome segments were designed *in silico* to create several single pieces of synthetic DNA, ligated into MMQCI vectors, and transformed to create stable frozen clone stocks. DNA plasmids were purified, quantified by 260/280 UV spec, and formulated in MMQCI's proprietary matrix.

**Table 1: ePlex BCID pathogens and resistance assays**

**a) ePlex BCID-GP Control M323 covers all 26 assays in the ePlex BCID-GP Panel.** The ePlex BCID-GP Panel has 20 Gram-positive assays, 4 resistance gene assays, and 2 Pan-assays. The Pan Gram-negative assay covers all GN pathogens in the ePlex BCID-GN panel<sup>†</sup>. The Pan-Candida assay covers four common Candida species (**bold**).

**b) ePlex BCID-GN Control M326 covers all 29 assays in the ePlex BCID-GN Panel.** The ePlex BCID-GN Panel has 21 Gram-negative assays, 6 resistance gene assays, and 2 Pan-assays. The Pan Gram-positive assay covers 13 GP pathogens in the ePlex BCID-GP panel\*. The Pan-Candida assay also covers the same four Candida species (**bold**).

**c) ePlex BCID-FP Control M320 covers all 15 assays in the ePlex BCID-FP Panel.** The ePlex BCID-FP Panel consists of 11 Candida species, 2 Cryptococcus species, Fusarium, and Rhodotorula.

## Results

**Table 2a: ePlex BCID-GP Control M323 External Testing at GenMark**

ePlex BCID-GP Positive A (M324)	No. Samples Tested	No. Valid Tests	No. Samples Detected	Percent Samples Detected
Bacillus cereus group	79	77	77	100%
Bacillus subtilis group	79	77	77	100%
Corynebacterium	79	77	77	100%
Enterococcus	79	77	77	100%
Enterococcus faecalis	79	77	77	100%
Enterococcus faecium	79	77	77	100%
Streptococcus	79	77	77	100%
Streptococcus agalactiae	79	77	77	100%
Streptococcus anginosus group	79	77	77	100%
Streptococcus pneumoniae	79	77	77	100%
Streptococcus pyogenes	79	77	77	100%
vanA	79	77	77	100%
vanB	79	77	77	100%

**Table 2b: ePlex BCID-GP Control M323 Internal Testing at MMQCI**

ePlex BCID-GP Positive A (M324)	No. Samples Tested	No. Valid Tests	No. Samples Detected	Percent Samples Detected
Bacillus cereus group	42	38*	38	100%
Bacillus subtilis group	42	38*	37	97%
Corynebacterium	42	39	39	100%
Enterococcus	42	39	39	100%
Enterococcus faecalis	42	39	39	100%
Enterococcus faecium	42	39	39	100%
Streptococcus	42	39	39	100%
Streptococcus agalactiae	42	39	39	100%
Streptococcus anginosus group	42	39	39	100%
Streptococcus pneumoniae	42	39	39	100%
Streptococcus pyogenes	42	39	39	100%
vanA	42	39	39	100%
vanB	42	39	39	100%

**Table 2: Verification of ePlex BCID-GP Control M323 at GenMark (a) and MMQCI (b).** a) MMQCI's ePlex BCID-GP Control M323 was tested a total of 122 times using 5 ePlex cartridge lots and 2 MMQCI control lots. Testing demonstrated robust and accurate results with 100% concordance across all Gram-positive targets and resistance gene targets in each control tubes. The Pan Gram-negative assay showed 98% concordance (42/43 runs detected). b) MMQCI performed a total of 84 runs across both positive controls and 40 negative control runs. Concordance of 100% was seen across 25/26 targets. \*We removed 1 valid run from analysis that had cartridge-related performance issues causing false negatives in our control panel for *mecA*, *B. subtilis*, and *B. cereus*.

**Table 3a: ePlex BCID-GN Control M326 External Testing at GenMark**

ePlex BCID-GN Positive A (M327)	No. Samples Tested	No. Valid Tests	No. Samples Detected	Percent Samples Detected
Acinetobacter baumannii	100	82	82	100%
Bacteroides fragilis	100	82	82	100%
Cronobacter sakazakii	100	82	82	100%
Enterobacter (Non-cloacae complex)	100	82	82	100%
Enterobacter cloacae complex	100	82	82	100%
Fusobacterium nucleatum	100	82	82	100%
Klebsiella pneumoniae	100	82	82	100%
Neisseria meningitidis	100	82	82	100%
Salmonella	100	82	82	100%
Serratia (spp.)	100	82	82	100%
Serratia marcescens	100	82	82	100%
OXA	100	82	82	100%
VIM	100	82	82	100%

**Table 3b: ePlex BCID-GN Control M326 Internal Testing at MMQCI**

ePlex BCID-GN Positive A (M327)	No. Samples Tested	No. Valid Tests	No. Samples Detected	Percent Samples Detected
Acinetobacter baumannii	11	10	10	100%
Bacteroides fragilis	11	10	10	100%
Cronobacter sakazakii	11	10	10	100%
Enterobacter (Non-cloacae complex)	11	10	10	100%
Enterobacter cloacae complex	11	10	10	100%
Fusobacterium nucleatum	11	10	10	100%
Klebsiella pneumoniae	11	10	10	100%
Neisseria meningitidis	11	10	10	100%
Salmonella	11	10	10	100%
Serratia (spp.)	11	10	10	100%
Serratia marcescens	11	10	10	100%
OXA	11	10	10	100%
VIM	11	10	10	100%

**Table 3c: ePlex BCID-GN Control M326 Internal Testing at MMQCI**

ePlex BCID-GN Positive B (M328)	No. Samples Tested	No. Valid Tests	No. Samples Detected	Percent Samples Detected
Escherichia coli	12	10	10	100%
Fusobacterium necrophorum	12	10	10	100%
Proteus (spp.)	12	10	10	100%
Pseudomonas aeruginosa	12	10	10	100%
Pan Candida	12	10	10	100%
Pan Gram-positive	12	10	10	100%
CTX-M	12	10	10	100%

**Table 3: Verification of ePlex BCID-GN Control M326 at GenMark (a) and MMQCI (b).** a) MMQCI's ePlex BCID-GN Control M326 was tested a total of 298 times using 12 ePlex cartridge lots and 3 MMQCI control lots. M326 tested was highly reproducible and robust with 100% concordance across 19/21 Gram-negative targets and resistance gene targets in each control tube. \*\*Discordant calls for *F. necrophorum* were due to cartridge-related performance issues. *E. coli* showed 99% concordance (90/91 runs detected). b) MMQCI performed a total of 46 runs across all positive and negative control and achieved 100% concordance.

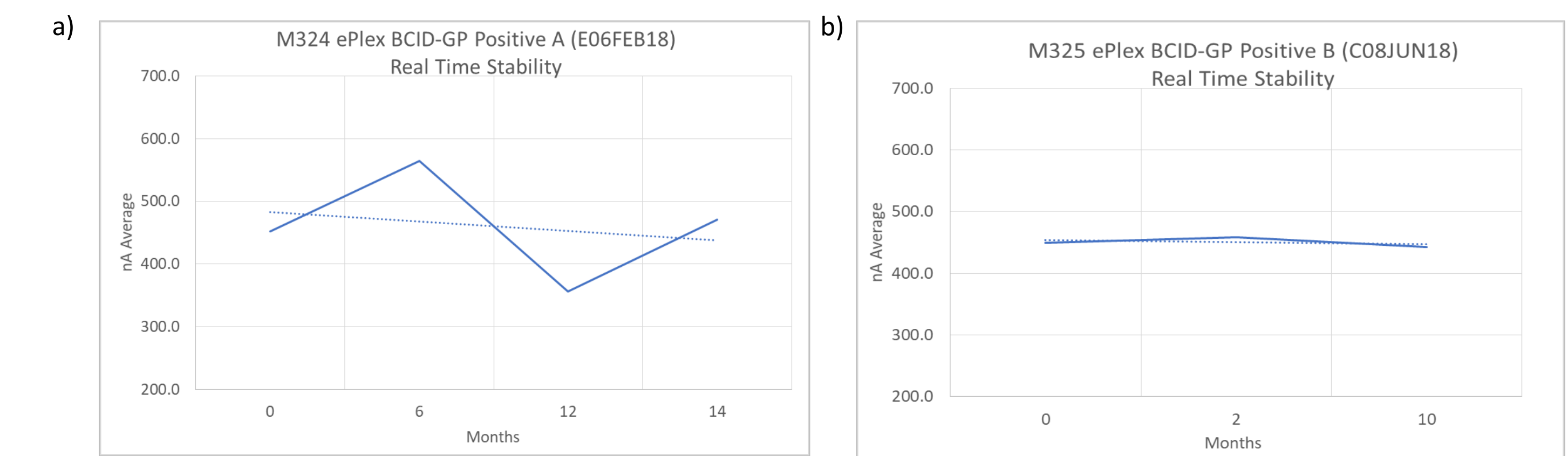
**Table 4a: ePlex BCID-FP Control M320 External Testing at GenMark**

ePlex BCID-FP Positive A (M321)	No. Samples Tested	No. Valid Tests	No. Samples Detected	Percent Samples Detected
Candida albicans	97	94	94	100%
Candida auris	97	94	94	100%
Candida guilliermondii	97	94	94	100%
Candida famata	97	94	94	100%
Candida glabrata	97	94	94	100%
Candida kefyr	97	94	94	100%
Cryptococcus gattii	97	94	94	100%

**Table 4b: ePlex BCID-FP Control M320 Internal Testing at MMQCI**

ePlex BCID-FP Positive A (M321)	No. Samples Tested	No. Valid Tests	No. Samples Detected	Percent Samples Detected
Candida albicans	13	12	12	100%
Candida dubliniensis	13	12	12	100%
Candida famata	13	12	12	100%
Candida glabrata	13	12	12	100%
Candida kefyr	13	12	12	100%
Cryptococcus gattii	13	12	12	100%

**Table 4: Verification of ePlex BCID-FP Control M320 at GenMark (a) and MMQCI (b).** a) The ePlex BCID-FP Control M320 was tested 222 times at GenMark and achieved 100% concordance across 14/15 targets. † The 2 false negatives seen for Rhodotorula occurred on the same cartridge lot; 1 false negative was due to an issue with an amplification pool. b) M320 testing at MMQCI showed 100% concordance across 14/15 targets. \*We observed 1 valid run that had multiple false negatives due to a bay/cartridge problem that was excluded from our analyses. The 1 Rhodotorula false negative was due to poor PCR pool amplification on that particular cartridge.



**Figure 2: Representative Real-time Functional Stability of ePlex BCID Products** a) BCID-GP Positive A (M324) was tested for real-time functional stability on the ePlex system over the course of 14 months. The graphs shows average nanoamp (nA) values across all targets, which is the signal generated by the ePlex instrument. b) BCID-GP Positive B (M325) was tested for real-time functional stability on the ePlex system over the course of 10 months. The graphs shows average nanoamp (nA) values across all targets, which is the signal generated by the ePlex instrument. Linear regression analysis shows no significant trending with significance of *F value* > 0.05. Testing is on-going.

## Conclusions

MMQCI's synthetic, multiplex controls are designed to be part of an essential clinical laboratory quality control program. The multiplex nature of the controls streamlines manufacture, thus making them affordable for clinical labs.

This study was performed using only GenMark RUO consumables. ePlex<sup>®</sup> In-vitro Diagnostic (IVD) material is expected to have higher validity upon launch.

MMQCI's proprietary matrix and stabilization buffers allow for stable, reliable controls that can be carried through the entire molecular diagnostic assay to accurately simulate all pathogens and corresponding resistance markers detected by GenMark's ePlex<sup>®</sup> Blood Culture Identification (BCID) Panels assay.

Controls performed robustly at both sites with ≥97% accuracy across all targets for all three panels.

MMQCI's ePlex BCID Controls are ready-to-use, non-infectious and well-characterized quality control panels for use in the clinical laboratory.

Current real time stability data indicate stability over 12 months, based on data from products with similar formulation, we predict stability of 24-months for all ePlex BCID controls.

Additional validation data resulted in a post-launch product improvement to M323.

## Acknowledgements

- ePlex<sup>®</sup> is a registered trademark of GenMark Diagnostics, Inc.
- Reagents for this study provided by GenMark Diagnostics, Inc. Carlsbad, CA