Unified amplicon-based whole-genome sequencing of influenza, RSV, and SARS-CoV-2 from routine diagnostics: performance and variant reporting

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Introduction

Influenza, RSV, and SARS-CoV-2 co-circulate and evolve under immune and therapeutic pressures, complicating decision-making for both vaccine formulation and antiviral use. Fragmented, pathogen-specific sequencing approaches limit cross-virus comparability. Genomic surveillance is essential but often fragmented due to pathogen-specific workflows, limiting cross-virus comparability. A unified sequencing strategy could streamline surveillance and enhance early detection of antigenic drift and resistance markers.

Methods

We applied a standardized, multiplexed, amplicon-based next-generation sequencing (NGS) workflow to 34 diagnostic specimens (Ct <35) positive for influenza A/B, RSV-A/B, or SARS-CoV-2. Whole-genome amplification used DeepChek® assays (ABL Diagnostics), followed by Illumina MiSeq 2×250 bp sequencing. Although the wet-lab workflow is standardized across pathogens, consensus generation and annotation utilized two different analysis environments: Geneious Prime for influenza and MicrobioChek® for RSV and SARS-CoV-2, with Nextclade used for RSV clade assignment. Quality metrics included genome breadth and depth of coverage. Phylogenetic reconstruction used maximum-likelihood inference with 1,000 bootstrap replicates.

Results

Near-complete genomes (mean coverage ≥98%) were recovered for all samples. Influenza A(H1N1) pdm09 sequences clustered in clade 6B.1A; A(H3N2) clustered in subclade 3C.2a1b.2a.2; and influenza B belonged to the Victoria lineage V1A.3a.2. RSV sequences were assigned to Nextclade clades A.D.5.1, A.D.1.10, A.D.2.1, and A.D.3 (RSV-A) and to B.D.4.1.3 and B.D.E.1 (RSV-B), consistent with the ON1 (RSV-A) and BA (RSV-B) genotypes prevalent in recent seasons. Clinically relevant mutations included influenza A/H1N1 HA substitutions (A186T, Q189E, K142R) and NA V453M; influenza B HA Δ162–164 deletion and NA D197N/H273Y; RSV-A F-protein T122A; and SARS-CoV-2 spike L455F/L455S and F456L with ORF8 truncation (G8*), consistent with recent Omicron sublineages. Sequencing quality metrics (mapped reads, Q30, uniformity) met thresholds for reliable full-genome reporting across all three viruses.

Table 1. NGS performance metrics by virus type. Mean genome coverage (%), sequencing depth (×), Mean % Mapped reads and Mean Q30 (%) are shown for each virus group.

Virus Type	Samples	Mean Coverage (%)	Mean Depth (×)	Mean % Mapped Reads	Mean Q30 (%)
Influenza A/H1N1	3	99.26	20,586	91.73	93.1
Influenza A/H3N2	4	99.8	12,034	90	93.22
Influenza B	3	98.29	3800	54.9	95.43
RSVA	11	100	10,082	86.66	90
RSVB	3	100	7411	88.08	90.23
SARS-CoV-2	10	99.98	1755	86.86	95.76

Table 2. Clinically relevant amino-acid substitutions detected in this cohort, with reported or suspected implications for antigenicity, antiviral susceptibility, or immune escape, based on published literature and curated databases.

Virus	Gene	Mutation	Medical Impact
A/H1N1	HA	A186T, Q189E, E224A K142R	Antigenic drift in Sa/Sb antigenic sites Drift in Ca antigenic region
	NA	V453M	Altered antigenicity; framework mutation
	M2	D21G	Adamantane resistance
	PA	N321K, V100I, I330V	Increased polymerase activity
	NS1	E55K, L90I, E125D	Increased IFN antagonism
Influenza B	HA	I117V, A127T, N129D, P144L Δ162–164	Antigenic drift Vaccine escape potential
	NA	D197N, H273Y	NA inhibitor reduced susceptibility
RSV-A	F	T122A	Frequent polymorphism in the fusion peptide/p27 region
SARS-CoV-2	Spike	L455F/L455S	Increased ACE2 binding affinity and reduced neutralization by monoclonal and polyclonal antibodies (immune escape)
		F456L	Strong immune escape
	ORF8	G8*	Modulates lung inflammation

Conclusions

A unified amplicon-NGS approach yields harmonized genomic data across respiratory viruses, enabling timely detection of antigenic drift and resistance markers while supporting integrated, cross-pathogen surveillance. By coupling standardized mutation profiling with phylogenetic reconstruction, the approach reduces reliance on pathogen-specific strategies and offers a scalable solution for strengthening early-warning systems and supporting informed vaccine and antiviral policies.