

Development of a Rapid Multiplex Nucleic Acid Amplification Diagnostic* Test for the Detection of Influenza Types A, B, C, and D with Influenza A Hemagglutinin-Subtype H1-H18 Delineation

Daniel FUJA, Brett SMITH, Jeff BARRETT, Wendy SMITH, Hailey DAKER, Makensie BANKS, Zowie GEBREMESKEL, Marianne KIM
BioFire Defense, LLC, Salt Lake City, Utah, USA

ABSTRACT

Background: Influenza is a priority pathogen with high risk for future pandemics. New virulence and capacity to infect novel hosts is rapidly and continuously evolving via mutation and genetic reassortment. As learned during the global Coronavirus pandemic, a comprehensive, easy-to-use, rapid diagnostic* test is needed immediately when a pandemic threat emerges. Employing automated multiplex PCR technology for simultaneous detection, we developed a diagnostic* test for influenza detection, including existing pathogenic and potential emerging subtypes on the BIOFIRE® FILMARRAY® TORCH and SPOTFIRE® systems.

Methods: Similar to the FDA-cleared BIOFIRE® Respiratory 2.1 (RP2.1) and SPOTFIRE® Respiratory/Sore Throat (R/ST) Panels, the design for this Emerging Influenza Panel utilizes a pouch-based nested, multiplex PCR capable of detecting and differentiating all four known genera of influenza and hemagglutinin subtypes H1-H18 within the genus *Alphainfluenzavirus*.

Influenza assays are designed principally to sequences deposited to the NCBI Virus database (previously NCBI Influenza Virus Resource). These assays are built using nested primers deliberately constructed to maximize sensitivity to the many disparate sequences for each influenza target and maintain on- and off-panel exclusivity.

Assays are evaluated *in silico* and on benchtop to ensure coverage and performance. Testing is conducted on both FDA-cleared platforms* with synthetic target transcripts based on common sequences and inactivated, whole-virus genomes (BEI Resources, NIAID, NIH) to confirm function with high multiplex under pouch conditions.

Results: *In silico* evaluation demonstrates detection of influenza A, B, C and D capturing 90% or more for each type, detecting over 99% of all available sequences of both human and non-human hosts. *In silico* data also establishes hemagglutinin-specific detection and demarcation with 88% sequence inclusivity or greater for subtypes H1-H18, with exclusivity to human, coronavirus, and other common respiratory pathogens. Continuous *in silico* surveillance for H5N1 and other currently circulating sequences confirms ongoing inclusivity.

For all type and subtype assays, detection of designed synthetic nucleic acid is robust, typically down to at least 1000 copies per test. Pilot studies with whole influenza viruses are detected on both platforms for influenza from both human and non-human hosts, including H1, H3, H5 (Bovine), H5 (Avian), Influenza B, and Influenza C.

Conclusions: Initial assay design and functional testing highlight feasibility of this influenza diagnostic* panel which detects all four genera of Influenza, as well as differentiates between Influenza A Hemagglutinin subtypes on both FDA-cleared BIOFIRE® FILMARRAY® TORCH and SPOTFIRE® systems. Following FDA clearance* and launch, this diagnostic* will provide a critical tool in pandemic preparedness to quickly and effectively differentiate between all types of influenza and hemagglutinin subtypes.



BIOFIRE SYSTEMS TECHNICAL BACKGROUND

- The BIOFIRE FILMARRAY TORCH platform is a rapid multiplex diagnostic system expandable from two to twelve modular units (Figure 1).
- The BIOFIRE SPOTFIRE platform is the next generation BIOFIRE system, with a smaller footprint, but maintaining expandability for one to four independent pouch-running modules.
- Both systems are compatible with a panel-specific consumable pouch (Figure 2). This pouch incorporates automated sample extraction (Figure 2A); nucleic acid purification (Figure 2B and 2C); reverse-transcription and nested multiplex first-stage PCR (Figure 2D and 2E); and individual second-stage PCR reactions in a PCR array (Figure 2G) to evaluate multiple targets in a single run. With minimal hands-on time, a comprehensive result is returned.

EMERGING INFLUENZA PANEL ASSAY DESIGN STRATEGY

- Prioritize inclusivity for use as an emerging threat response tool capable of detecting all Influenza types and H-subtypes (Table 1).
- In-house and commercially available tools were used to identify sequences for each target and design multiple candidate primer sets.
- Each primer set was evaluated to maximize inclusivity (Figure 3).
- H1 requires three primer sets to capture multiple human and non-human H1 sequences.
- A broadly inclusive H1 assay was designed (H1E) that overlaps with other, more specific assays (Figure 4).
- For all assays, favorable primer candidates were tested within pouch in multiplex. Results were used to inform selection and optimization until all Influenza A subtypes were incorporated. The general location of each assay on the hemagglutinin gene is shown in Figure 5.

*The Emerging Influenza Panel has not been approved by the FDA or any other regulatory body. Use of the term “diagnostic” refers to the intended use as defined in the contract for development and future potential in that regard. No approval request has been submitted, nor should approval be assumed or projected.

Table 1. Panel Targets

Panel Assay	Interpretation
H1-2009	H1, H1-2009
H1	H1
H1E	H1
H2b	H2
H3	H3
H4	H4
H5b	H5
H6	H6
H7	H7
H8	H8
H9a	H9
H9b	H9
H10	H10
H11	H11
H12	H12
H13	H13
H14	H14
H15	H15
H16	H16
H17	H17
H18	H18
pIAV_1	Influenza A
pIAV_2	Influenza A
pIBV	Influenza B
pICV	Influenza C
pIDV	Influenza D

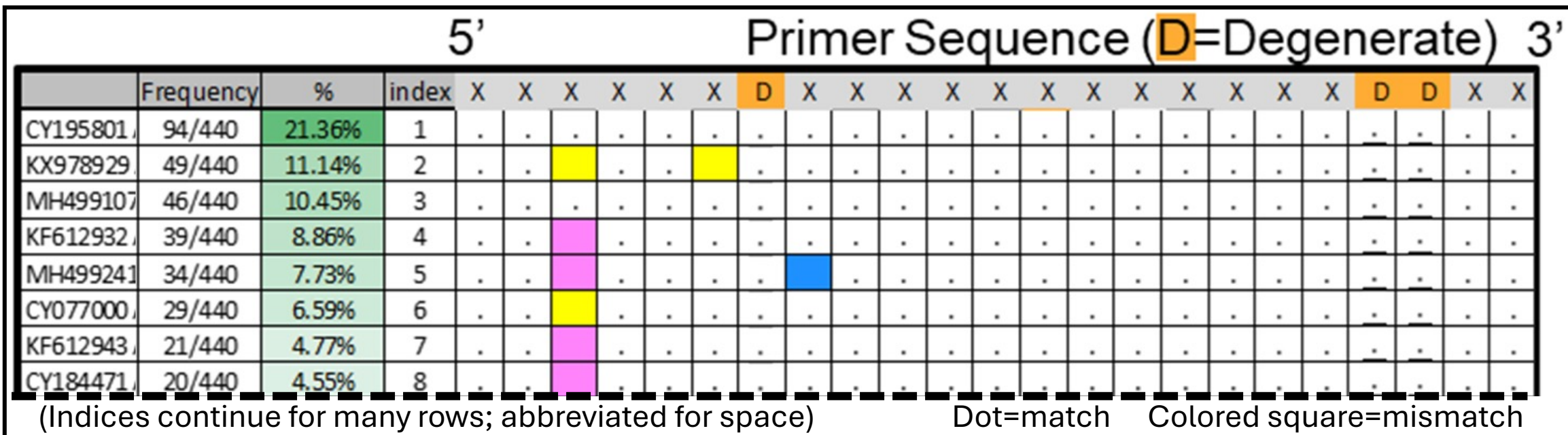


Figure 3. Example of Primer Inclusivity (H13 Subtype Assay)

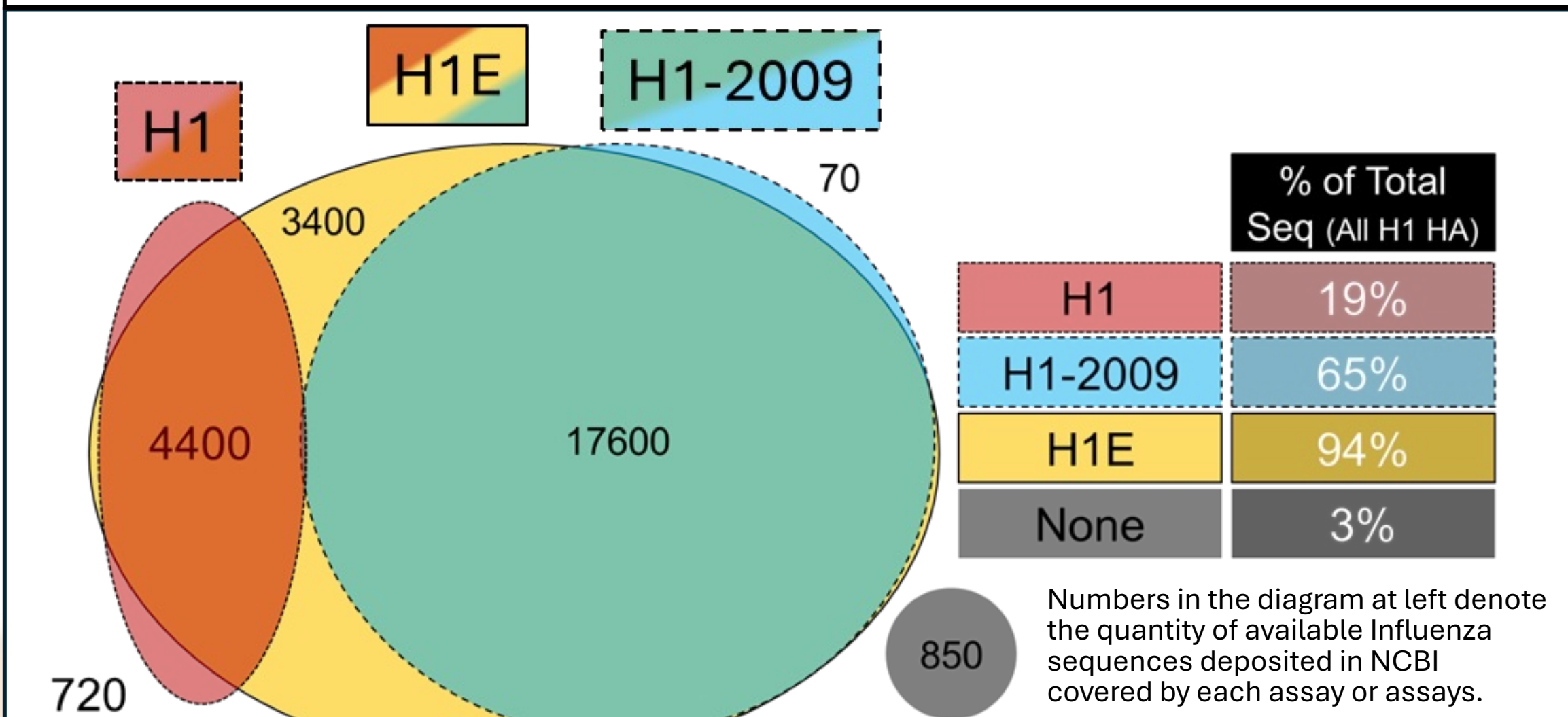


Figure 4. H1 Inclusivity Schematic

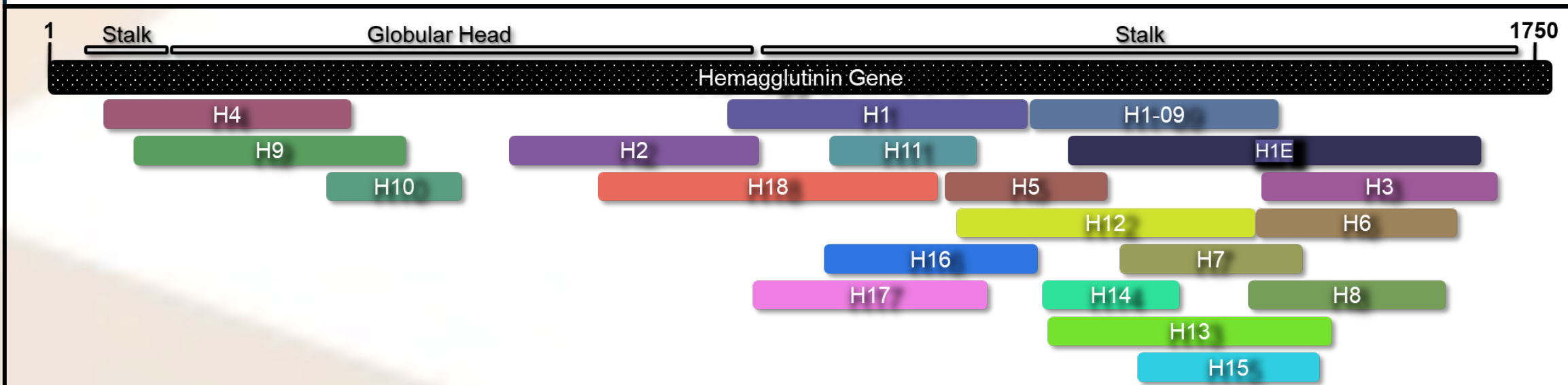


Figure 5. HA Schematic With All Subtype Target Regions

RESULTS

- Primers were continually evaluated throughout design, as new sequences of interest were deposited (e.g., H5 outbreak or death). *In silico* analysis has demonstrated inclusivity with the designed assays, as illustrated in Figure 6.

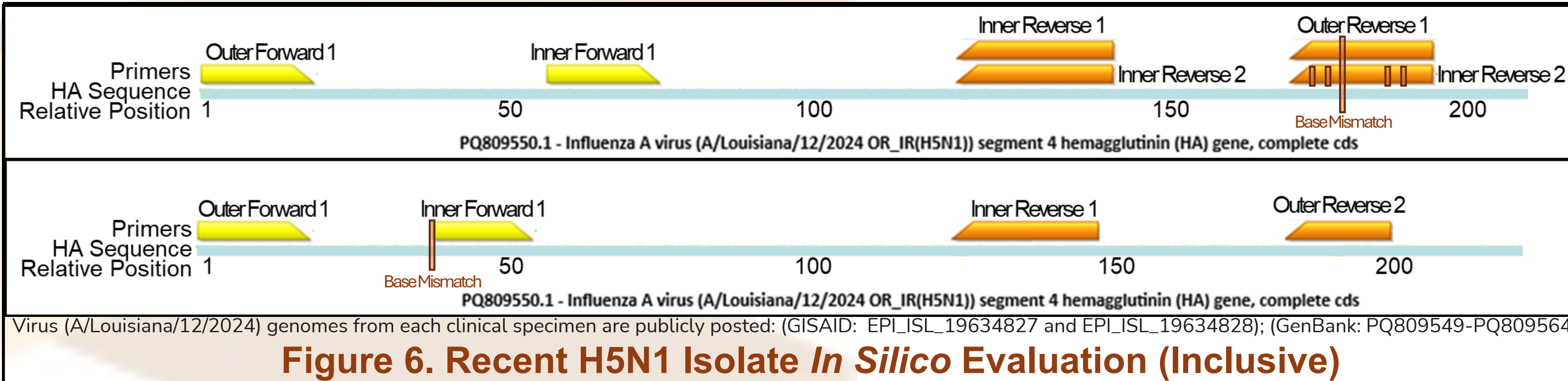


Figure 6. Recent H5N1 Isolate *In Silico* Evaluation (Inclusive)

- Evaluation with synthetic RNA (Figure 7) and select organism (Table 2) demonstrated robust detection with high concentration sample pools.

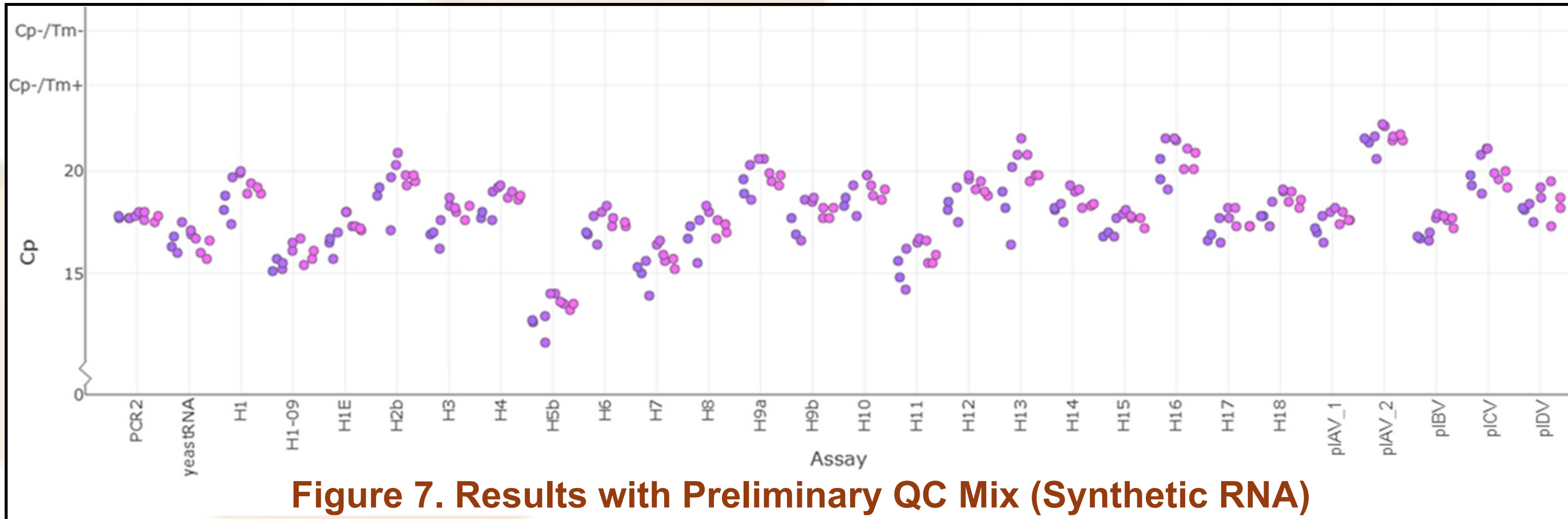


Figure 7. Results with Preliminary QC Mix (Synthetic RNA)

Table 2. Organism Detection in Pouch

Assay Sample						Influenza	Influenza	Influenza	Influenza
	H1	H1-2009	H1E	H3	H5	A (1)	A (2)	B	C
H1N1	6/6	—	6/6	—	—	6/6	6/6	—	—
H3	—	—	—	6/6	—	6/6	6/6	—	—
H5N1-Bovine	—	—	—	—	6/6	6/6	6/6	—	—
H5N1-Mallard	—	—	—	—	6/6	6/6	6/6	—	—
Influenza B	—	—	—	—	—	—	—	6/6	—
Influenza C	—	—	—	—	—	—	—	—	6/6
Legend	detection					no detection			
Conclusion	All detected by appropriate assay(s), respectively								

CONCLUSION

- The Emerging Influenza Panel has been successfully designed to detect all four influenza types and 18 hemagglutinin subtypes.
- The Emerging Influenza Panel functions appropriately on both the BIOFIRE® FILMARRAY® TORCH and SPOTFIRE® platforms.
- The Emerging Influenza Panel successfully detected all Flu types and subtypes in pilot testing (H1N1, H3, H5N1, Flu B, and Flu C).
- Regulatory and clinical evaluation will be conducted following the conclusion of panel design and optimization.

Presented at the European Scientific Working Group on Influenza (ESWI) October 20 – 23, 2025



See all BioFire
Defense's Scientific
posters by Scanning
the QR Code

DISCLAIMER: The conclusions contained herein are those of the authors and should not be interpreted as representing the official policies or endorsements, either expressed or implied, of BARDA, the Department of Defense or the U.S. Government.

This project has been funded in whole or in part with Federal funds from the U.S. Department of Health and Human Services; Office of the Administration for Strategic Preparedness and Response; Biomedical Advanced Research and Development Authority (BARDA), under Contract No. 75A50123C00071.