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

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About Us

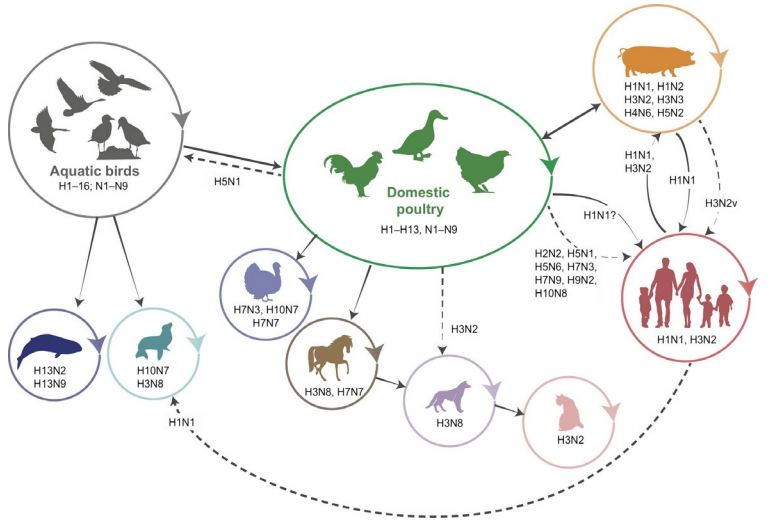
ATCC is a global leader in providing authenticated, high-quality biological resources and standards for industry, academia, and government.

- Founded in 1925, ATCC is a private, nonprofit, global biological resource center and standards organization that provides scientists with the biomaterials and resources they need to conduct critical life science research.
- World's trusted, premier biological materials resource and standards development organization:
  - 4,000+ cell lines
  - 80,000+ microorganisms
  - Genomic and synthetic nucleic acids
  - Media, sera, and reagents
  - Advanced cell models
  - Standards

Background & Introduction

The Need for Analytical Reference Materials (ARMs)

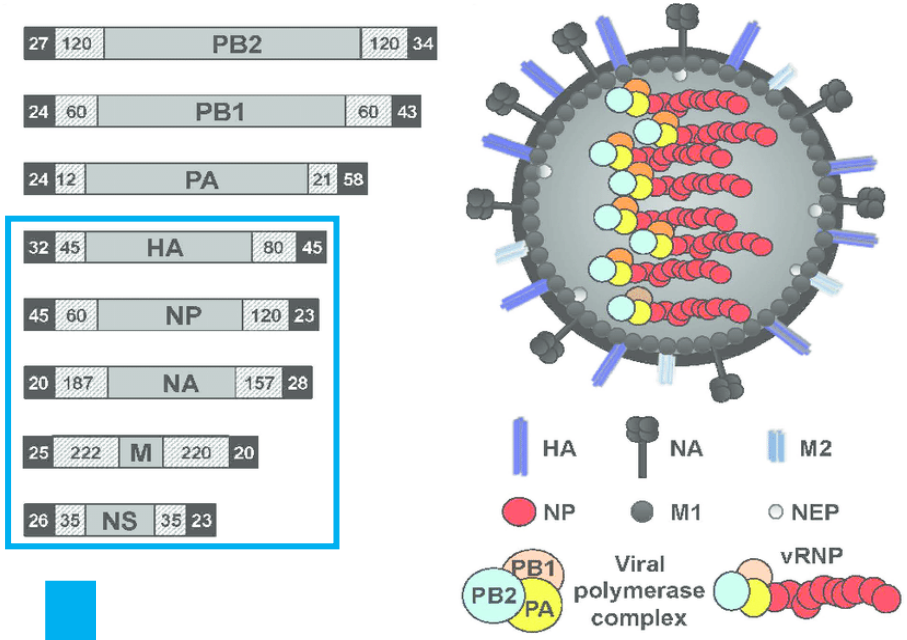
- Human influenza and highly pathogenic avian influenza viruses pose a significant public health risk due to their potential for widespread illness and economic consequences.
- Early detection and control of outbreaks rely on effective surveillance and diagnostic testing.
- ATCC® has developed a comprehensive suite of quantitative synthetic analytical reference materials for the following:
  - Avian flu virus serotypes H5N1, H5N6, H7N7, H7N9, and H9N2
  - Human flu A virus serotypes H1N1, H3N2, and H1N1 2009 pandemic
  - Influenza B virus strains



Joseph U, et al. Influenza Other Respir Viruses 11(1): 74-84, 2016. PubMed: 27426214

ATCC® Catalog Number	Influenza Subtype
ATCC® VR-3384SD™	B (Victoria)
ATCC® VR-3385SD™	B (Victoria)
ATCC® VR-3386SD™	H1N1
ATCC® VR-3387SD™	H3N2
ATCC® VR-3388SD™	H1N1 pdm09
ATCC® VR-3436SD™	H5N1
ATCC® VR-3437SD™	H7N9
ATCC® VR-3438SD™	H7N7
ATCC® VR-3439SD™	H5N6
ATCC® VR-3440SD™	H9N2

Design of the Products



Breen M, et al. Viruses 8(7): 179, 2016. PubMed: 27347991

- Based upon a systematic literature review of over 260 influenza PCR assays we identified the diagnostically relevant genome segments HA, NP, NA, M1/M2, and NEP/NS1.
- We implemented a two-transcript design to accommodate diagnostically relevant segments of the influenza genome
  - Transcript A → HA and NP genes
  - Transcript B → M1/M2, NA, and NEP/NS1 genes
- The products are manufactured using reliable synthetic biology technology, verified through next-generation sequencing, and quantitated via digital PCR.
- Both transcripts fall within the range of 1×10^5 and 1×10^6 copies/µL.

Application Data

Applications & Data Generation

- Generation of a standard curve for quantitative PCR (qPCR)
- Positive control for qPCR assays
- Assay verification and validation studies
- Monitor assay-to-assay and lot-to-lot variation
- Molecular diagnostics assay development

The qPCR data that follow were generated on the CFX Opus Real-Time PCR System (Bio-Rad). Amplification was achieved using the Invitrogen SuperScript III Platinum One-Step qRT-PCR Kit.

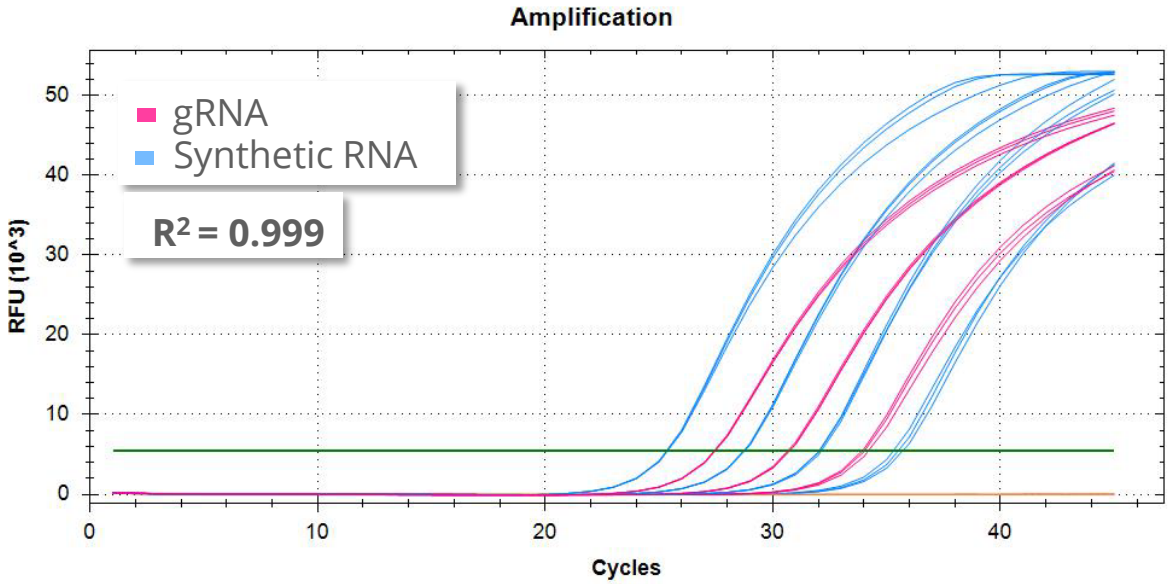
The following assays were used to validate the products:

Influenza Subtype	Publication Source	Assay Target
H5N1	Hoffmann, et al., 2016	HA
	CDC Flu SC2 Multiplex Assay, 2020	M
	FDA Milk Assay, 2024	HA
H7N9	WHO, Molecular Detection of Influenza viruses, 2021	HA
	CDC Flu SC2 Multiplex Assay, 2020	M
H5N6	Hoffmann, et al., 2016	HA
	CDC Flu SC2 Multiplex Assay, 2020	M
H9N2	Hassan, et al., 2022	HA
	CDC Flu SC2 Multiplex Assay, 2020	M
H1N1 pdm09	WHO, Molecular Detection of Influenza viruses, 2021	HA
	WHO, Molecular Detection of Influenza viruses, 2021	NA

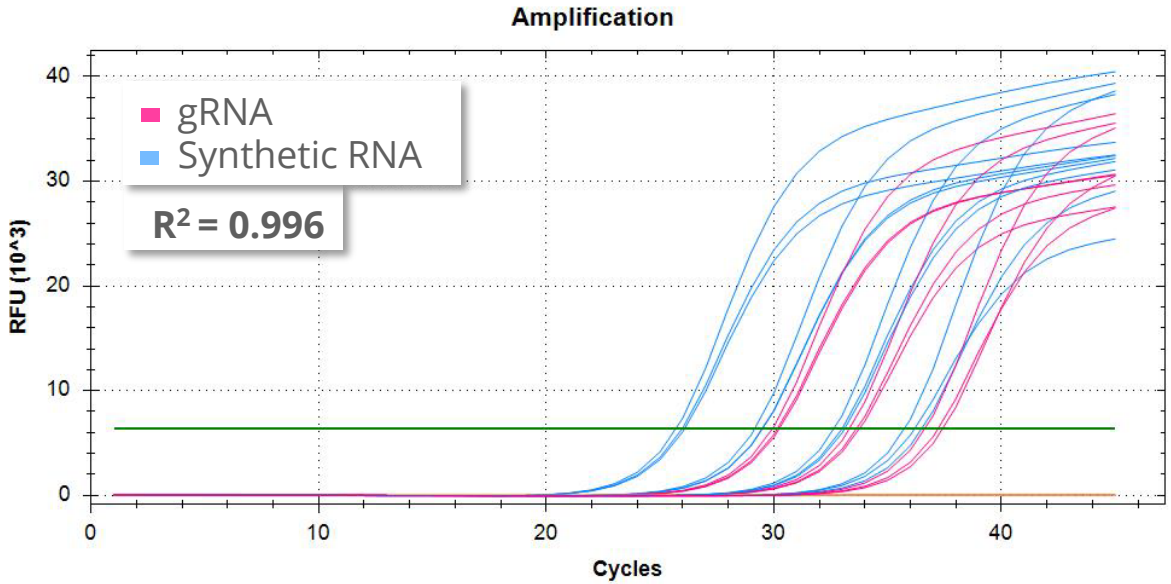
qPCR Amplification

Influenza A H5N1, ATCC® VR-3436SD™

Hoffmann *et al*., 2016 assay targeting **HA**



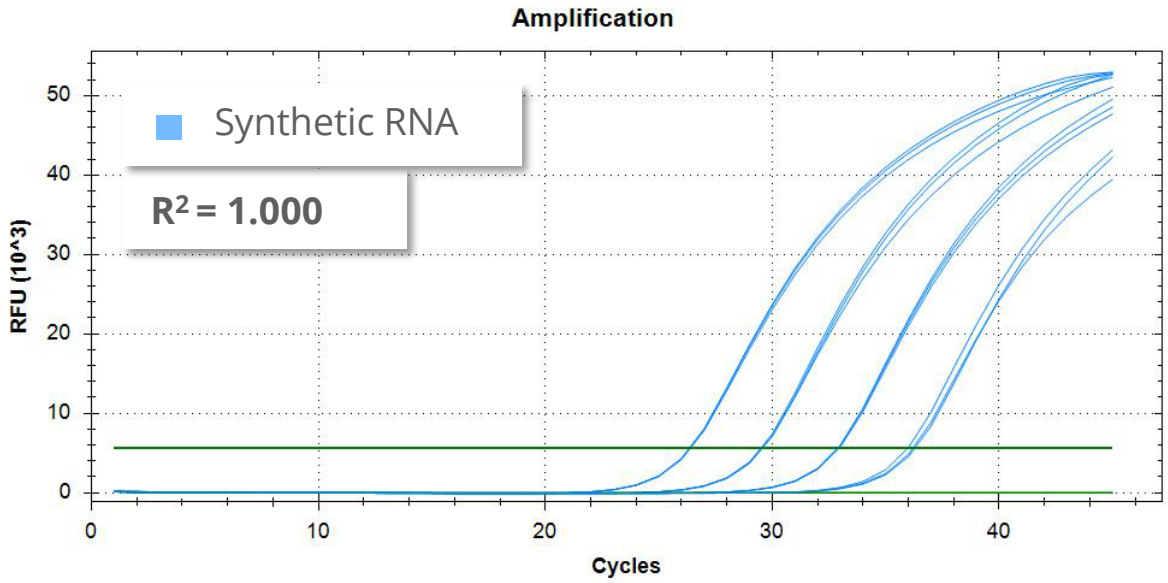
CDC Flu SC2 Multiplex assay targeting **M**



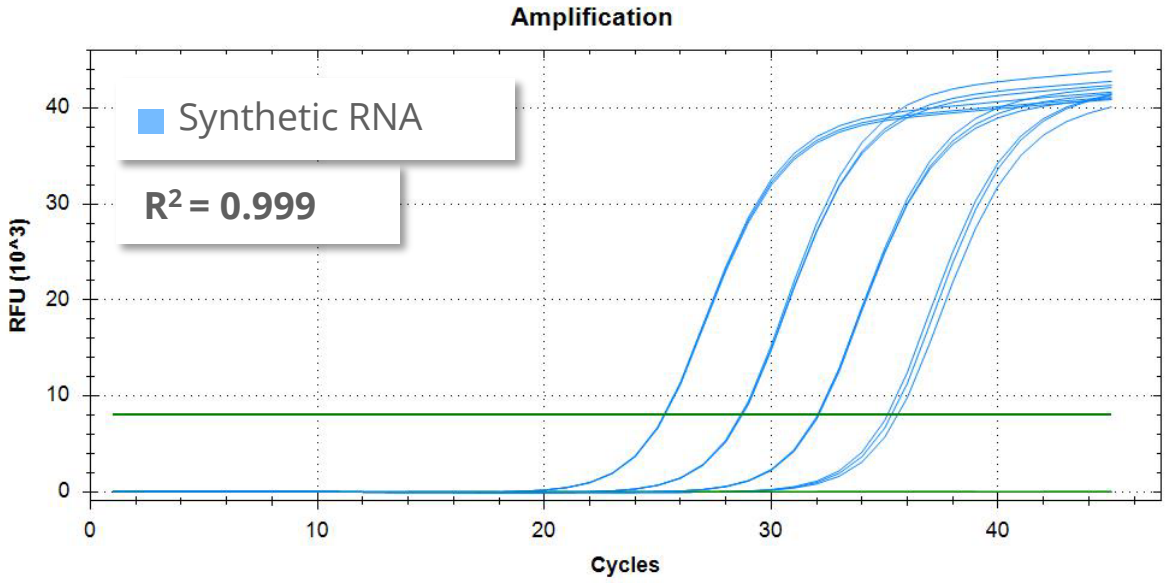
qPCR Amplification

Influenza A H5N6, ATCC® VR-3439SD™

Hoffmann *et al*., 2016 assay targeting **HA**



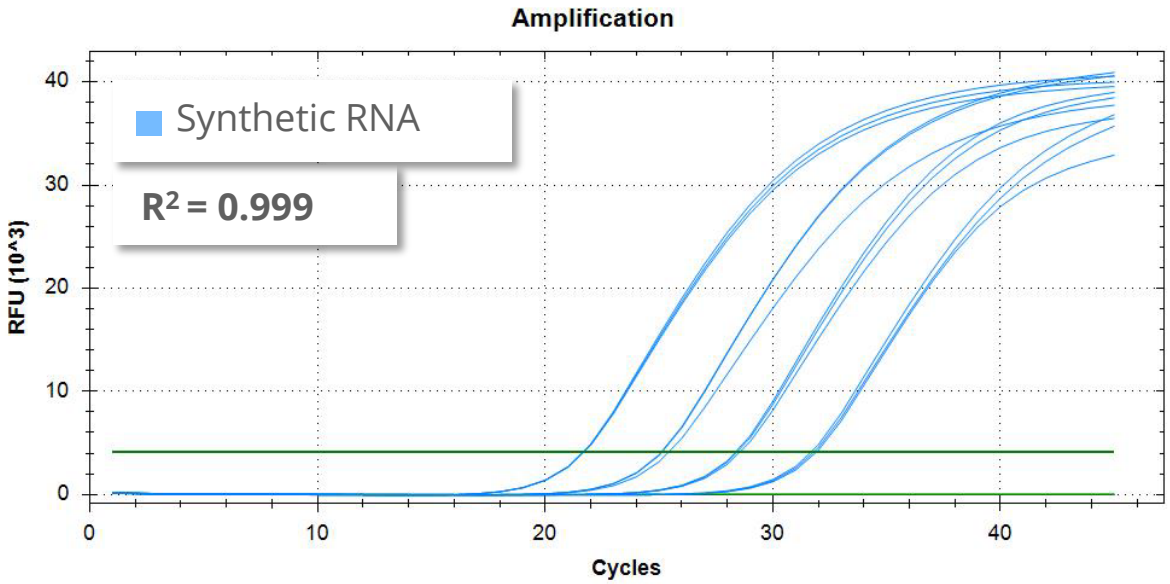
CDC Flu SC2 Multiplex assay targeting **M**



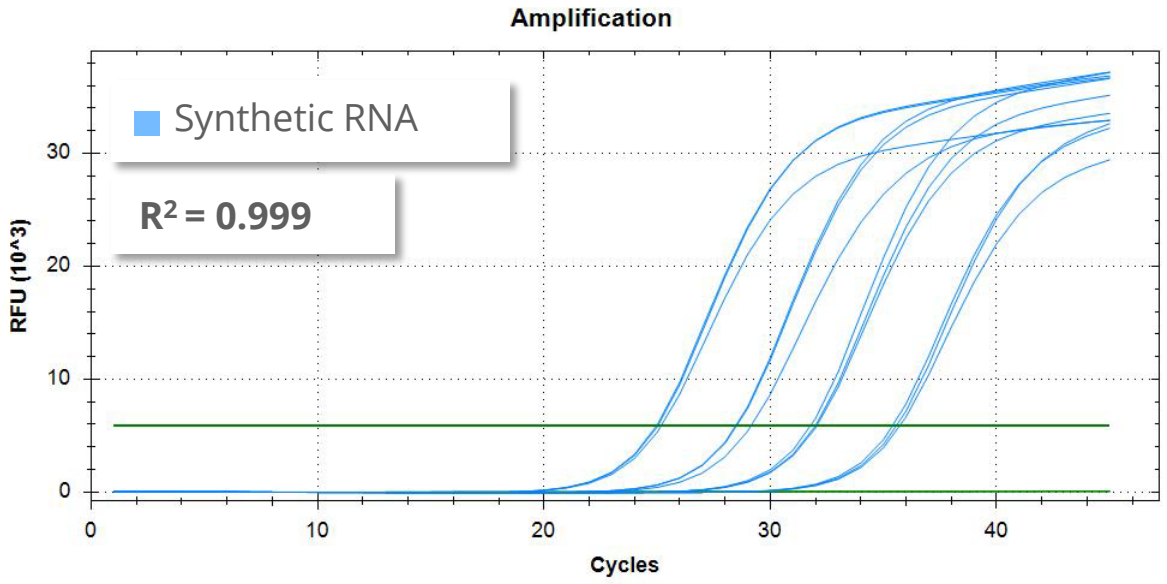
qPCR Amplification

Influenza A H7N9, ATCC® VR-3437SD™

WHO assay targeting **HA**



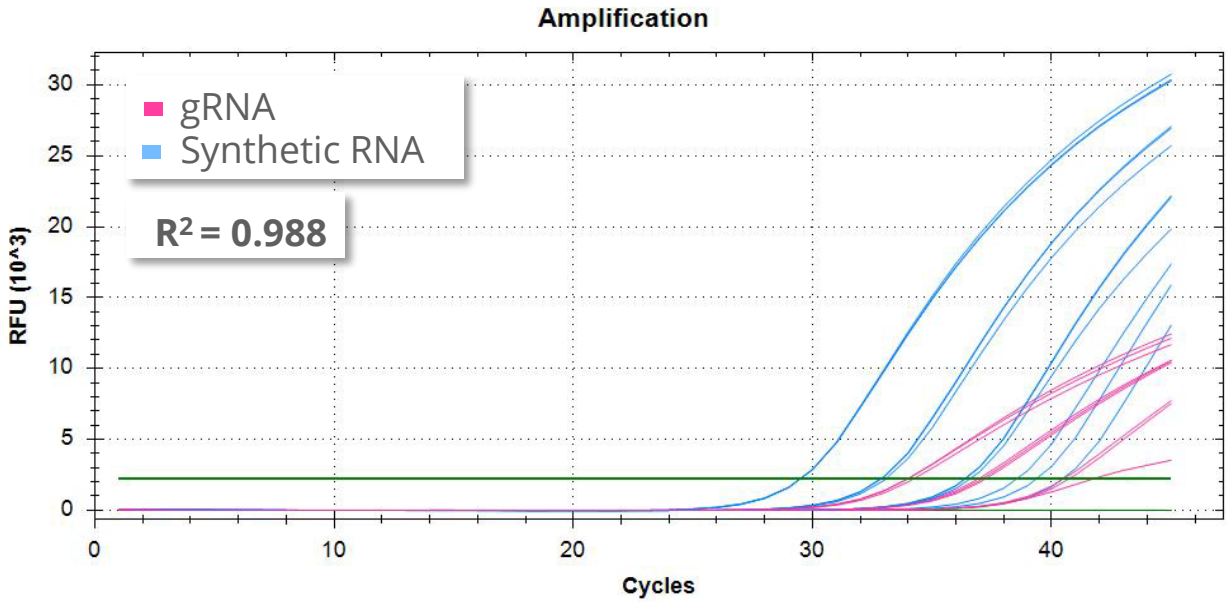
CDC Flu SC2 Multiplex assay targeting **M**



qPCR Amplification

Influenza A H5N1, ATCC® VR-3436SD™

FDA assay targeting **HA**

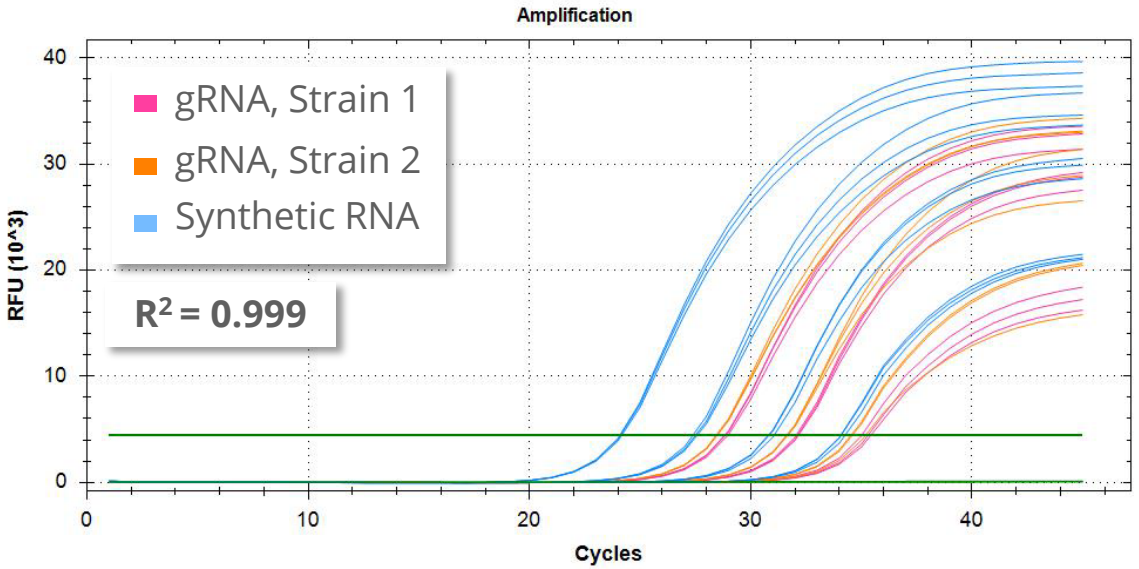


- Per the FDA protocol, the QIAGEN One-Step RT qPCR kit was used to achieve this amplification.
- Cycling conditions were 50°C for 50 min and 95°C for 15 min, followed by 45 cycles of 95°C for 15 sec, 64°C for 1 min, and 90°C for 1 min and 10 sec.

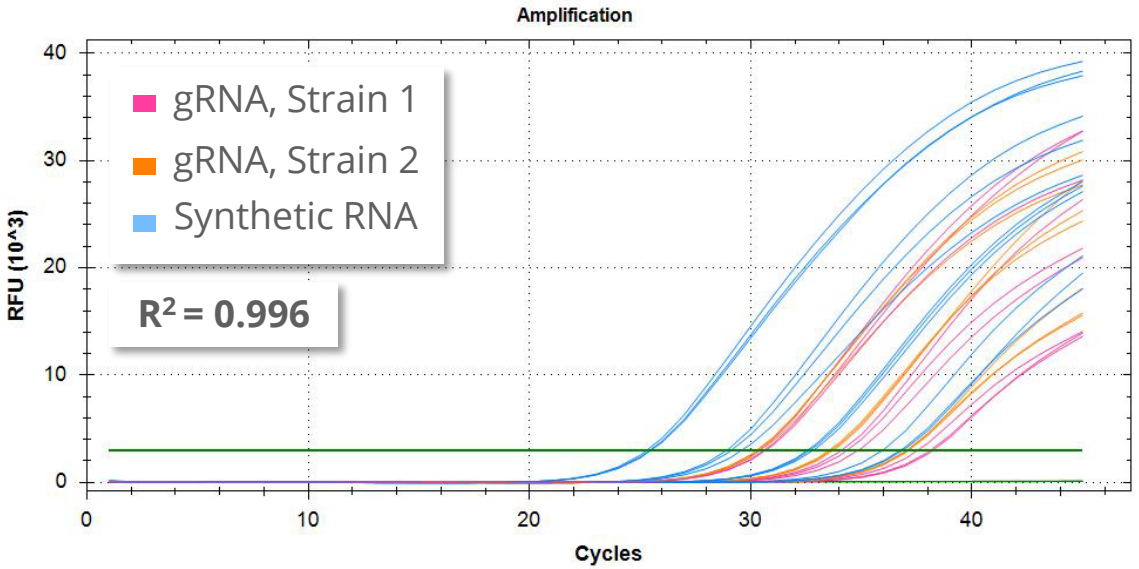
qPCR Amplification

Influenza A H1N1 pdm09, ATCC® VR-3388SD™

WHO assay targeting **HA**



WHO assay targeting **NA**



Conclusions

- Our data demonstrate that the ATCC® quantitative synthetic influenza viral RNA products can be used as reliable analytical reference materials for assay development, verification, and validation.
- The products can be used to generate a standard curve with qPCR assays to determine the viral load of samples.
- These analytical reference materials are compatible with numerous published assays and are shown here to serve as useful controls for viral detection and quantification.
- Lists of known compatible assays from primary literature and public health organizations are available on each product page in the technical data sheet.

ATCC Influenza Resources

References

- Breen M, *et al.* Viruses 8(7): 179, 2016. PubMed: 27347991.
- CDC, Research Use Only CDC Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay Real-Time RT-PCR Primers and Probes, CDC, 2020.
- FDA, HPAI H5 Subtyping in Milk and Milk Products Using RT-qPCR, 2024.
- Hassan KE, *et al.* Viruses 14(2): 415, 2022. PubMed: 35216008.
- Hoffmann B, *et al.* Sci Rep 6: 27211, 2016. PubMed: 27256976.
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- WHO Information for the Molecular Detection of Influenza Viruses, 2021.

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