Development of Synthetic Multiplexed External Controls for Monitoring the Performance of a Qualitative Laboratory Nucleic Acid Testing Panel Used for Rapid Identification of Respiratory Pathogens

Joan Gordon[1], Daniel Magoon[1], Ivy Mead[1], Nizar Mohamed[2], Steve Nesbitt[1], Trinh Nguyen[1], Daren Osato[2], Clark Rundell[1], Astrid Schroeder[2], Tania Spenlinhauer[1] and Ken Wong[2]

[1]Maine Molecular Quality Controls Inc., Saco, ME., [2] GenMark Diagnostics, Inc., Carlsbad, CA.

Background

Acute respiratory infections represent a challenge for clinicians and a substantial burden to the healthcare system; therefore, rapid diagnosis is important to providing appropriate patient care. Advanced molecular diagnostic panels 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 (MMQCI) Inc. has developed a unique, extractable multiplex control panel consisting of one negative control and four positive controls, designed to monitor all viral and bacterial organisms detected by GenMark's ePlex® Research Use Only (RUO) Respiratory Pathogen Panel which is currently in development and subject to change.

Materials and Methods

The synthetic, multiplex molecular controls contain genome segments of all viral and bacterial pathogens detected by the GenMark ePlex® (RUO) Respiratory Pathogen Panel assay. The pathogens detected 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 and RNA transcripts were generated, quantified by 260/280 UV spec and formulated in MMQCI's proprietary matrix. The controls were optimized to a concentration close enough to LOD to provide sensitive monitoring yet not so close that false negatives would occur.
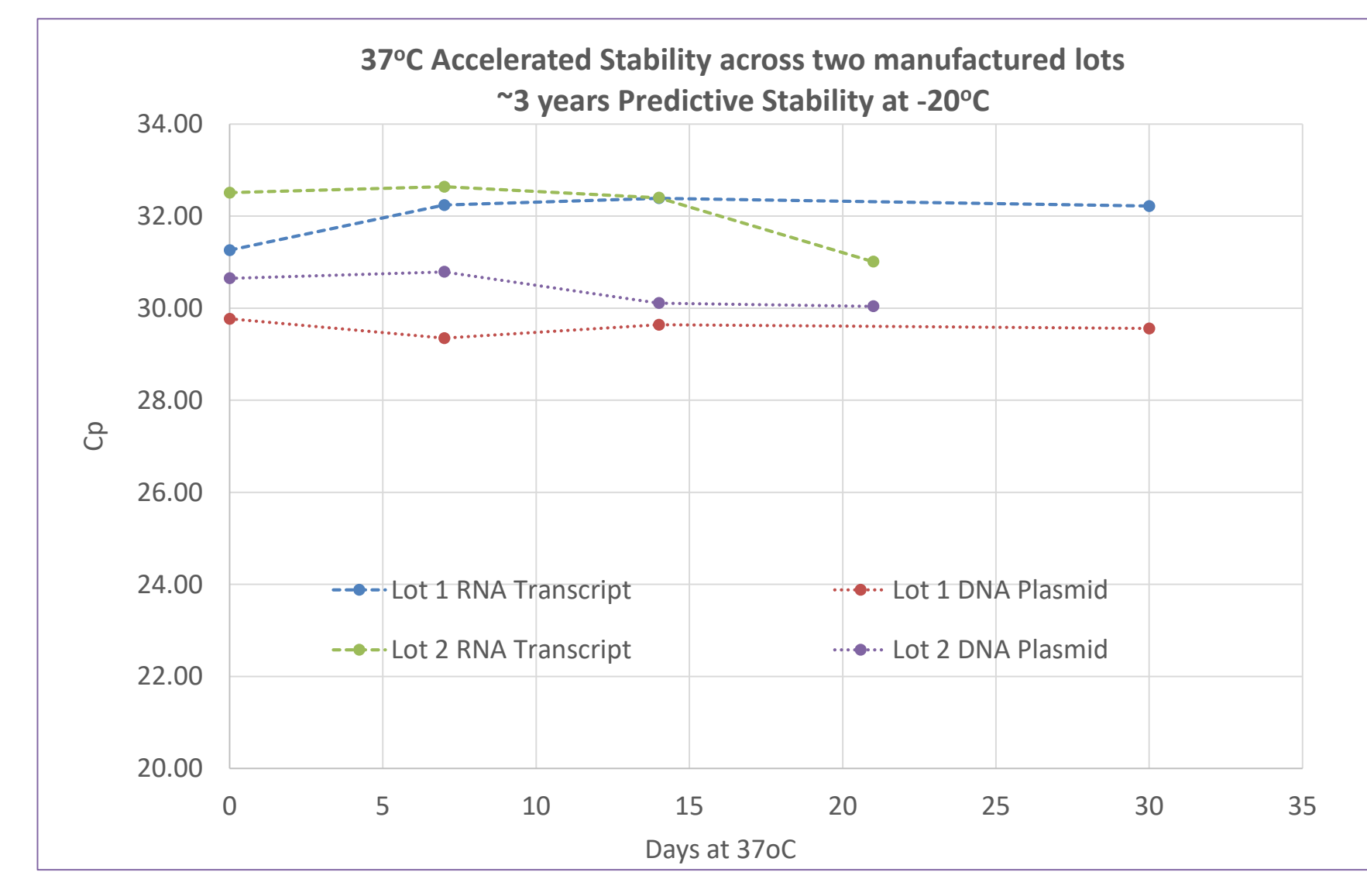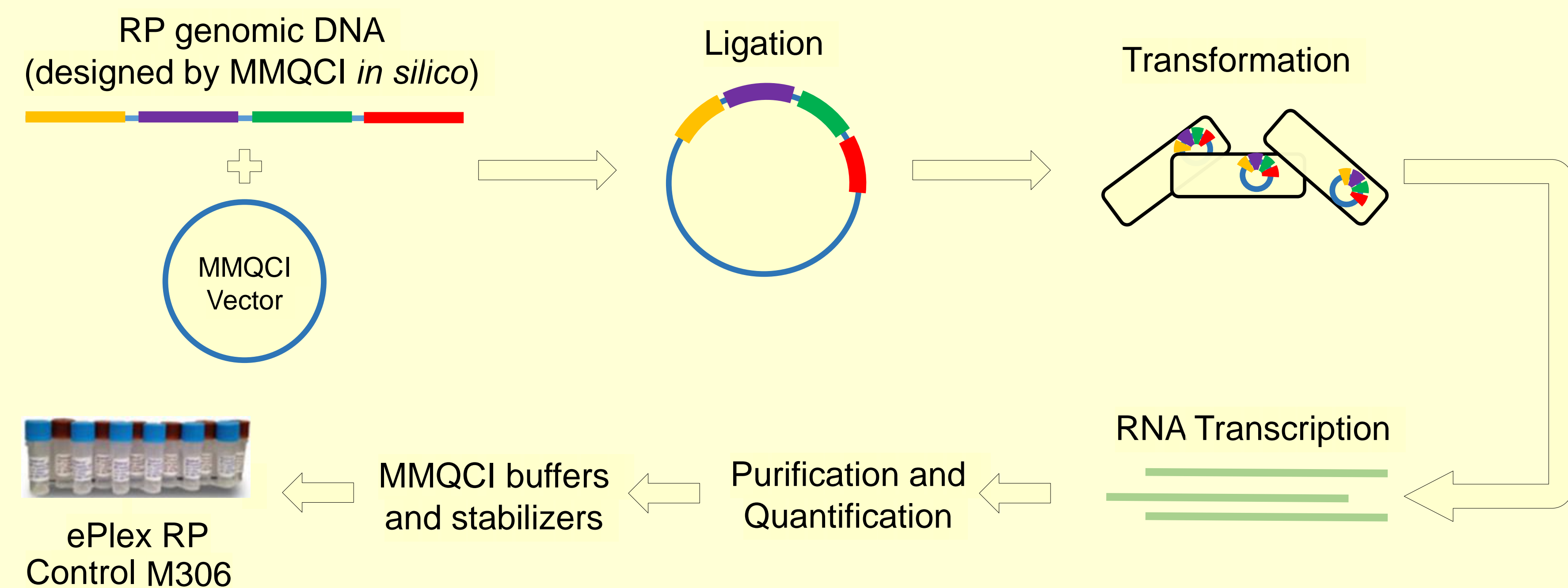

Figure 1: Accelerated stability at 37°C predict MMQCI controls containing buffers and stabilizers are stable for a 24 month period: MMQCI ePlex RP Control M306 was tested for accelerated stability using MMQCI in-house qPCR assays. Two lots of MMQCI ePlex RP Control M306 were stored at 37°C for over 21 days. Both the RNA transcript and DNA plasmid had Cp values with standard deviations less than 1 over the entire 21 day period. Using the Arrhenius temperature coefficient equation with a Q10 value of 2, this would be predictive of a ~3 year stability when stored at -20°C.
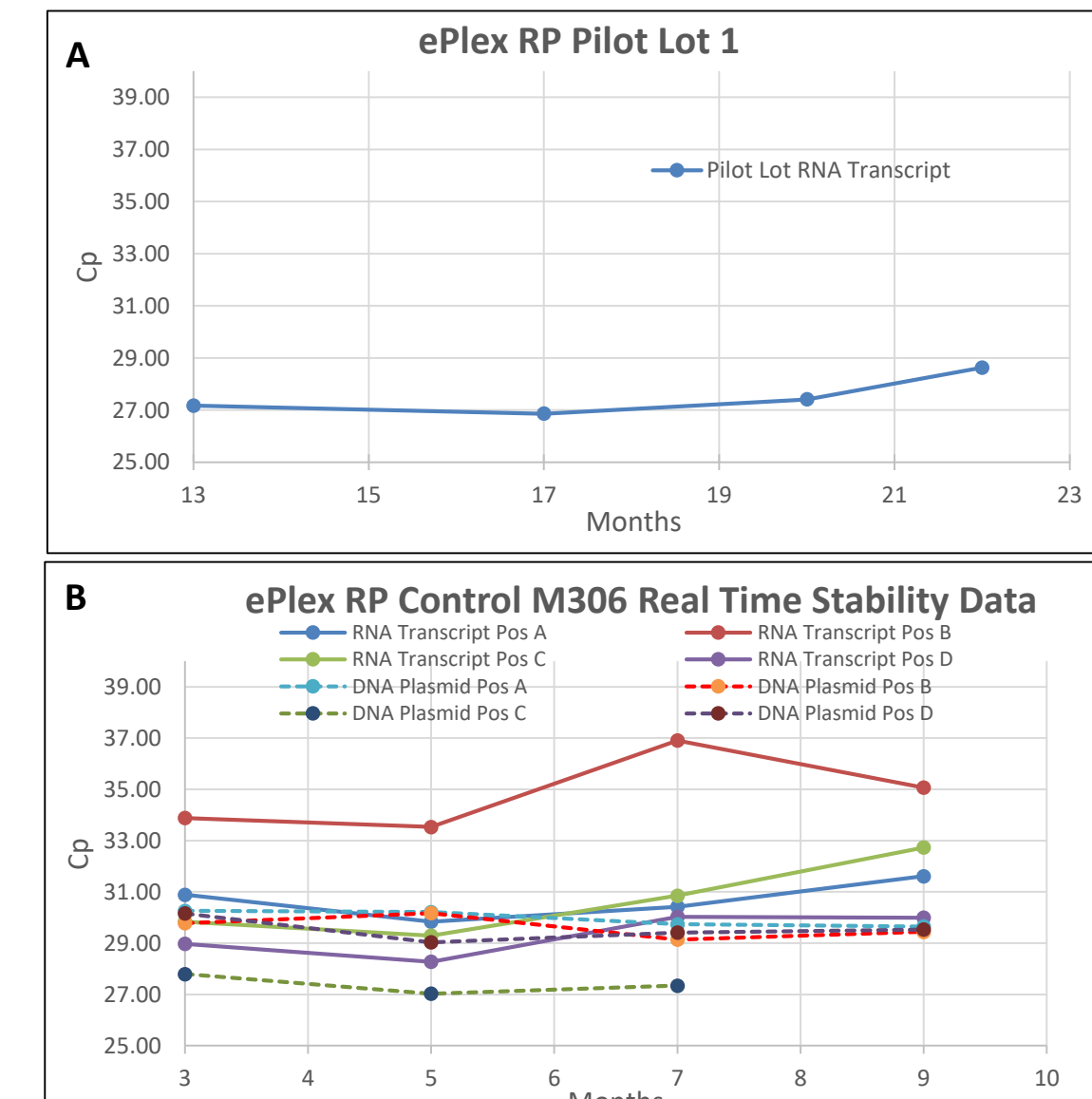

Figure 2: Real-time stability of MMQCI ePlex RP Control M306: Two lots of MMQCI ePlex RP controls were tested over a 22 and 9 month period using MMQCI in-house qPCR assays. Both the RNA transcript and DNA plasmid had Cp values with standard deviations less than 2 indicating controls are stable when stored at -20°C with no trending.

Table 1: ePlex RP Control M306

Viruses
- Adenoviruses
- Coronavirus NL63
- Coronavirus HKU1
- Coronavirus 229E
- Coronavirus OC43
- MERS*
- Human Bocavirus*
- Human Metapneumovirus
- Human Rhinovirus/Enterovirus
- Respiratory Syncytial Virus A
- Respiratory Syncytial Virus B
- Influenza A
- Influenza A subtype H1
- Influenza A subtype H3
- Influenza A subtype H1-2009
- Influenza B
- Parainfluenza Virus 1
- Parainfluenza Virus 2
- Parainfluenza Virus 3
- Parainfluenza Virus 4

Bacteria
- *Bordetella pertussis*
- *Legionella pneumophila**
- *Mycoplasma pneumoniae*
- *Chlamydophila pneumoniae*

* Targets included on CE-IVD product which will not be included on the US ePlex RP panel currently in development.

Table 2: MMQCI ePlex RP Control M306 tested externally at GenMark: MMQCI controls tested on GenMark ePlex® Respiratory Panel showed 100% detection of all targets (n = 10) when tested by GenMark.

Table 3: MMQCI ePlex RP Control M306 tested internally at MMQCI: MMQCI controls tested on GenMark ePlex® Respiratory Panel showed 100% detection of all targets (n = 6) when test by MMQCI.

Table 2. ePlex RP Control M306 Tested Externally at GenMark

ePlex Positive A	No. Samples Tested	No. Valid Tests	No. Samples Detected	Percent Samples Detected
Adenovirus	10	10	10	100%
Coronavirus NL63	10	10	10	100%
Influenza A	10	10	10	100%
Influenza A H3	10	10	10	100%
Parainfluenza Virus 4	10	10	10	100%
Respiratory Syncytial Virus A	10	10	10	100%
Chlamydophila pneumoniae	10	10	10	100%
ePlex Positive B	No. Samples Tested	No. Valid Tests	No. Samples Detected	Percent Samples Detected
Adenovirus	10	10	10	100%
Coronavirus HKU1	10	10	10	100%
Human Metapneumovirus	10	10	10	100%
Influenza A 2009 H1N1	10	10	10	100%
Parainfluenza Virus 2	10	10	10	100%
Respiratory Syncytial Virus B	10	10	10	100%
Legionella pneumophila	10	10	10	100%
ePlex Positive C	No. Samples Tested	No. Valid Tests	No. Samples Detected	Percent Samples Detected
Adenovirus	10	9	9	100%
Coronavirus 229E	10	9	9	100%
Influenza A H1	10	9	9	100%
Parainfluenza Virus 1	10	9	9	100%
Parainfluenza Virus 3	10	9	9	100%
Bordetella pertussis	10	9	9	100%
ePlex Positive D	No. Samples Tested	No. Valid Tests	No. Samples Detected	Percent Samples Detected
Coronavirus OC43	10	8	8	100%
MERS	10	8	8	100%
Human Bocavirus	10	8	8	100%
Human Rhinovirus/Enterovirus	10	8	8	100%
Influenza B	10	8	8	100%
Mycoplasma pneumoniae	10	8	8	100%
ePlex Negative	No. Samples Tested	No. Valid Tests	No. Samples Detected	Percent Samples Detected
No Targets	10	9	0	100%

Table 3. ePlex RP Control M306 Tested Internally at MMQCI

ePlex Positive A	No. Samples Tested	No. Valid Test	No. Samples Detected	Percent Samples Detected
Adenovirus	6	6	6	100%
Coronavirus NL63	6	6	6	100%
Influenza A	6	6	6	100%
Influenza A H3	6	6	6	100%
Parainfluenza Virus 4	6	6	6	100%
Respiratory Syncytial Virus A	6	6	6	100%
Chlamydophila pneumoniae	6	6	6	100%
ePlex Positive B	No. Samples Tested	No. Valid Test	No. Samples Detected	Percent Samples Detected
Adenovirus	6	6	6	100%
Coronavirus HKU1	6	6	6	100%
Human Metapneumovirus	6	6	6	100%
Influenza A 2009 H1N1	6	6	6	100%
Parainfluenza Virus 2	6	6	6	100%
Respiratory Syncytial Virus B	6	6	6	100%
Legionella pneumophila	6	6	6	100%
ePlex Positive C	No. Samples Tested	No. Valid Test	No. Samples Detected	Percent Samples Detected
Adenovirus	6	6	6	100%
Coronavirus 229E	6	6	6	100%
Influenza A H1	6	6	6	100%
Parainfluenza Virus 1	6	6	6	100%
Parainfluenza Virus 3	6	6	6	100%
Bordetella pertussis	6	6	6	100%
ePlex Positive D	No. Samples Tested	No. Valid Test	No. Samples Detected	Percent Samples Detected
Coronavirus OC43	6	6	6	100%
MERS	6	6	6	100%
Human Bocavirus	6	6	6	100%
Human Rhinovirus/Enterovirus	6	6	6	100%
Influenza B	6	6	6	100%
Mycoplasma pneumoniae	6	6	6	100%
ePlex Negative	No. Samples Tested	No. Valid Test	No. Samples Detected	Percent Samples Detected
No Targets	9	9	0	100%

Table 4 . ePlex RP Control M306 Clinical Study Data

ePlex Positive A	No. Samples Tested	No. Valid Test	No. Samples Detected	Percent Samples Detected
Adenovirus	27	27	27	100%
Coronavirus NL63	27	27	27	100%
Influenza A	27	27	27	100%
Influenza A H3	27	27	27	100%
Parainfluenza Virus 4	27	27	27	100%
Respiratory Syncytial Virus A	27	27	27	100%
Chlamydophila pneumoniae	27	27	27	100%
ePlex Positive B	No. Samples Tested	No. Valid Test	No. Samples Detected	Percent Samples Detected
Adenovirus	29	29	29	100%
Coronavirus HKU1	29	29	29	100%
Human Metapneumovirus	29	29	29	100%
Influenza A 2009 H1N1	29	29	29	100%
Parainfluenza Virus 2	29	29	29	100%
Respiratory Syncytial Virus B	29	29	29	100%
Legionella pneumophila	29	29	29	100%
ePlex Positive C	No. Samples Tested	No. Valid Test	No. Samples Detected	Percent Samples Detected
Adenovirus	28	28	28	100%
Coronavirus 229E	28	28	28	100%
Influenza A H1	28	28	28	100%
Parainfluenza Virus 1	28	28	28	100%
Parainfluenza Virus 3	28	28	28	100%
Bordetella pertussis	28	28	28	100%
ePlex Positive D	No. Samples Tested	No. Valid Test	No. Samples Detected	Percent Samples Detected
Coronavirus OC43	25	25	25	100%
MERS	25	25	25	100%
Human Bocavirus	25	25	24	96%
Human Rhinovirus/Enterovirus	25	25	25	100%
Influenza B	25	25	25	100%
Mycoplasma pneumoniae	25	25	25	100%

Table 4: A manufacture lot of ePlex RP Control M306 was tested across 6 clinical sites on multiple cartridge lots. 100% concordance of positive calls was seen for three of the positive controls (ePlex Positive A, ePlex Positive B and ePlex Positive C) and 96% concordance for positive calls for one positive control (ePlex Positive D).

Table 5. Summary of each control component for Clinical Study Test Results

	Total test	No. invalid	Correct Positive Control Result	Incorrect Positive Control Result	Percent Correct Positive Control	Correct Negative Control Result	Incorrect Negative Control Result	Percent Correct Negative Control
ePlex Positive A	27	0	27	0	100%	26	1	96%
ePlex Positive B	29	0	29	0	100%	28	1	97%
ePlex Positive C	28	0	28	0	100%	28	0	100%
ePlex Positive D	25	0	24	1	96%	25	0	100%
ePlex Negative	109	0	NA	NA	NA	104	5	95%
Total	**218**	**0**	**108**	**1**	**99%**	**211**	**7**	**97%**

Table 6. Summary of all Clinical Study Test Results

	Total test	No. invalid	Total Correct Control Result	Total Incorrect Control Result	Percent Correct Control Result
ePlex Negative Control	109	0	104	5	95%
ePlex Positive Control	109	0	106	3	97%

Tables 5 & 6: 218 external controls were tested across 6 clinical sites. There was a single false negative for all positive controls and 7 false positives across all positive and negative controls (Table 5). A total of 109 positive controls and 109 negative controls were tested and resulted in 97% concordance for all positive controls, and 95% concordance for all negative controls (Table 6).

Results Summary

🔖 The single false negative result indicates a concentration below the limit of detection. A small change in concentration has been made and further testing will be completed to confirm robust detection for this pathogen.

🔖 False Positives are most likely due to environmental contamination, which was resolved with additional training.

🔖 Preliminary stability studies across two ePlex RP Control M306 lots indicated robust performance for a minimum of 12 months. Stability studies are ongoing to extend shelf life.

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.

🔖 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 detected by GenMark's ePlex® (RUO) Respiratory Pathogen Panel assay.

🔖 Controls performed robustly at all sites with 99% accurate positive detection for all targets.

🔖 The ePlex RP Control M306 is a ready-to-use, non-infectious and well-characterized quality control panel for use in the clinical laboratory.