Validation of a multiplexed synthetic control panel to monitor the performance of simultaneous detection of multiple respiratory viral, bacterial pathogens, and antimicrobial resistance genes in lower respiratory infections

Session III
#35

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Introduction

Lower respiratory infections are one of the leading causes of illness and death in adults and children worldwide. Advanced molecular diagnostic tests facilitate rapid and accurate identification and detection of the organism. External controls assist in detection of analytical errors to ensure optimal performance of the testing system enabling paramount patient care. When used routinely, external quality controls are designed to assist in identifying shifts, trends, and random errors caused by variations in the test system, such as failing reagent lots and/or operator errors. Maine Molecular Quality Controls Inc (MMQCI) has developed a stable, multiplexed synthetic control to be used as an external quality control to simultaneously monitor all 26 pathogens (or 27 pathogens, when using the Pneumonia Panel *plus)* and 7 antibiotic resistance markers detected by BioFire® FilmArray® Pneumonia Panel and BioFire® FilmArray® Pneumonia Panel *plus* assays**.**

Materials and Methods

FilmArray Pneumonia/Pneumonia*plus* Control encompasses genome segments for all viral and bacterial pathogens, as well as, antibiotic resistance markers, detected by the BioFire Pneumonia Panel and BioFire Pneumonia Panel *plus* assays. All genome segments were designed *in silico* to create synthetic DNA segments, ligated into MMQCI vectors, and transformed to create stable frozen clone stocks. DNA plasmid and RNA transcripts were generated, quantified by 260/280 UV spec and formulated in MMQCI's proprietary matrix. Traceability of each target was established by performing bidirectional, quality-scored Sanger sequencing of all target sequences. Validation studies were conducted to demonstrate reproducibility across multiple sites and within-run precision. Performance studies, including failure mode, shipping delay and stability studies, were conducted to demonstrate robustness and reliability as an external control.

Figure 1. Design Strategy for FilmArray Pneumonia/Pneumonia*plus* Control

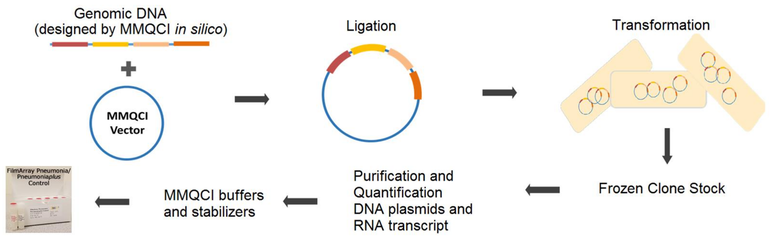

Table 1. FilmArray Pneumonia/Pneumonia*plus* Analytes

Table 1: BioFire Pneumonia and BioFire Pneumonia Panel *plus* Analytes		
Viruses		
• Adenovirus • Coronavirus • Human Metapneumovirus • Human Rhinovirus/Enterovirus	• Influenza A • Influenza B • Parainfluenza Virus • Respiratory Syncytial Virus	• Middle East Respiratory Syndrome Coronavirus* (Pneumonia Panel *plus* only)
Bacteria		
Quantitative Bacteria • *Acinetobacter calcoaceticus-baumannii* complex • *Enterobacter cloacae* complex • *Escherichia coli* • *Haemophilus influenzae* • *Klebsiella aerogenes* • *Klebsiella oxytoca* • *Klebsiella pneumoniae* group	• *Moraxella catarrhalis* • *Proteus spp.* • *Pseudomonas aeruginosa* • *Serratia marcescens* • *Staphylococcus aureus* • *Streptococcus agalactiae* • *Streptococcus pneumoniae* • *Streptococcus pyogenes*	*Qualitative Bacteria* • *Chlamydia pneumoniae* • *Legionella pneumophila* • *Mycoplasma pneumoniae*
Antimicrobial Resistance Genes		
Carbapenemases: • KPC • NDM • Oxa-48-like • VIM • IMP	Methicillin resistance: • *mec*A/C and MREJ	ESBL: • CTX-M

Results

Validation of FilmArray Pneumonia/Pneumonia*plus* Control

Table 2. Summary Table of Clinical and MMQCI Internal Data
A total of 308 tests of BioFire Pneumonia/Pneumonia *plus* Control (n=156 positive controls, n=152 negative controls) across 4 sites, correct results were reported in 300 tests on the first attempt. There were 2 negative controls with false positive results and 6 positive controls with false negative results. All produced correct results upon a single retest for an overall correct result rate of 97.4%.

Sites	Total Tests	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	Total Percent Correct
4	308	0	150	6	96.2%	150	2	98.7%	97.4%

Table 3. Internal Reproducibility Study
Internal reproducibility was performed at MMQCI, by testing 3 lots over 60 days across 3 operators on multiple instruments (n=63 positive control samples, n=60 negative control samples). All positive controls gave correct results except for 3 which gave false negative results. Repeat tests gave correct results on the first retest. All negative controls gave correct results. The overall correct result rate at MMQCI was 97.6%.

Control	Control Lot #	No. of Tests	Invalid	Correct Results	Incorrect Results	Percent Correct
FilmArray Pneumonia/Pneumonia*plus* Negative	E30APR18A	20	0	20	0	100%
FilmArray Pneumonia/Pneumonia*plus* Negative	C18MAY18A	20	0	20	0	100%
FilmArray Pneumonia/Pneumonia*plus* Negative	D30MAY18A	20	0	20	0	100%
FilmArray Pneumonia/Pneumonia*plus* Positive	D04MAY18A	21	0	20	1	95.2%
FilmArray Pneumonia/Pneumonia*plus* Positive	A29MAY18A	21	0	20	1	95.2%
FilmArray Pneumonia/Pneumonia*plus* Positive	C12JUN18A	21	0	20	1	95.2%
	TOTAL	***123***	***0***	***120***	***3***	***97.6%***

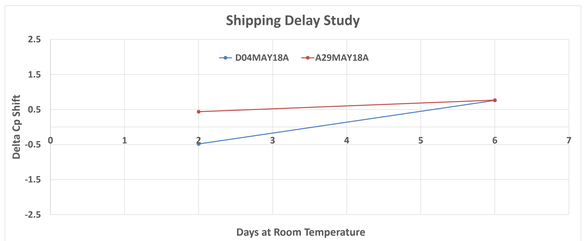

Figure 4: MMQCI Pneumonia/Pneumonia*plus* Positive lots D04MAY18A and A29MAY18A were placed on dry ice in MMQCI's standard shipping box, packaged according to MMQCI's standard protocol for 2 days at ambient temperature. To simulate a shipping delay, samples were tested at 2 and 6 days at ambient temperature after being stored on dry ice for 2 days. All FilmArray Pneumonia/Pneumonia*plus* Positive controls were stable after 2 days packaged in dry ice followed by 6 days at ambient temperatures of approximately 19-21°C.

Table 4. CLIA-certified External Clinical Site Results
FilmArray Pneumonia/Pneumonia*plus* Control performed reproducibly across 3 clinical sites using multiple pouch lots and incorporating multiple operators. Three positive controls and 3 negative controls were run per day/per site over a period of 10 days. Correct results were obtained on the first test of 175 controls. Two negative controls gave initial false positive results and 3 positive controls gave initial false negative results. All produced the correct results upon a single retest for an overall correct result rate of 97.3%.

Site	Total Tests	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	Total Percent Correct
1	61	0	30	0	100%	30	1	96.8%	60
2	63	0	30	2	93.8%	30	1	96.8%	60
3	61	0	30	1	96.8%	30	0	100%	60
All Sites	***185***	***0***	***90***	***3***	***96.8%***	***90***	***2***	***97.8%***	***97.3%***

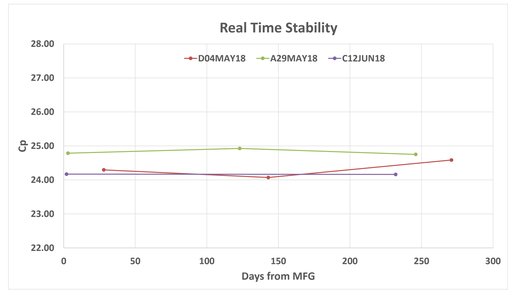

Figure 2: Three lots of FilmArray Pneumonia/Pneumonia*plus* Positive controls were tested over a 10 month period using MMQCI in-house qPCR assay. Linear regression was performed for all three lots and no significant trend or shift was observed. Testing is on-going.

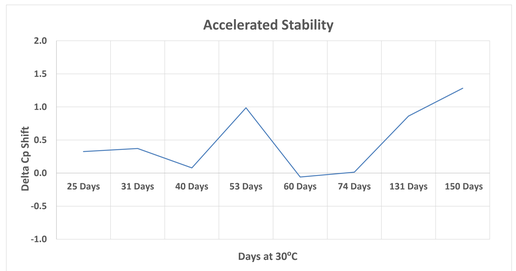

Figure 3: MMQCI Pneumonia/Pneumonia*plus* Positive lot C12JUN18A was tested for accelerated stability using FilmArray Pneumonia Panel *plus* assay. MMQCI control lot C12JUN18A was stored at 30°C for 131 days. Linear regression was performed and no significant trend or shift was observed. Using the Arrhenius temperature coefficient equation with a conservative Q10 value of 2, this would be predictive of a ~11 year stability when stored at -20°C.

Acknowledgements

- Reagents for this study were kindly provided by BioFire® Diagnostics, Salt Lake City, Utah.
- Patient Samples were kindly provided by NorDx, Scarborough, Maine.

Table 5. Summary of Within-run Precision at MMQCI
Within-run precision (repeatability) was demonstrated by 1 operator testing 1 lot each of FilmArray Pneumonia/Pneumonia*plus* Positive and FilmArray Pneumonia/Pneumonia*plus* Negative, with 1 lot of BioFire Pneumonia Panel *plus* pouches on one FilmArray 2.0, all within one day at MMQCI.

Control	Control Lot #	Number of Tests	Correct Results
FilmArray Pneumonia / Pneumonia*plus* Positive	D04MAY18A	6	6/6
FilmArray Pneumonia / Pneumonia*plus* Negative	E30APR18A	6	6/6

Table 6. Failure Mode of MMQCI Positive control and Patient Sample positive for *M. catarrhalis*. Failure modes tested, 1.) mimic poor hydration, ¼ volume of hydration buffer (n=3 for each sample), and 2.) mimic operator error, pouch inserted incorrectly on loading dock (n=3 for each sample). All runs performed under failure mode analysis were reported as invalids for both MMQCI and patient samples.

Analyte	Tested Correctly		Inadequate Hydration		Pouch inserted Incorrectly	
	M342	Patient Sample	M342	Patient Sample	M342	Patient Sample
Mcatarrhalis	Detected	Detected	Invalid	Invalid	Invalid	Invalid

Conclusions

- The FilmArray Pneumonia/Pneumonia*plus* Control performed with greater than 97% accuracy when tested with the FilmArray Pneumonia Panel assay.
- FilmArray Pneumonia/Pneumonia*plus* Control can be used as a reliable quality control with a current shelf life of 12 months when stored frozen, with preliminary studies indicating stability for over 24 months.
- MMQCI can reproducibly manufacture FilmArray Pneumonia/Pneumonia*plus* Control, to create a stable, robust control to be carried through the entire molecular diagnostic assay.
- FilmArray Pneumonia/Pneumonia*plus* Control has been submitted to FDA for review.