



Evaluation of Ready-to-Use External Controls for Molecular Detection of SARS-CoV-2, Influenza, and RSV Using the Xpert® Xpress CoV-2/Flu/RSV Plus Assay

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INTRODUCTION

Accurate and timely detection of respiratory viruses such as SARS-CoV-2, Influenza A/B, and RSV is critical for clinical decision-making, infection control, and public health surveillance. Molecular diagnostics, especially RT-PCR, are the preferred method in clinical labs due to their sensitivity, specificity, and rapid turnaround time.

The Cepheid Xpert® Xpress CoV-2/Flu/RSV plus assay enables simultaneous detection of all four pathogens in a single automated test. Reliable external controls are essential for monitoring assay performance and ensuring result accuracy. This study evaluated Microbiologics® ready-to-use control swabs (Catalog #8246), designed as single-use external controls for the nucleic acid amplification tests (NAATs) for SARS-CoV-2, Influenza A/B, and RSV.

Results support the qualification of these controls for use in assay verification, stability testing, and routine quality control, reinforcing their value in supporting reliable respiratory virus diagnostics in clinical settings.

METHODS

Microbiologics® Flu/RSV/SARS-CoV-2 Control Panel (Catalog #8246) was evaluated for compatibility with the Cepheid Xpert® Xpress SARS-CoV-2/Flu/RSV Plus assay. Positive controls (Kit Component #6121) and negative controls (Kit Component #6122) were tested across multiple lot numbers (see Table 1). Swabs were hydrated in either eNAT™ Molecular Collection and Preservation Medium (Copan, 2 mL) or 0.85% saline (Hardy Diagnostics, 3mL), then vortexed and processed. Testing was performed using the GeneXpert® Dx System utilizing the Xpert® Xpress SARS-CoV-2/Flu/RSV Plus assay. Each sample was processed by pipetting 300 µL of the hydrated swab suspension into the cartridge sample chamber. Materials and reagents used are summarized in Table 2. Expected results for positive controls were SARS-CoV-2, Flu A, Flu B, and RSV detected; negative controls were expected to be negative for all analytes.

Table 1: External Controls

Control	Strains	Lot Numbers
6121	Human Lung Epithelial Cells, A549	6121-126 6121-129 6121-130 6121-131
	Influenza A (H3N2) Virus	
	Influenza A (H1N1) Virus Strain California/04/09	
	Influenza B Virus Strain Hong Kong/5/72	
	Respiratory Syncytial Virus A Strain Long	
	SARS-CoV-2, Isolate USA/WA1/2020	

Table 2: Test Materials

Item	Part Number	Lot Number
eNAT™ Transport Buffer, Copan	6U073S01	210472401
Xpress SARS-CoV-2/Flu/RSV plus, Cepheid	XP3COV2/FLU/RSV-10	1000321346
		1000394988
		1000362367
Saline, 0.85%, Hardy	K59	504092

RESULTS

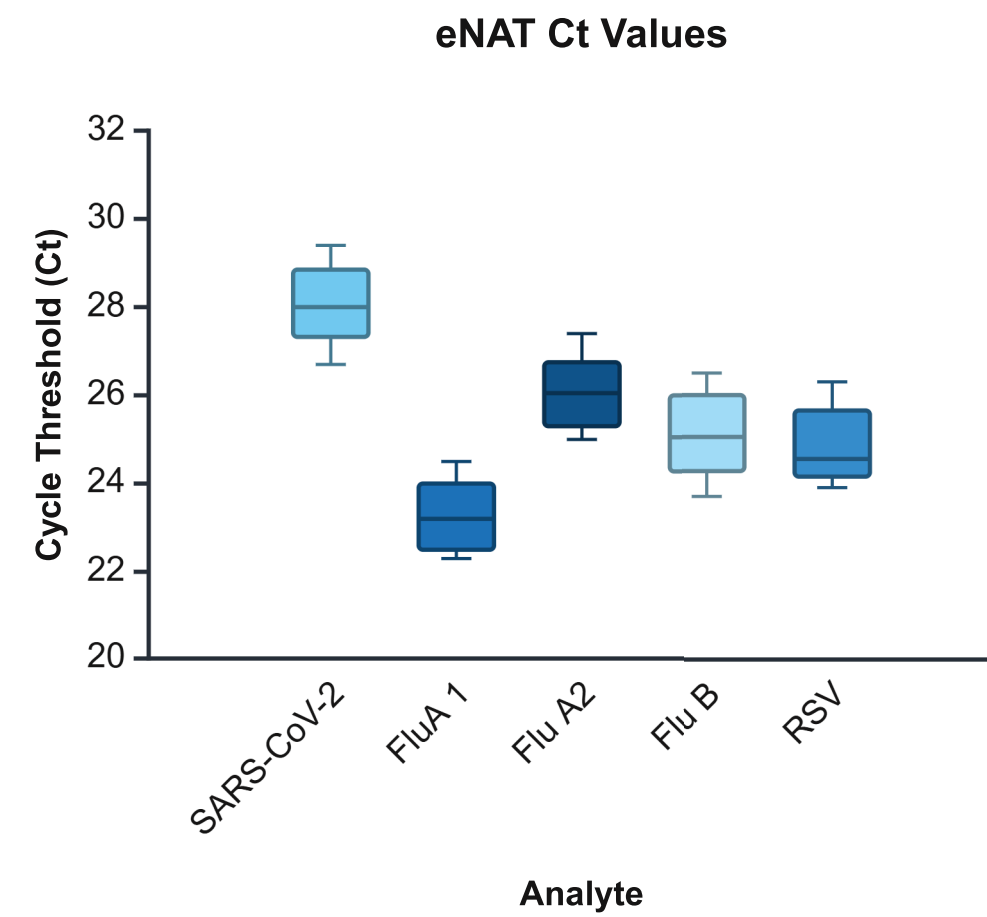


Figure 1. Distribution of cycle threshold (Ct) values for SARS-CoV-2, Flu A1, Flu A2, Flu B, and RSV targets across three lots (6121-126, 6121-129, 6121-131) of positive control swabs (6121) hydrated in eNAT™ transport medium. The results demonstrate consistent detection across all targets with minimal variability, supporting the reproducibility and reliability of the control material for use with the Xpert® Xpress CoV-2/Flu/RSV plus assay.

Table 3. Mean Ct and Positive Rate for External Positive Control with eNAT™

External Control	Lot Number	N	% Agreement with Expected Results	SARS-CoV-2 Mean Ct and % CV	Flu A 1 Mean Ct and % CV	Flu A2 Mean Ct and % CV	Flu B Mean Ct and % CV	RSV Mean Ct and % CV	SPC Mean Ct and % CV
6121	6121-126	5	5/5	28.9	24	26.9	26	25.6	31.1
			100%	1.3	1.6	1.6	1.3	2	4
	6121-129	5	5/5	28	23.1	25.9	25	24.7	32.3
			100%	2.3	2.7	2.3	2.6	3	11.7
	6121-131	4	4/4	27.1	22.5	25.2	24.1	24.2	29.6
			100%	1	1	0.7	1.2	0.8	2.8

Table 4. Mean Ct and Positive Rate for External Positive Control with Saline

External Control	Lot Number	N	% Agreement with Expected Results	SARS-CoV-2 Mean Ct and % CV	Flu A 1 Mean Ct and % CV	Flu A2 Mean Ct and % CV	Flu B Mean Ct and % CV	RSV Mean Ct and % CV	SPC Mean Ct and % CV
6121	6121-130	5	5/5	29	24.8	27.2	26.6	26.2	27.9
			100%	2.5	1.6	1.4	1.6	1.3	0.6
	6121-131	5	5/5	30	25.1	27.5	26.8	26.6	27.9
			100%	1.1	1.1	1.2	1.3	1.3	1.4

Results

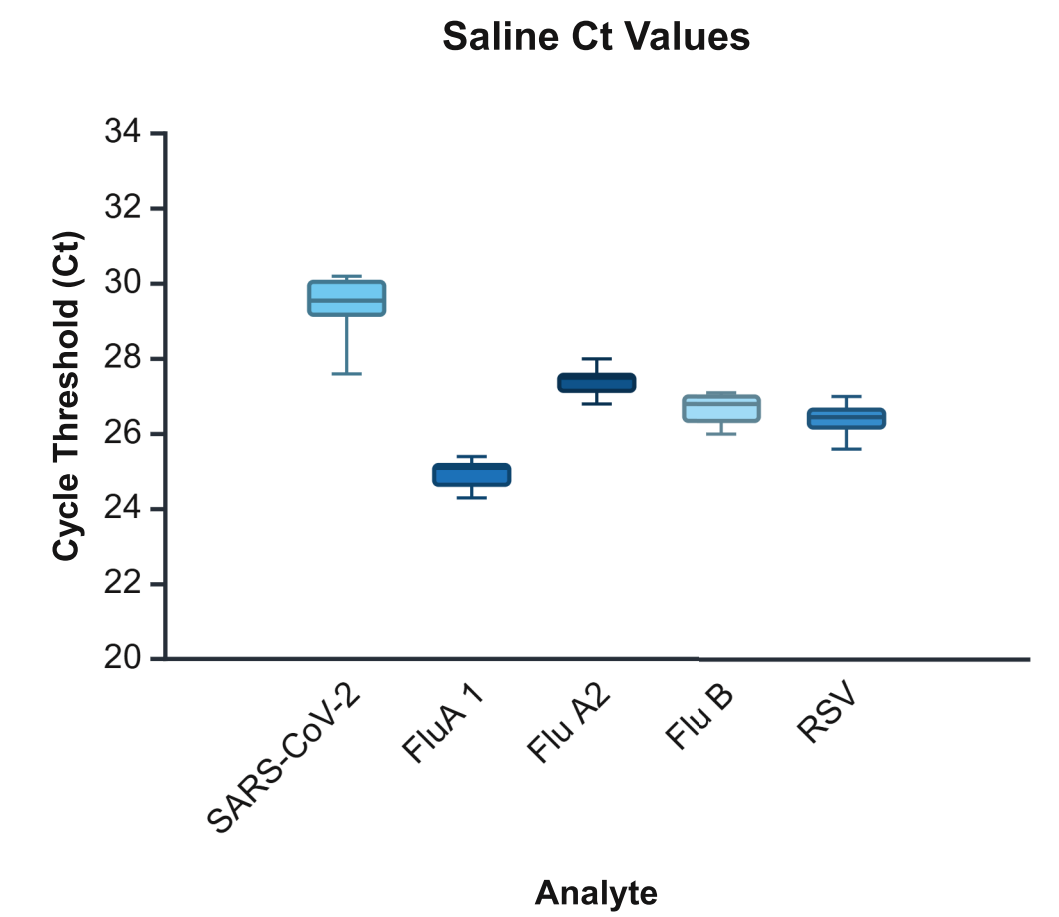


Figure 2. Distribution of cycle threshold (Ct) values for SARS-CoV-2, Flu A1, Flu A2, Flu B, and RSV targets across three lots (6121-126, 6121-129, 6121-131) of positive control swabs (6121) hydrated in 0.85% saline. The results demonstrate consistent detection across all targets with minimal variability, supporting the reproducibility and reliability of the control material for use with the Xpert® Xpress CoV-2/Flu/RSV plus assay.

CONCLUSION

Positive control swabs (Kit Component #6121) consistently produced expected results across all tested lots, hydration volumes, and transport media. All target analytes, SARS-CoV-2, Influenza A, Influenza B, and RSV, were detected 100% of the time, with Ct values showing low variability and falling within expected ranges.

Negative control swabs (Kit Component #6122) also performed as expected, yielding 100% negative results for all targets across multiple lots and conditions, with no false positives or indeterminate results observed.

Together, these findings confirm that the Microbiologics® Flu/RSV/SARS-CoV-2 Control Panel external control swabs (Catalog #8246) are reliable tools for routine quality control, assay verification, and stability monitoring when used with the Cepheid Xpert® Xpress CoV-2/Flu/RSV plus assay.