

# Multiplexed AccuPlex™ Recombinant Viral Reference Materials Enable Assessment of Sensitivity and Detection Range of SARS-CoV-2, FluA, FluB and RSV Diagnostic Assays



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## Introduction

As countries worldwide have reopened after the COVID-19 lockdown and children returned to schools, flu and other respiratory infections have significantly increased. Many multiplexed diagnostic tests that distinguish SARS-CoV-2, Influenza A, B and RSV have recently been introduced, and clinical labs are working to implement them for the flu season. One critical need in test implementation is multiplexed, quantitated, whole process reference materials to verify assay sensitivity and reportable range for ongoing QC. LGC Clinical Diagnostics has developed AccuPlex SARS-CoV-2, Flu A/B and RSV reference materials and verification panels to meet this need. These materials require extraction but are replication-deficient are non-infectious. The AccuPlex reference materials are quantitated by digital PCR (dPCR) to assess sensitivity on various multiplexed PCR-based diagnostic assays.

## Materials & Methods

LGC SeraCare developed AccuPlex SARS-CoV-2, FluA/B and RSV Molecular controls kit using the AccuPlex recombinant virus technology (Figure 1). The RNA genome of SARS-CoV-2 is split across multiple AccuPlex recombinant virus constructs, and the viral particles are combined. Multiple recombinant virus constructs were designed to contain the entire genome from influenza A (H1N1) pdm09 lineage, Influenza B/Victoria lineage, and ~90% of the genomic RNA sequences from RSV subtype A strain. Recombinant viruses encompassing the sequences for these four respiratory pathogens were each quantitated by dPCR and blended to equal copies/mL in a viral transport media, then serially diluted. Testing was performed on Cepheid Xpert Xpress SARS-CoV-2, Flu and RSV Assay. A comparison of AccuPlex FluA virus was performed with native FluA virus obtained from ATCC and tested on Hologic Panther Fusion FluA/B/RSV assay and Roche cobas 6800/8800 to determine if the performance of AccuPlex viruses are similar to native influenza viruses.

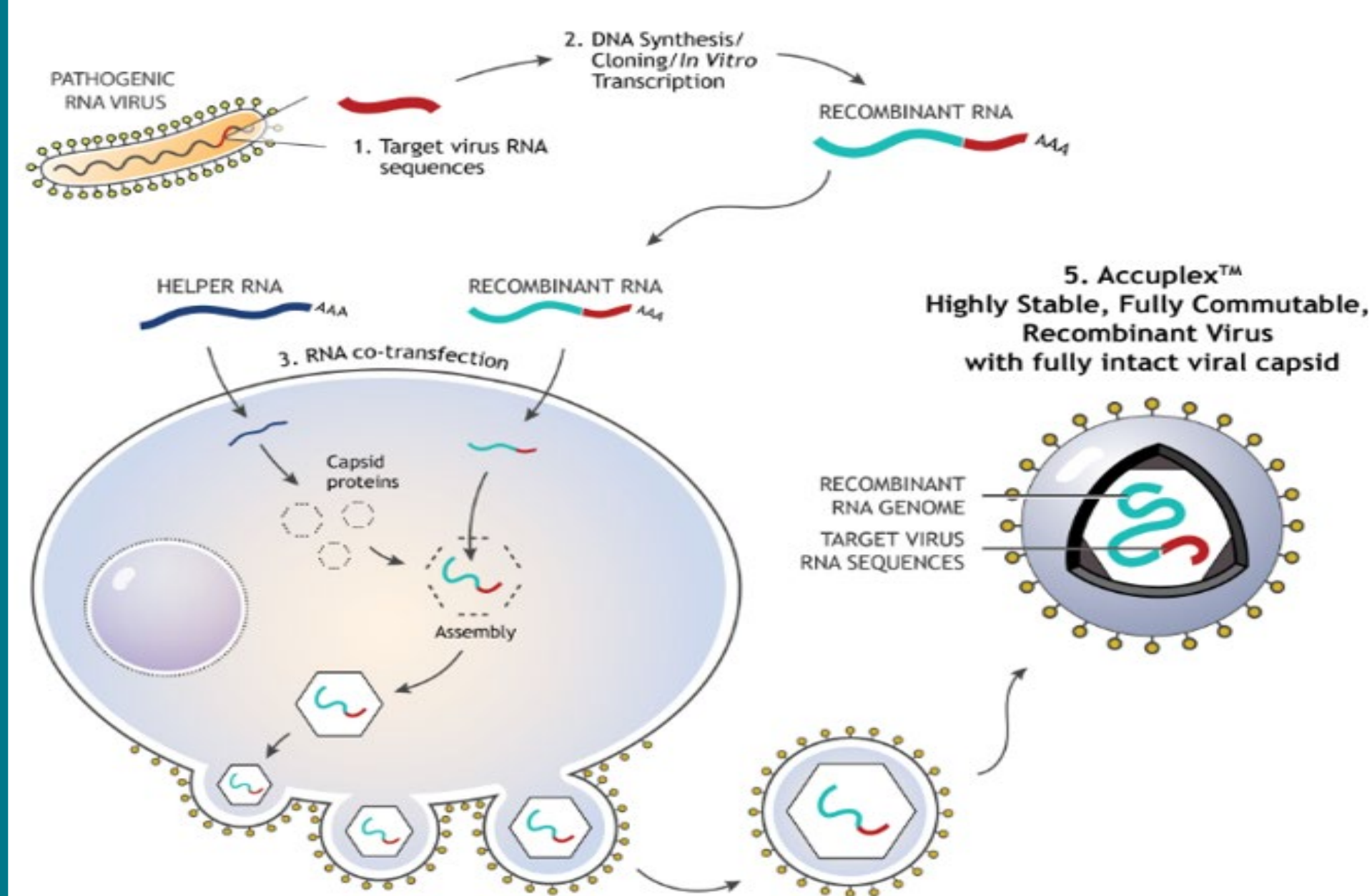


Figure 1: Process for AccuPlex recombinant virus production.

AccuPlex SARS-CoV-2, FluA/B and RSV Molecular Controls Kit contains 5 vials of positive reference material at 5000 cp/mL with the following sequence coverage:

Virus	Genbank Accession Number	Regions Included
Flu A	KU933490 - KU933497	Full Genome
Flu B	CY236601.1 - CY236608.1	Full Genome
RSV	NC_001803	1.4380; 8460..15191
SARS-CoV-2	NC_045512.2	Full Genome

Table 1: AccuPlex SARS-CoV-2, Flu A/B and RSV kit components.

There are also 5 vials of negative controls that contain recombinant virus particles with sequences from human RNase P gene. The sequences are based on the Genbank accession number NC\_000010.11

## Results & Discussion

The AccuPlex SARS-CoV-2, Flu A/B, and RSV Reference Material at ~5000 copies/mL gave cycle threshold (Ct) values from ~30 - 33 depending on analyte and qualitative calls using the Cepheid Xpert Xpress assay.

AccuPlex SARS-CoV-2, FluA/B and RSV Reference Material			Qualitative Result
AccuPlex SARS-CoV-2, FluA/B and RSV Reference Material - Positive Vial	SARS-CoV-2 Avg Ct	31.6	SARS-CoV-2 POSITIVE; Flu A POSITIVE; Flu B POSITIVE; RSV POSITIVE
	FluA 1 Avg Ct	30.7	
	FluA 2 Avg Ct	30.9	
	FluB Avg Ct	30.0	
	RSV Avg Ct	32.8	
AccuPlex SARS-CoV-2, FluA/B and RSV Reference Material - Negative Vial	SARS-CoV-2 Avg Ct	0.0	SARS-CoV-2 NEGATIVE; Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE
	FluA 1 Avg Ct	0.0	
	FluA 2 Avg Ct	0.0	
	FluB Avg Ct	0.0	
	RSV Avg Ct	0.0	

Figure 2: Cepheid Xpert Xpress results showing Ct values and qualitative results for positive and negative vials of AccuPlex SARS-CoV-2, FluA/B and RSV Molecular Controls Kit.

## Comparison of AccuPlex FluA with Native Influenza virus

AccuPlex Influenza A virus had similar sensitivities as the native influenza A virus on the Hologic Panther Fusion FluA/B and RSV Assay. Results from the Roche cobas 6800/8800 also show similar performance across AccuPlex FluA and native influenza A virus. Representative results are shown in Table 2 and Figure 3.

Sample	Lot #	Concentration (copies/mL)	Influenza A by PCR
AccuPlex FluA	107182	1.00E+04	Detected
	107183	1.00E+03	Detected
	107184	1.00E+02	Detected
ATCC Influenza A virus	107185	1.00E+04	Detected
	107186	1.00E+03	Detected
	107187	1.00E+02	Not Detected

Table 2: Hologic Panther Fusion FluA/B/RSV assay results showing detection for both AccuPlex FluA and ATCC influenza virus.

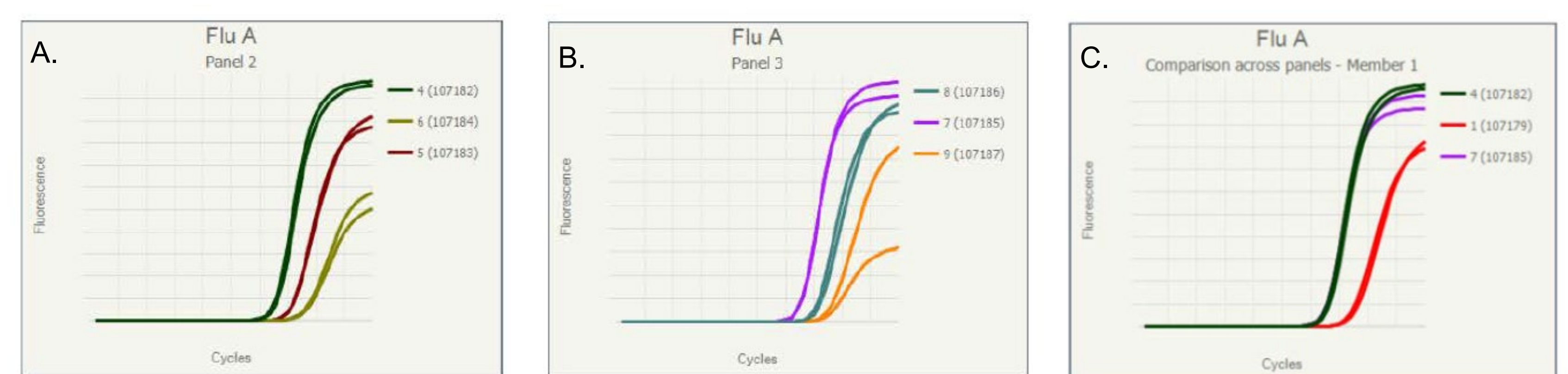


Figure 3: A-B] Roche cobas 6800/8800 results showing similar performance for panel 2 (AccuPlex FluA) and panel 3 (ATCC Influenza virus). C] Comparison between amplification curves for Panel 2 and 3 (Samples 4 and 7) at 1.00E+04 cp/mL show very similar performance.

## Conclusions

- AccuPlex SARS-CoV-2, FluA/B and RSV Molecular Controls kit contain the full SARS-CoV-2, FluA and Flu B genome and ~90% of the RSV genome.
- AccuPlex recombinant viral products are non-infectious and replication deficient. Use of these reference materials ensures safe handling in contrast to viral samples.
- AccuPlex SARS-CoV-2, FluA/B and RSV Molecular Controls are fully extractable with a real viral protein coat; superior to “naked” transcribed RNA.
- AccuPlex SARS-CoV-2, FluA/B and RSV Molecular Controls has been designed to use with *in vitro* diagnostic test methods that detect and differentiate SARS-CoV-2, FluA/B and RSV virus.