2024 – 2025 Influenza A Sequence Surveillance Assessment for BIOFIRE® FILMARRAY® and BIOFIRE® SPOTFIRE® Respiratory Solutions

Introduction

The BIOFIRE® FILMARRAY® and BIOFIRE® SPOTFIRE® Respiratory Solutions include multiplexed PCR-based in vitro diagnostic tests for the detection of nucleic acids of viruses and bacteria in upper and lower respiratory specimens and are intended to aid in the diagnosis of respiratory infections, including pneumonia.

The BIOFIRE FILMARRAY Respiratory Solutions, including the BIOFIRE® Respiratory 2.1 (RP2.1) Panel, BIOFIRE® Respiratory 2.1*plus* (RP2.1*plus*) Panel, BIOFIRE® FILMARRAY® Pneumonia (PN) Panel, and BIOFIRE® FILMARRAY® Pneumonia *plus* (PN*plus*) Panel, are intended to be used as indicated in moderate- and high-complexity laboratories. The BIOFIRE SPOTFIRE Respiratory Solutions, including BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel, BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel Mini, BIOFIRE® SPOTFIRE® Respiratory (R) Panel and BIOFIRE® SPOTFIRE® Respiratory (R) Panel Mini, are intended to be used as indicated in moderate- and high-complexity laboratories. The BIOFIRE SPOTFIRE Respiratory Solutions are also cleared for use in CLIA-waived settings.

Each BIOFIRE Panel includes one or more assays for the detection of influenza A viruses. The influenza A virus results reported by each panel (including haemagglutinin (HA) subtype, if applicable) are indicated in Table 1.

Table 1. Influenza A Virus Reporting for BIOFIRE FILMARRAY and BIOFIRE SPOTFIRE Respiratory Solutions Products.

System	Product	Influenza A Virus Reporting
BIOFIRE FILMARRAY	**BIOFIRE RP2.1/RP2.1*plus* Panels**	**Influenza A virus** Influenza A virus A/H1 Influenza A virus A/H1-2009 Influenza A virus A/H3
	BIOFIRE PN/PN*plus* Panels	**Influenza A virus**
BIOFIRE SPOTFIRE	**SPOTFIRE R/ST Panel**	**Influenza A virus** Influenza A virus A/H1-2009 Influenza A virus A/H3
	SPOTFIRE R Panel	**Influenza A virus** Influenza A virus A/H1-2009 Influenza A virus A/H3
	SPOTFIRE R/ST Panel Mini	**Influenza A virus**
	SPOTFIRE R Panel Mini	**Influenza A virus**

Influenza A viruses have a single-stranded segmented RNA genome and the low-fidelity viral RNA polymerase causes continuous genetic mutation and evolution as the virus replicates. Consequently, the genome sequence of influenza A viruses circulating and infecting humans changes over time. Seasonal or global pandemics can arise

Attachment 2 of LLDC
069952 - Rev 02.A
Old Document Reference
QS-339J-01
BFR0002-5862-03

when antigenic drift (small mutations in viral genes that can lead to changes in surface proteins) and antigenic shift (major mutations resulting in new surface proteins in viruses that infect humans) lead to the emergence of a novel influenza A virus in a population where there is little to no immunity. Viral genetic variation and evolution can also affect the ability of sequence-based diagnostic tests to accurately identify the virus in clinical specimens. Therefore, it is important for manufacturers of influenza A virus diagnostic tests to monitor the genetic changes in the virus over time to assess whether the test continues to be safe and effective for its intended purpose.

Surveillance of 2024-2025 Influenza A Virus Sequences

To monitor for emerging variant viruses with genetic changes that may alter detection of influenza A viruses by the BIOFIRE Panels, bioMérieux regularly (at least annually) assesses newly available influenza A virus sequence data from the Global Initiative on Sharing All Influenza Data (GISAID) database and other sources where appropriate. Sequences are aligned to the influenza A virus assay primers, allowing for sequence-based predictions of reactivity with each assay and identification of potential sequence-dependent limitations on reactivity (referred to as in-silico analysis). Sequence-based assay specificity (risk of cross-reactivity with non-influenza A virus sequences) is also evaluated annually.

In silico analysis for pan-influenza A assays include all available sequences of the targeted genes from human subtypes (H1N1/H1N1pdm09, H1N2, and H3N2 virus subtypes) as well as other virus subtypes that may have been deposited in the analysis timeframe. The analysis also includes sequences of influenza viruses of avian, swine, and bovine origin. The most recent surveillance in silico reactivity assessment for influenza A virus was performed on approximately 70,000 pan-influenza A virus and over 60,000 haemagglutinin subtype sequences deposited to the GISAID database from July 1, 2024 to July 31, 2025. Predicted assay reactivity for each panel (percent (%) and number of total sequences predicted to be efficiently amplified by an assay) is presented in Table 2.

Table 2. Predicted Reactivity of Influenza A Virus Sequences for BIOFIRE FILMARRAY and BIOFIRE SPOTFIRE Respiratory Solutions Products.

Influenza A assay/assay group	BIOFIRE Respiratory Solutions			
	BIOFIRE FILMARRAY RP2.1/RP2.1*plus* Panels	BIOFIRE FILMARRAY PN/PN*plus* Panels	BIOFIRE SPOTFIRE R/ST and R Panels	BIOFIRE SPOTFIRE R Panel Mini and R/ST Panel Mini
FluA	-	**99.7%** (72,043/72,236[a])	-	-
Pan-FluA assays	**99.7%** (72,043/72,236[a])	-	**99.7%** (72,043/72,236[a])	-
	99.8% (68,034/68,200[b])	-	**99.8%** (68,034/68,200[b])	-
H1 subtype[c]	**N/A** (0/0)	-	-	-
H1-2009 subtype	**98.1%** (36,717/37,421)	-	**98.1%** (36,716/37,420)	-
H3 subtype	**99.9%** (28,195/28,221)	-	**99.9%** (28,195/28,221)	-
Group 1 assays	-	-	-	**99.7%** (72,043/72,236[a])
	-	-	-	**99.8%** (68,034/68,200[b])
Group 2 assays	-	-	-	**98.1%** (36,716/37,420)

Attachment 2 of LLDC 069952 - Rev 02.A
Old Document Reference
QS-339J-01
BFR0002-5862-03

Influenza A assay/assay group	BIOFIRE Respiratory Solutions			
	BIOFIRE FILMARRAY RP2.1/RP2.1*plus* Panels	BIOFIRE FILMARRAY PN/PN*plus* Panels	BIOFIRE SPOTFIRE R/ST and R Panels	BIOFIRE SPOTFIRE R Panel Mini and R/ST Panel Mini
	-	-	-	**99.9%** (28,195/28,221)

[a] Including, but not limited to, 35,806 sequences of influenza A H1 subtype, 27,262 sequences of influenza A H3 subtype, 71 sequences of Influenza A H5N1 subtype, 118 sequences of swine origin, 6,184 sequences of avian origin, and 2,758 sequences of bovine origin.
[b] Including, but not limited to, 33,687 sequences of influenza A H1 subtype, 25,330 sequences of influenza A H3 subtype, 75 sequences of Influenza A H5N1 subtype, 118 sequences of swine origin, 6,180 sequences of avian origin, and 2,779 sequences of bovine origin.
[c] No sequences for the H1 subtype were deposited into the GISAID database during the surveillance period (July 1, 2024 – July 31, 2025); however, in the most recent surveillance period in which sequences were deposited into the GISAID database (October 1, 2022 – May 31, 2023), the Influenza A H1 assay was predicted to react with 97.7% (127/130) of the deposited sequences.

This in silico analysis reveals that the pan-influenza A virus assays (FluA, Pan-FluA, and Group1) in the BIOFIRE Respiratory Solutions panels are predicted to be reactive with >99% of the influenza A virus sequences (of human, avian, swine, and bovine origin) deposited to the GISAID database during the 2024 - 2025 respiratory season. The subtype assay for Influenza A H1-09 is predicted to be reactive with 98.1% of the total H1-09 database sequences. The subtype assay for Influenza A H3 is predicted to be reactive with >99% of the total H3 database sequences.

In future annual analyses, if a variant sequence that is predicted to impact reactivity (>10-fold) represents 5% or more of the annual deposited sequences, the potential impact on amplification, detection and reporting by each panel will be investigated. If the investigation confirms a limitation on reactivity with one or more assays that would alter panel performance, a notification about the impact on test performance will be released and distributed.

NOTE: Testing with BIOFIRE FILMARRAY and BIOFIRE SPOTFIRE Panels containing assays for the detection of influenza A virus (and viral subtype sequences) is not intended to monitor for or identify novel variant viral strains of public health concern nor potential zoonotic transmission events.

In Silico Reactivity Assessment of Influenza A Virus Strains Recommended for 2024-2025 Influenza Vaccine

The World Health Organization (WHO) Global Influenza Surveillance and Response System (GISRS) recommends the composition of influenza virus vaccines biannually based on global surveillance data. The recommended influenza A virus strains to include in vaccines for use in the 2025-2026 northern hemisphere influenza season are:

H1N1pdm09:	A/Victoria/4897/2022 (egg-based vaccines)
	A/Wisconsin/67/2022 (cell-based or recombinant vaccines)
H3N2:	A/Croatia/10136RV/2023 (egg-based vaccines)
	A/District of Columbia/27/2023 (cell-based or recombinant vaccines)

bioMérieux evaluated each of the recommended influenza A virus vaccine strain sequences against respective BIOFIRE FILMARRAY and BIOFIRE SPOTFIRE Panel pan-influenza A or subtype assay(s) and no reactivity limitations for vaccine strains were predicted by in silico analysis. Consequently, the panels are predicted to detect nucleic acids from vaccines if present in the specimens being tested.

Attachment 2 of LLDC
069952 - Rev 02.A
Old Document Reference
QS-339J-01
BFR0002-5862-03

Conclusion

- Influenza A virus assays in BIOFIRE FILMARRAY and BIOFIRE SPOTFIRE Panels are predicted to react with >99% of influenza A virus sequences deposited to the GISAID database from July 1, 2024 to July 31, 2025.
- bioMérieux has a surveillance program in place to evaluate newly deposited influenza A virus sequences. This active annual sequence surveillance program, along with other post-market monitoring activities, allows bioMérieux to maintain claims of state-of-the-art performance for detection and (subtyping) of influenza A virus in upper and lower respiratory specimens with BIOFIRE FILMARRAY and BIOFIRE SPOTFIRE Panels and to notify users if new deficiencies or limitations on influenza A virus detection are identified.

Technical Support Contact Information

bioMérieux is dedicated to providing the best customer support available. If you have any questions or concerns about this process, please contact your local bioMérieux representative or your authorized distributor.

Attachment 2 of LLDC
069952 - Rev 02.A
Old Document Reference
QS-339J-01
BFR0002-5862-03