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Development of Human and Avian Influenza Analytical Reference Materials for Surveillance and Diagnostics

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Background

Influenza viruses pose a significant public health threat, with the potential to cause widespread illness and economic losses. Early detection and control of outbreaks depend on effective surveillance and diagnostic testing using accurate analytical reference materials (ARMs). Without ARMs, diagnostic tests may yield false results that undermine surveillance and public health efforts. ATCC® developed a comprehensive suite of quantitative synthetic ARMs for avian influenza serotypes H5N1, H5N6, H7N7, H7N9, and H9N2; human influenza A serotypes H1N1, H3N2, and H1N1 2009 pandemic; and Influenza B strains. These were developed to serve as high-quality ARMs suitable for use in BSL-1 facilities. In the following study, we experimentally verified the utility of the ARMs with published quantitative PCR assays from sources such as the U.S. Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO) as well as published RT-LAMP assays and the BioFire FilmArray RP2.1 Respiratory Panel, demonstrating that these ARMs can serve as reliable and safe controls for molecular assays used in diagnostics and surveillance.

Table 1: Synthetic and genomic RNA materials used in this study.

Source	Item Number	Subtype	RNA Type	Strain/Reference Strain
ATCC®	VR-3384SD™	B/Victoria	Synthetic	B/Malaysia/2506/2004
ATCC®	VR-3385SD™	B/Victoria	Synthetic	B/Brisbane/60/2008
ATCC®	VR-1931DQ™	B/Victoria	Genomic	B/Florida/78/2015
ATCC®	VR-3386SD™	A/H1N1	Synthetic	A/Brisbane/59/2007
ATCC®	VR-1893DQ™	A/H1N1	Genomic	A/Florida/3/2006
ATCC®	VR-3387SD™	A/H3N2	Synthetic	A/Hiroshima/52/2005
ATCC®	VR-1882DQ™	A/H3N2	Genomic	A/Wisconsin/15/2009
ATCC®	VR-3388SD™	A/H1N1pdm09	Synthetic	A/Netherlands/2629/2009
ATCC®	VR-1736DQ™	A/H1N1pdm09	Genomic	A/Virginia/ATCC1/2009
ATCC®	VR-3436SD™	A/H5N1	Synthetic	A/white-tailed eagle/Japan/OU-1/2022
BEIR	NR-59885	A/H5N1	Genomic	A/bovine/Ohio/B24OSU-439/2024
ATCC®	VR-3437SD™	A/H7N9	Synthetic	A/Shanghai/4664T/2013
ATCC®	VR-3438SD™	A/H7N7	Synthetic	A/chicken/Wenzhou/334b/2013
ATCC®	VR-3439SD™	A/H5N6	Synthetic	A/goose/Guangdong/GS018/2015
ATCC®	VR-3440SD™	A/H9N2	Synthetic	A/ostrich/Yunnan/438/2014
BEIR	NR-43016	A/H9N2	Genomic	A/turkey/Wisconsin/1/1966

Methods

qRT-PCR

- Synthetic influenza RNA items and when available, genomic RNA (gRNA) items were tested at concentrations ranging from 50-50,000 GC/rxn.
- The assays used were published by the CDC,[1] WHO,[2] FDA,[3] or from highly cited papers.[4]
- Amplification was achieved using Invitrogen SuperScript III Platinum One-Step qRT-PCR Kit (Thermo Fisher Scientific) (Figures 1, 2A-B, and 3) or QIAGEN One-Step RT qPCR Kit (Figure 2C) on the CFX Opus Real-Time PCR System (Bio-Rad).

RT-LAMP

- Synthetic avian influenza A/H5 and A/H7 RNA items were evaluated with RT-LAMP assays from Ahn *et al.* 2019[5] using the WarmStart Colorimetric LAMP 2X Master Mix with UDG (New England Biolabs).

BioFire Film Array RP2.1 Panel

- Synthetic avian influenza RNA items were tested at 100×, 10×, and 3× limit of detection (LoD) for influenza A detection, which is reported in the kit details as 1.4 × 10^2 copies/mL.[6]

Results

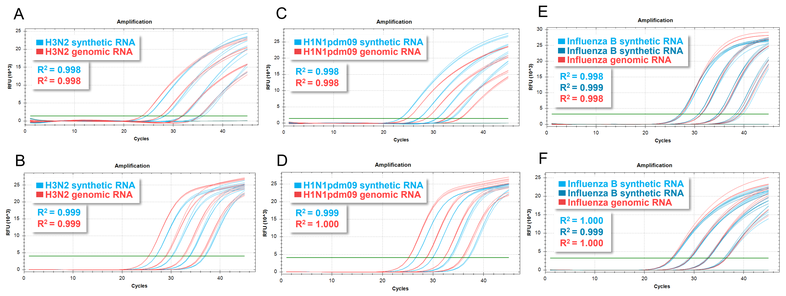

Figure 1: Comparative qRT-PCR amplification of synthetic and genomic human influenza virus RNA. Amplification curves generated with (A-B) synthetic A/H3N2 RNA (ATCC® VR-3387SD™, blue) and genomic A/H3N2 RNA (ATCC® VR-1882DQ™, red) using (A) a WHO assay targeting HA[2] and (B) the CDC Flu A assay targeting the M gene from the Flu-SC2 Multiplex assay.[1] Amplification curves generated with (C-D) synthetic A/H1N1pdm09 RNA (ATCC® VR-3388SD™, blue) and genomic A/H1N1pdm09 RNA (ATCC® VR-1736DQ™, red) using (C) a WHO assay targeting HA[2] and (D) the CDC Flu A assay targeting the M gene from the Flu-SC2 Multiplex assay.[1] Amplification curves generated with (E-F) synthetic influenza B RNA (ATCC® VR-3384SD™, light blue; ATCC® VR-3385SD™, dark blue) and genomic influenza B RNA (ATCC® VR-1931DQ™, red) using (E) a WHO assay targeting HA[2] and (F) the CDC Flu B assay targeting NS1 from the Flu-SC2 Multiplex assay.[1]

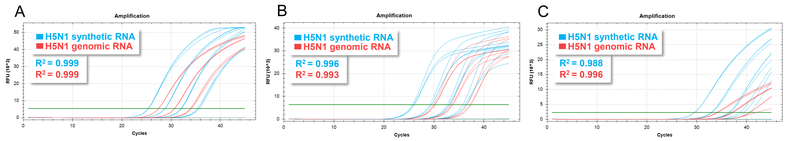

Figure 2: Comparative qRT-PCR amplification of synthetic and genomic H5N1 RNA. Amplification curves generated with synthetic A/H5N1 RNA (ATCC® VR-3436SD™, blue) and genomic A/H5N1 RNA (BEIR NR-59885, red) using (A) a Hoffmann *et al.* 2016[4] assay targeting HA, (B) the CDC Flu A assay targeting the M gene from the Flu-SC2 Multiplex assay[1], and (C) the HA assay from the FDA's 2024 protocol "HPAI H5 Subtyping in Milk and Milk Products Using RT-qPCR."[3]

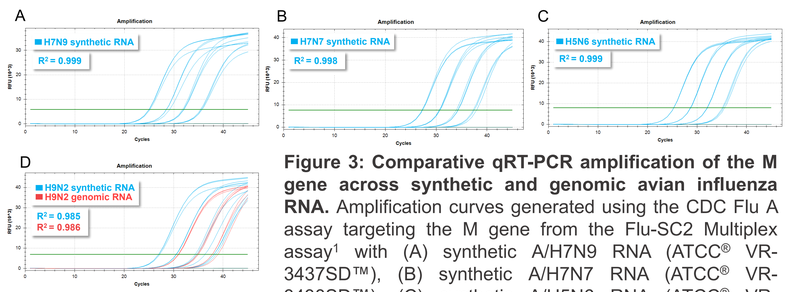

Figure 3: Comparative qRT-PCR amplification of the M gene across synthetic and genomic avian influenza RNA. Amplification curves generated using the CDC Flu A assay targeting the M gene from the Flu-SC2 Multiplex assay[1] with (A) synthetic A/H7N9 RNA (ATCC® VR-3437SD™), (B) synthetic A/H7N7 RNA (ATCC® VR-3438SD™), (C) synthetic A/H5N6 RNA (ATCC® VR-3439SD™), and (D) synthetic H9N2 RNA (ATCC® VR-3440SD™) and genomic A/H9N2 RNA (BEIR NR-43016).

Results (continued)

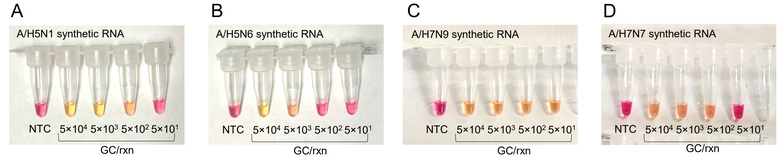

Figure 4: RT-LAMP results using assays from Ahn *et. al.* 2019.[5] The H5 assay from the paper was tested with (A) synthetic A/H5N1 RNA (ATCC® VR-3436SD™) and (B) synthetic A/H5N6 RNA (ATCC® VR-3439SD™), and the H7 assay was tested with (C) synthetic A/H7N9 RNA (ATCC® VR-3437SD™) and (D) synthetic A/H7N7 (ATCC® VR-3438SD™). From left to right, the PCR strip tubes contain a no-template control (NTC), 5 ×10^4 GC/rxn, 5 ×10^3 GC/rxn, 5 ×10^2 GC/rxn, and 5 ×10^1 GC/rxn of synthetic RNA.

Table 2: Detection of ATCC® Synthetic Avian Influenza RNA Products with the BioFire Diagnostics FilmArray RP2.1 Respiratory Panel (bioMérieux)

ATCC® Item	Subtype	Diluent	× LOD		
			100	10	3
VR-3436SD™	A/H5N1	0.25 mg/mL Poly(A)	+	+	+
		Molecular-grade water	+	+	-
VR-3437SD™	A/H7N9	0.25 mg/mL Poly(A)	+	+	-
VR-3438SD™	A/H7N7	0.25 mg/mL Poly(A)	+	+	+*
VR-3439SD™	A/H5N6	0.25 mg/mL Poly(A)	+	+	+
VR-3440SD™	A/H9N2	0.25 mg/mL Poly(A)	+	+	+

*Detection failed on first attempt, successfully detected in retest of same sample

Conclusions

- ATCC® quantitative synthetic influenza RNA function as reliable, well-characterized ARMs across multiple molecular detection platforms.
- These ARMs are compatible with numerous published qRT-PCR assays and RT-LAMP assays and performed robustly with the BioFire FilmArray RP2.1 Respiratory Panel.
- The synthetic RNA ARMs are equivalent to their corresponding genomic RNA, making them a valuable BSL-1 alternative to BSL-3–derived materials.

ATCC Influenza Resources

References

1. CDC, Research Use Only CDC Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay Real-Time RT-PCR Primers and Probes, CDC, 2020.
2. WHO Information for the Molecular Detection of Influenza Viruses, 2021.
3. FDA, HPAI H5 Subtyping in Milk and Milk Products Using RT-qPCR, 2024.
4. Hoffmann B, *et al.* Riems influenza a typing array (RITA): An RT-qPCR-based low density array for subtyping avian and mammalian influenza a viruses. Sci Rep 6: 27211, 2016. PubMed: 27256976.
5. Ahn SJ, *et al.* BMC Rapid and simple colorimetric detection of multiple influenza viruses infecting humans using a reverse transcriptional loop-mediated isothermal amplification (RT-LAMP) diagnostic platform. Infect Dis 19(1):676, 2019. PubMed: 31370782.
6. BIOFIRE Respiratory Panel 2.1 (RP2.1). BFR0000-8579-03, 2023.

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