DEVELOPMENT OF A MULTIPLEXED EXTERNAL CONTROL FOR MONITORING THE PERFORMANCE OF QUALITATIVE LABORATORY NUCLEIC ACID TESTING PANELS USED FOR IDENTIFICATION OF RESPIRATORY PATHOGENS INCLUDING SARS-COV-2, FLU A/B AND RSV A/B

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Introduction

Influenza, RSV and SARS-CoV-2 are a significant burden on healthcare during the seasonal epidemics each year. Influenza viruses are a major cause of upper respiratory tract infections in older adults, while RSV is in young children. However, in this COVID-19 pandemic era; coinfection of influenza and COVID-19 is a major concern for public health authorities. Patients infected with SARS-CoV-2, Influenza A, Influenza B, or RSV have overlapping clinical presentations, but the approaches to treatment and management of each of these viruses are different.

Rapid, specific and sensitive test systems are important for medical decision making and prevention of transmission. Their performance must be closely monitored to identify shifts, trends, and random errors caused by the test system. A non-infectious synthetic control, manufactured precisely containing specific gene sequences for SARS-CoV-2, Flu A/B and RSV A/B can be used across multiple platforms, thus increasing the confidence and accuracy of patient results.

Materials and Methods

Several synthetic constructs containing multiple conservative gene sequences for SARS-CoV-2, Flu A/B, and RSV A/B detected by multiple assays were designed *in silico*, ligated and transformed to create stable frozen clone stocks. *In vitro* RNA transcripts were generated, quantified and formulated in a proprietary matrix to stabilize and carry the RNA through the entire test process, including extraction.

Figure 1. Design Strategy for Control

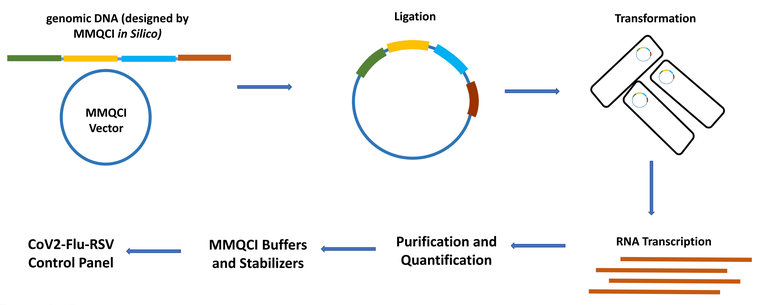

Table 1. Analytes

Table 1: MMQCI CoV2-Flu-RSV Control Panel Analytes	
Viruses	**Genes**
SARS-CoV-2	S gene (Spike Surface Glycoprotein)
	E gene (Small Envelope Protein)
	M gene (Matrix Protein)
	ORF7ab
	ORF8
	N gene (Nucleocapsid)
	ORF 10
	RdRP (ORF 1ab) (RNA-dependent RNA Polymerase)
Influenza A	PA gene (Polymerase Acidic Protein)
	M gene (Matrix Protein)
	PB2 gene (Polymerase Protein)
Influenza B	M gene (Matrix Protein)
	NS1 gene (Non-structural protein)
Respiratory Syncytial Virus A	N gene (Nucleocapsid)
	M gene (Matrix Protein)
	M2 gene (Matrix 2 Protein)
Respiratory Syncytial Virus B	N gene (Nucleocapsid)
	M gene (Matrix Protein)
	M2 gene (Matrix 2 Protein)

Results

Internal and External testing of CoV2-Flu-RSV Positive Control

Table 2. Summary Table of MMQCI Internal Testing Data on Xpert Xpress CoV-2/Flu/RSV *plus*

One lot of CoV2-Flu-RSV Positive Control was tested 10 times, on one reagent lot of Xpert Xpress CoV-2/Flu/RSV *plus* assay. Of the 10 controls tested, there was 1 failed run due to cartridge error which was repeated for a total of 10 controls with valid results and 100% correct results.

CoV2-Flu-RSV POS Control Lot# H09JUN23 testing data with Xpert Xpress CoV-2/Flu/RSV *plus* assay								
				Cycle threshold (Ct) value				
Run Date	**Sample ID**	**Reagent Lot #**	**Module**	**SARS-CoV-2**	**Flu A 1**	**Flu A 2**	**Flu B**	**RSV**
8/10/2023	H09JUN23	27712	A1	24.6	23.0	24.4	22.1	23.5
8/10/2023	H09JUN23	27712	A2	24.2	22.5	23.9	21.5	23.2
8/10/2023	H09JUN23	27712	A3	24.3	22.9	24.4	22.1	23.4
8/10/2023	H09JUN23	27712	A4	24.1	22.6	23.9	21.8	23.2
8/10/2023	H09JUN23	27712	B1	23.8	22.3	23.7	21.6	23.1
8/10/2023	H09JUN23	27712	B3	23.8	22.2	23.7	21.4	22.9
8/10/2023	H09JUN23	27712	B4	24.7	23.2	24.6	22.3	23.9
8/10/2023	H09JUN23	27712	C2	24.7	23	24.4	22.1	23.5
8/10/2023	H09JUN23	27712	C3	24.3	22.6	24.1	21.7	23.3
8/10/2023	H09JUN23	27712	A1	24.3	22.6	24.1	21.8	23.4
			Avg	**24.3**	**22.7**	**24.1**	**21.8**	**23.3**
			SD	**0.3**	**0.3**	**0.3**	**0.3**	**0.3**

Table 3. Summary Table of MMQCI Internal Testing Data on SARS-CoV-2 qPCR assay

Three lots of CoV2-Flu-RSV Positive Control were tested internally using an internal SARS-CoV-2 qPCR assay, extracted with QIAamp Viral Extraction kit, Reverse Transcription performed using ABI Taqman Reverse Transcription kit and run on the LC480 II which detects the SARS-CoV-2 N1 gene. 3 Positive control lots were tested in duplicate using the SARS-CoV-2 N1 qPCR. All positive lots had amplification in the expected range for the N1 assay. Testing demonstrated controls are consistent across lots for the gene target.

Internal testing data with SARS-CoV-2 qPCR assay			
		N1 Assay	
Product	**Lot**	**Result**	**Ct Value**
CoV2-Flu-RSV Pos control	B02MAR23	Positive	28.19
	B02MAR23	Positive	28.18
	H09JUN23	Positive	28.55
	H09JUN23	Positive	28.49
	K19JUL23	Positive	28.45
	K19JUL23	Positive	28.32

Table 4. Summary Table of ABBOTT ALINITY m SARS-COV-2 Data

Two lots of CoV2-Flu-RSV Positive Control were tested externally on one pouch lot of Alinity m SARS-CoV-2 assay with valid results and 100% correct calls.

External testing data with Alinity m SARS CoV-2 assay				
				Cycle Number (CN) Value
Assays	**Run Date**	**Reagent Lot #**	**Lot #**	**SARS-CoV-2**
Alinity m SARS-CoV-2 Assay	3/16/2023	A031023 /1	B02MAR23	20.43 CN (Positive)
Alinity m SARS-CoV-2 Assay	4/19/2023	A031023 /3	B30MAR23	20.49 CN (Positive)

Table 5. Summary Table of LDT qPCR assay at External Site

One lot of CoV2-Flu-RSV Positive Control was tested 10 times externally on three separate laboratory developed Real-Time qPCR assays, extracted with Thermo Scientific KingFisher Flex. Of the 10 controls tested, all had correct calls with valid results.

External testing data with Influenza A/B Real-Time PCR		
CoV2-Flu-RSV Pos control	Lot: B02MAR23	
Target: Flu A/B	**Result**	**Ct Value**
TUBE 1	POSITIVE FLU A	22.15
TUBE 2	POSITIVE FLU A	22.01
TUBE 3	POSITIVE FLU A	22.22
TUBE 4	POSITIVE FLU A	22.27
TUBE 5	POSITIVE FLU A	22.29
TUBE 6	POSITIVE FLU A	22.15
TUBE 7	POSITIVE FLU A	22.24
TUBE 8	POSITIVE FLU A	22.51
TUBE 9	POSITIVE FLU A	22.54
TUBE 10	POSITIVE FLU A	22.75
Flu A (+) Control	POSITIVE FLU A	26.31
TUBE 1	POSITIVE FLU B	21.29
TUBE 2	POSITIVE FLU B	21.39
TUBE 3	POSITIVE FLU B	21.26
TUBE 4	POSITIVE FLU B	21.20
TUBE 5	POSITIVE FLU B	21.21
TUBE 6	POSITIVE FLU B	21.44
TUBE 7	POSITIVE FLU B	21.49
TUBE 8	POSITIVE FLU B	21.41
TUBE 9	POSITIVE FLU B	21.47
TUBE 10	POSITIVE FLU B	21.59
Flu B (+) Control	POSITIVE FLU B	32.46

External testing data with SARS-CoV-2 Real-Time PCR		
CoV2-Flu-RSV Pos control	Lot: B02MAR23	
Target: COVID	**Result**	**Ct Value**
TUBE 1	POSITIVE	19.45
TUBE 2	POSITIVE	19.19
TUBE 3	POSITIVE	19.20
TUBE 4	POSITIVE	19.27
TUBE 5	POSITIVE	19.64
TUBE 6	POSITIVE	19.52
TUBE 7	POSITIVE	19.30
TUBE 8	POSITIVE	19.33
TUBE 9	POSITIVE	19.46
TUBE 10	POSITIVE	19.66
COVID (+) Control	POSITIVE	24.83

External testing data with RSV Real-Time PCR		
CoV2-Flu-RSV Pos control	Lot: B02MAR23	
Target: RSV	**Result**	**Ct Value**
TUBE 1	POSITIVE	21.77
TUBE 2	POSITIVE	21.43
TUBE 3	POSITIVE	21.88
TUBE 4	POSITIVE	21.94
TUBE 5	POSITIVE	21.95
TUBE 6	POSITIVE	21.85
TUBE 7	POSITIVE	21.90
TUBE 8	POSITIVE	20.63
TUBE 9	POSITIVE	22.25
TUBE 10	POSITIVE	22.36
RSV (+) Control	POSITIVE	29.10

Conclusion

Accurate detection across multiple extraction technologies and various diagnostic assays and common clinical instruments including: Cepheid Xpert® Xpress CoV-2/Flu/RSV *plus* in GenXpert Dx system, Abbott Alinity m SARS-CoV-2 Assay in Alinity m system and three internal lab developed Real-Time PCR assays with correct detected calls.

Testing across QIAstat-Dx Respiratory SARS-CoV-2 Panel in QIAstat-Dx instrument, and BioFire® Respiratory 2.1 (RP2.1) panel in BioFire® FilmArray® Torch System was completed and resulted in correct calls for the known target gene sequences covered in control panel.

MMQCI's multiplex CoV2-Flu-RSV Control Panel offers a ready-to use, non-infectious, robust solution to monitor all pathogens detected by several comprehensive, integrated multiplex respiratory assays.

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