ATCC Influenza Resources:

Supporting Science from Basic Research to Diagnostics, Surveillance, and Vaccines

Leka Papazisi, DVM, PhD, Principal Scientist, ATCC

Holly A. Asbury, BS, Senior Biologist, ATCC

Introducing today's speakers

Leka Papazisi, DVM, PhD
Principal Scientist, ATCC
Dr. Papazisi joined ATCC in 2019. His main responsibility is product development, from asset inception through lifecycle management. While at ATCC, Dr. Papazisi led the Microbiology R&D team in developing several new products, including a proprietary nucleic acid storage buffer formulation and various diagnostics control materials. In addition to technical leadership, his responsibilities include talent management, new product innovation, and management of internal and external cross-functional activities.

Holly A. Asbury, BS
Senior Biologist, ATCC
Holly joined the Microbiology R&D team in 2020. She focuses on developing and supporting analytical reference material products in the ATCC catalog. Holly has participated in several stability studies to determine product shelf-life and works on developing and troubleshooting real-time PCR and digital PCR assays for new product development, manufacturing, and quality control.

About ATCC

- Founded in 1925, ATCC® is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's premier biological materials resource and standards development organization
- 5,000+ cell lines
- 80,000 microorganisms
- Genomic & synthetic nucleic acids
- Media/reagents

- ATCC® collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Growing portfolio of products and services
- Sales and distribution in 150 countries, 20 international distributors
- Talented team of 600+ employees, over one-third with advanced degrees

bsi. ISO 9001 Quality Management

bsi. ISO 13485 Medical Devices Quality Management

ANAB ACCREDITED ISO/IEC 17025 TESTING LABORATORY

ANAB ACCREDITED ISO 17034 REFERENCE MATERIAL PRODUCER

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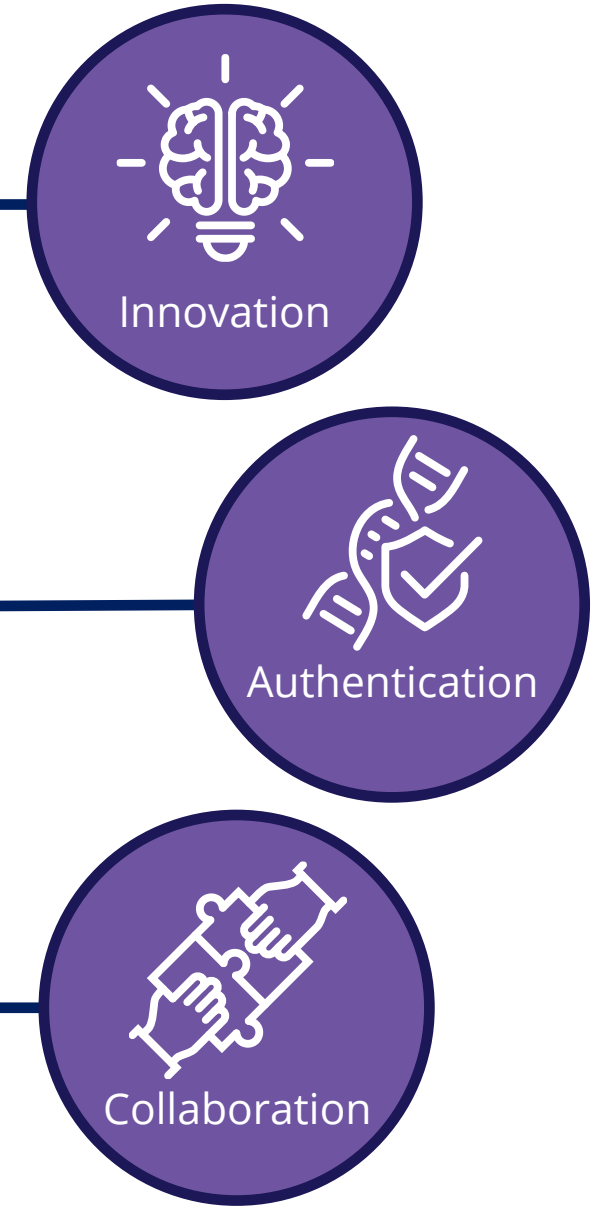
ATCC®

ATCC® 100 YEARS

We are inspired by customer needs for credibility and efficiencies in research



Agenda

- Human and avian influenza
- Influenza virus
- Prophylactics and therapeutics
- Diagnostics and surveillance

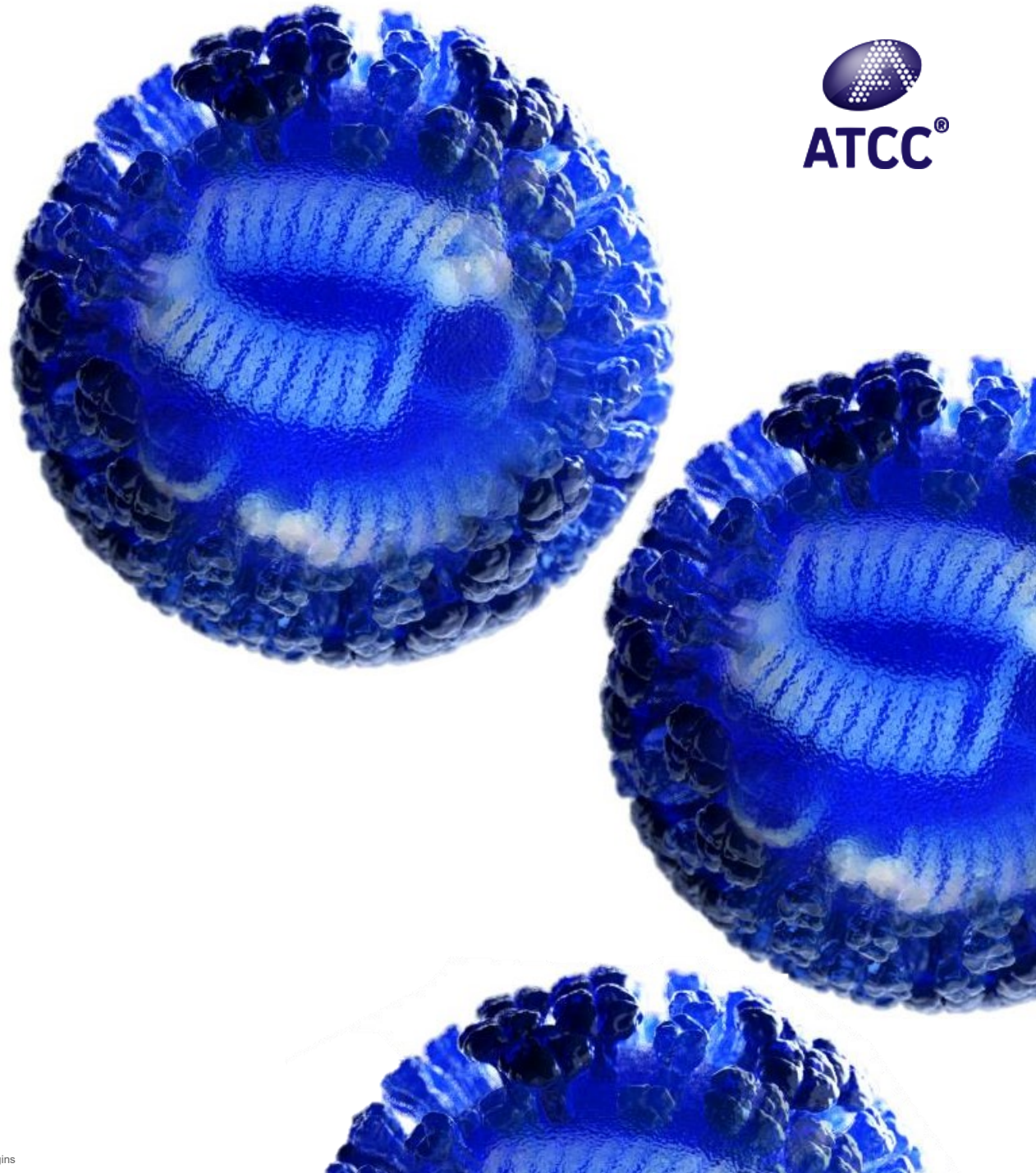


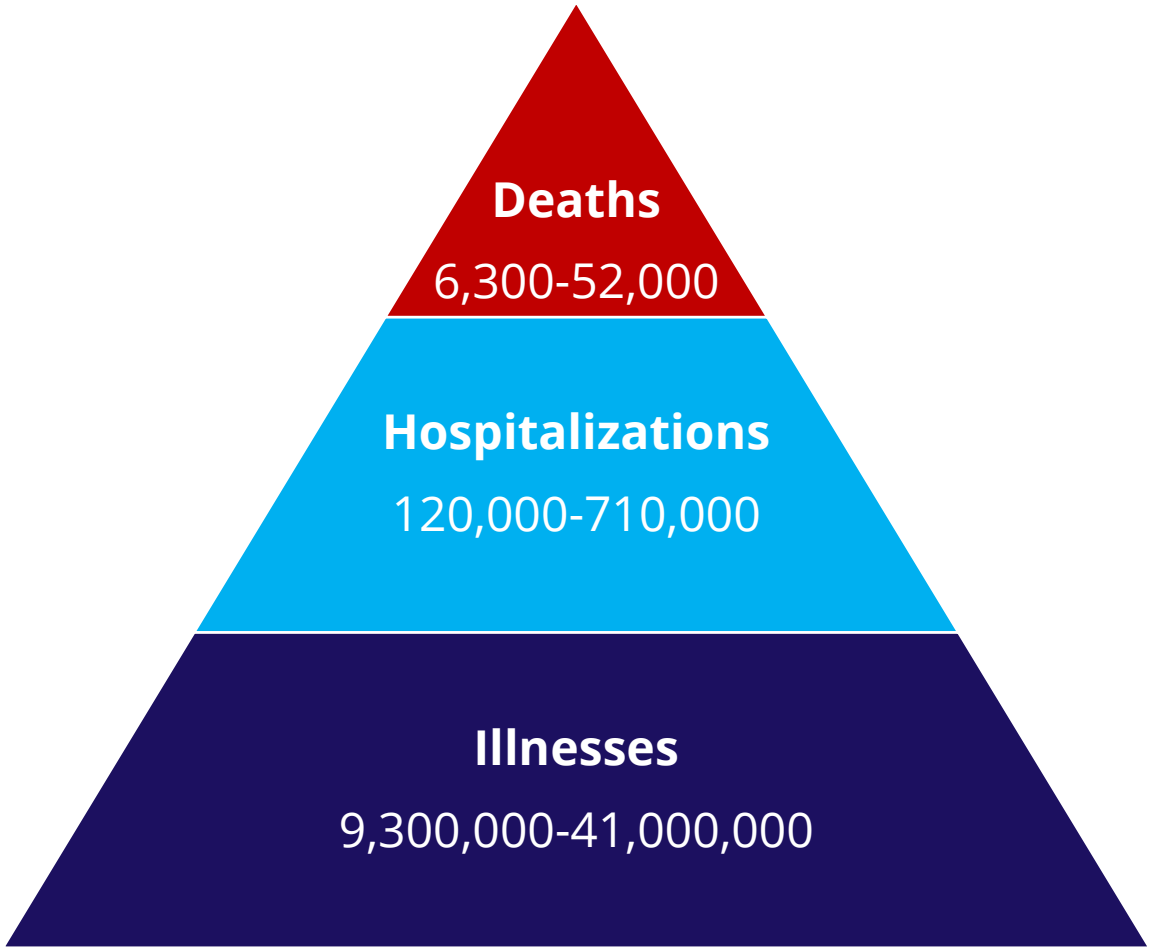
Photo credit: Dan Higgins

Human and avian influenza

Human influenza

"The flu"

- Acute respiratory infection caused by influenza virus types A, B, and C
- Symptoms are typically mild but can be severe in immunocompromised individuals
- Predominant health concern worldwide
- Affects 5-15% of the population annually in the northern hemisphere
- Transmitted through a respiratory route
- Prevention and control measures: vaccination, social distancing, and proper hygiene



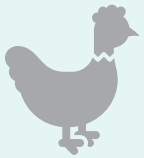
CDC's estimated range of annual burden of flu in the US from 2010-2024 (cdc.gov/flu-burden).

Avian influenza

The “bird flu”

- Highly contagious viral disease caused by influenza A viruses
- Originates in wild waterfowl
- Can be transmitted quickly to susceptible bird species (e.g., domestic livestock such as chickens and turkeys)
- Prevention and control measures:
  - Early detection and reporting
  - Separation of poultry and wild birds
  - Clean habitat conditions for poultry
  - Culling of infected flocks
  - Flock vaccination programs

400+ bird species globally affected with significant mortality



160+ million poultry birds have died or been culled in the United States

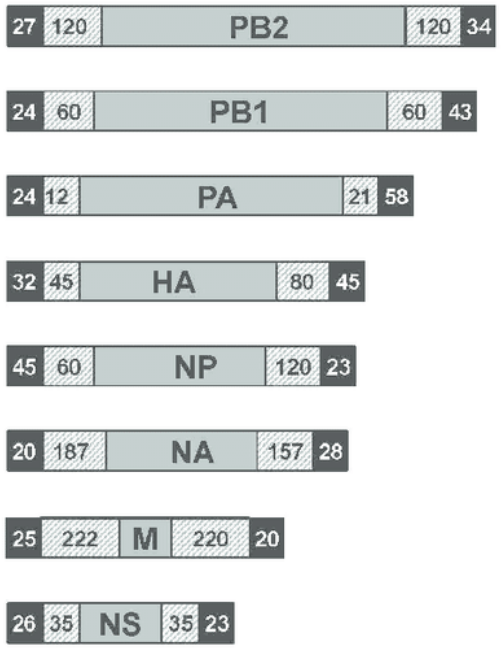
Sporadic human cases in people who had exposure to infected dairy cows

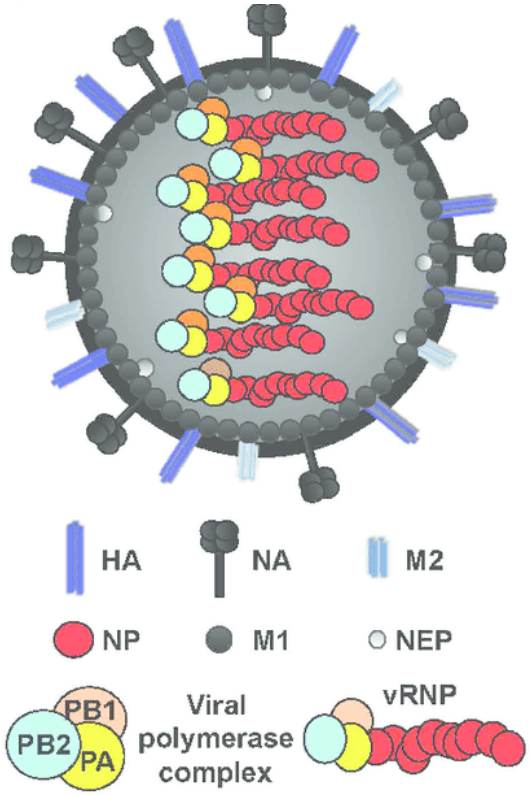
70 confirmed human cases of H5N1 in the United States with one reported death

Influenza virus

Influenza viruses

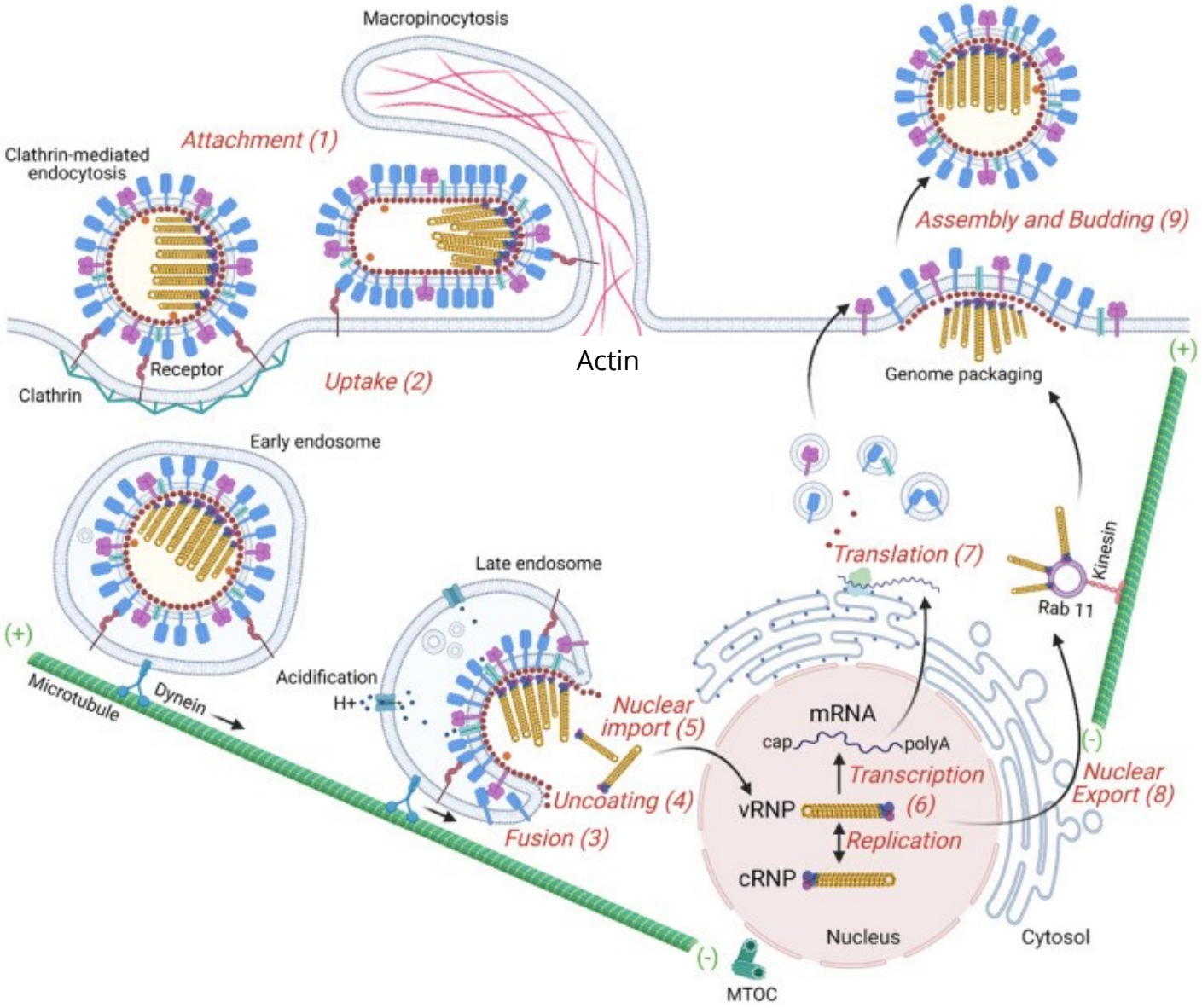
- Orthomyxoviridae family
- Segmented, single-stranded RNA genome with 8 segments
- 4 viral species/types:
  - **Influenza A and B** – mutates frequently causing epidemics and outbreaks every year as the seasonal flu
  - **Influenza C** – mutates much less frequently, causes mild disease, and rarely causes epidemics
  - **Influenza D** – similar to Influenza C, although it is a pathogen of cattle not humans





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

How influenza viruses infect a host cell

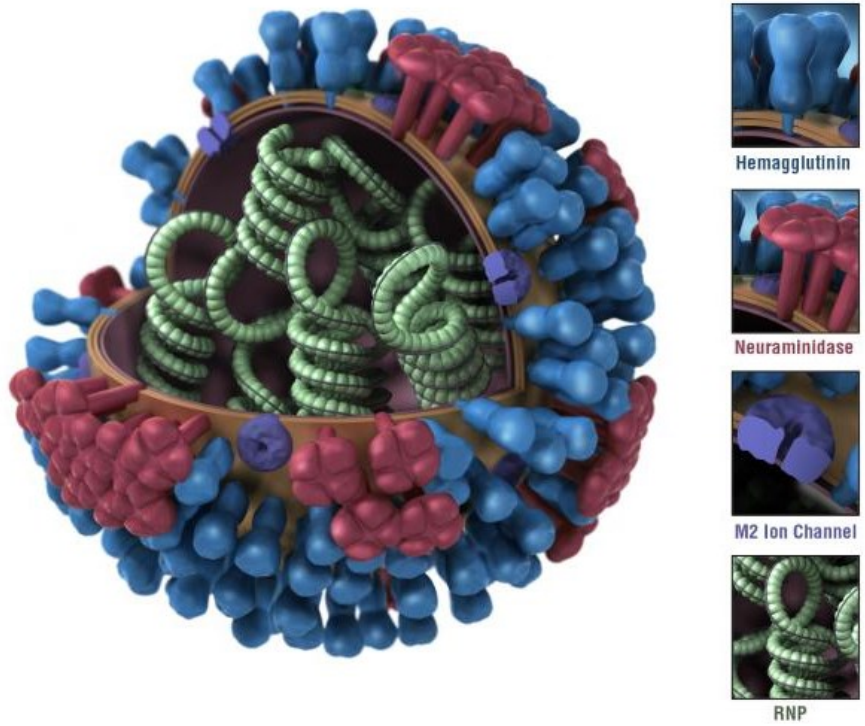


Moreira EA, et al. Cells 10(7): 1722, 2021. PubMed: 34359892

Influenza A viruses (IAV)

- Influenza A virus strains are named according to type, host, place of first isolation, year of isolation, and subtype

- The subtype designations are derived from two surface proteins:
  - Hemagglutinin (H1-H18)
  - Neuraminidase (N1-N11)
- Current seasonal human-affecting subtypes are H3N2 and H1N1pdm09 (descendant from the 2009 H1N1 “swine flu” pandemic)
- Some avian-origin influenza virus subtypes (H5N1, H7N9, H9N2) sporadically infect humans

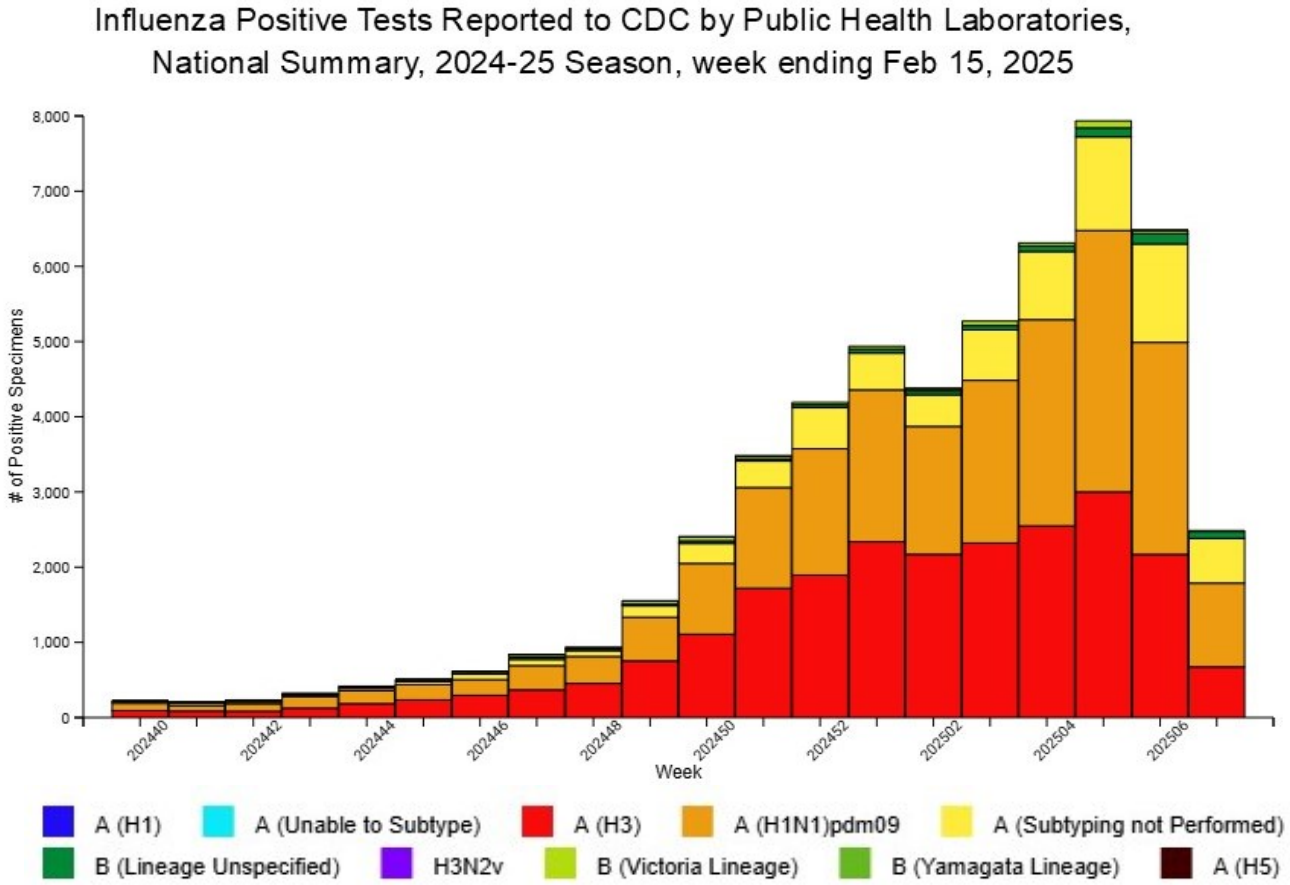


CDC. Types of influenza viruses. www.cdc.gov/flu/about/viruses-types.html

Influenza B viruses

Human pathogens causing seasonal outbreaks

- Two lineages characterized by differences in the hemagglutinin gene:
  - Victoria (globally prevalent)
  - Yamagata (diminished)
- Less pandemic potential than influenza A viruses
- Do not jump between host species



CDC data as of week 7 of 2025

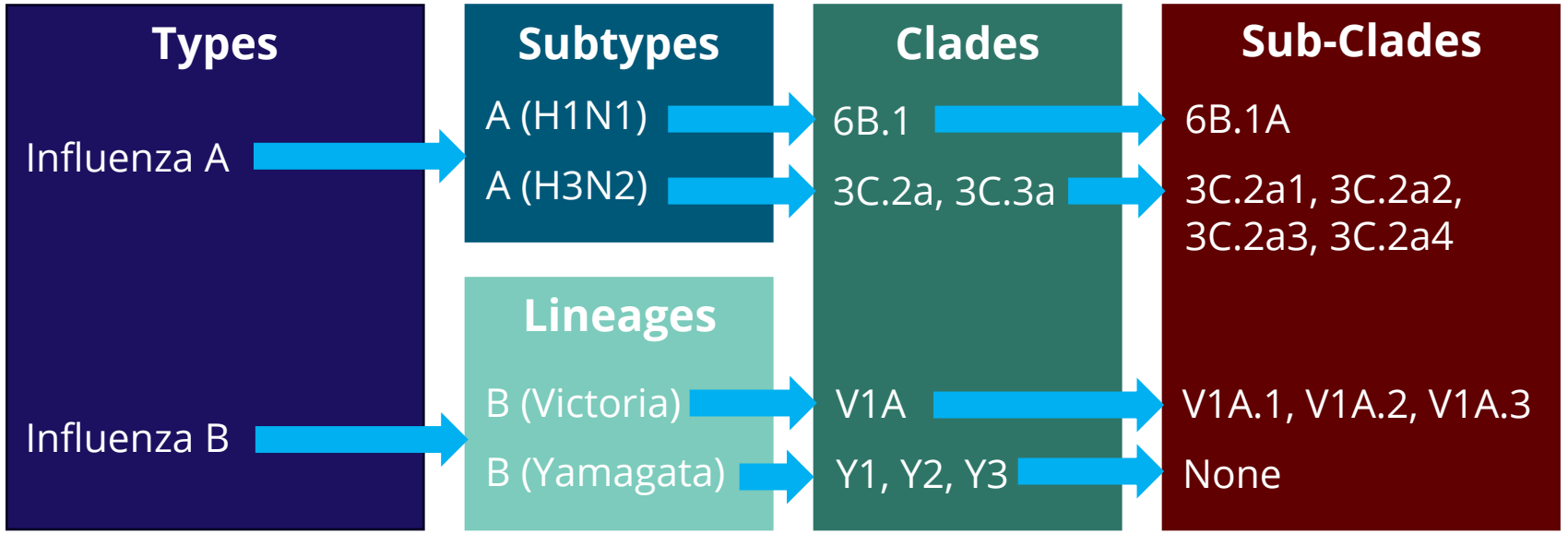
CDC FluView. https://www.cdc.gov/fluview/surveillance/2024-week-50.html

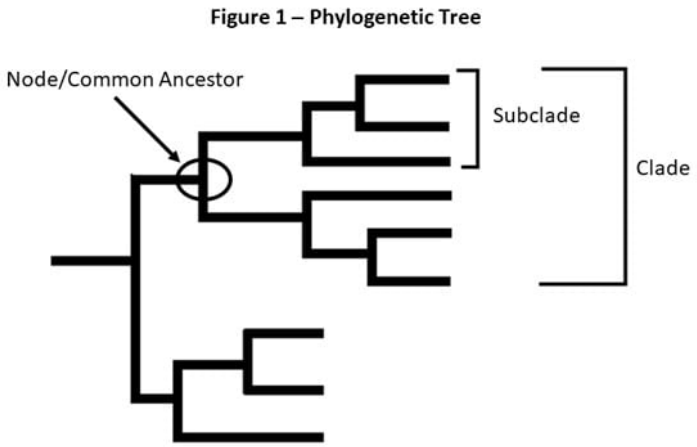
Clades and subclades

What are they?

Influenza A subtypes are further classified into clades and subclades based on genomic similarity among strains

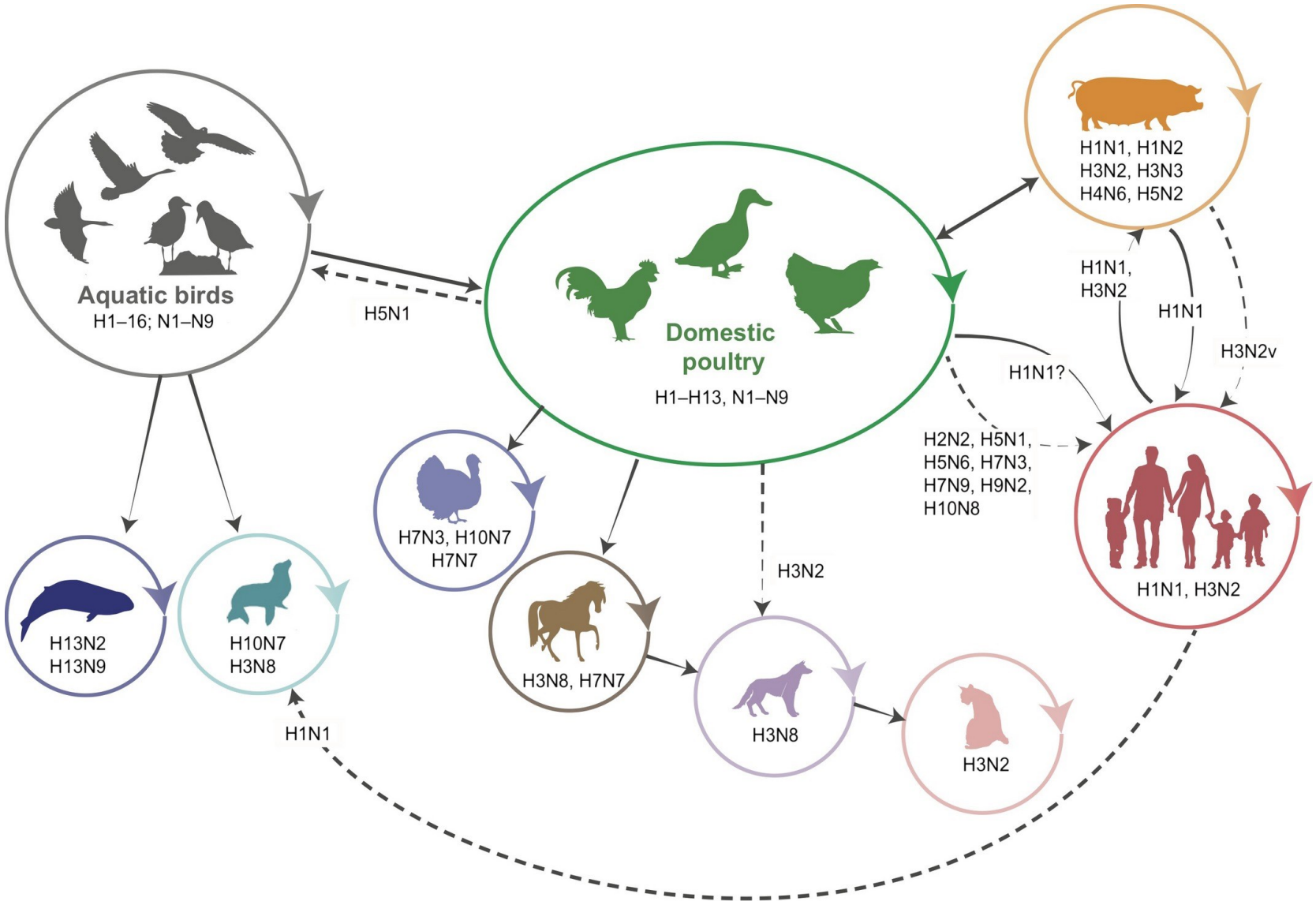
Human Seasonal Influenza Viruses (Select examples)





CDC. Types of influenza viruses. www.cdc.gov/flu/about/viruses-types.html

The ecology and interspecies transmission of IAV



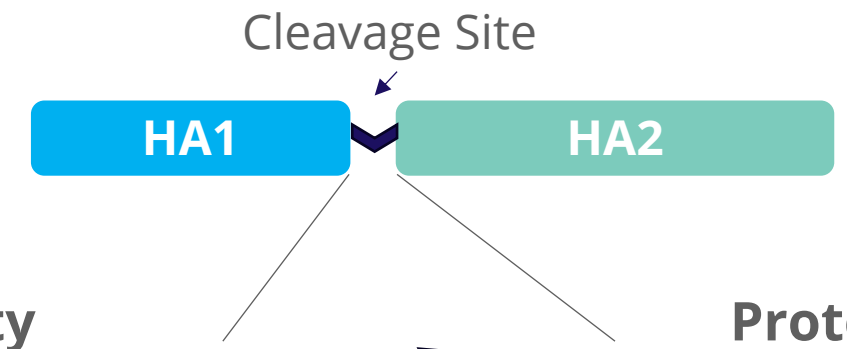
Joseph U, et al. Influenza Other Respir Viruses 11(1): 74-84, 2016. PubMed: 27426214

Antigenic drift & antigenic shift

How can the flu jump from species to species?

HPAI versus LPAI

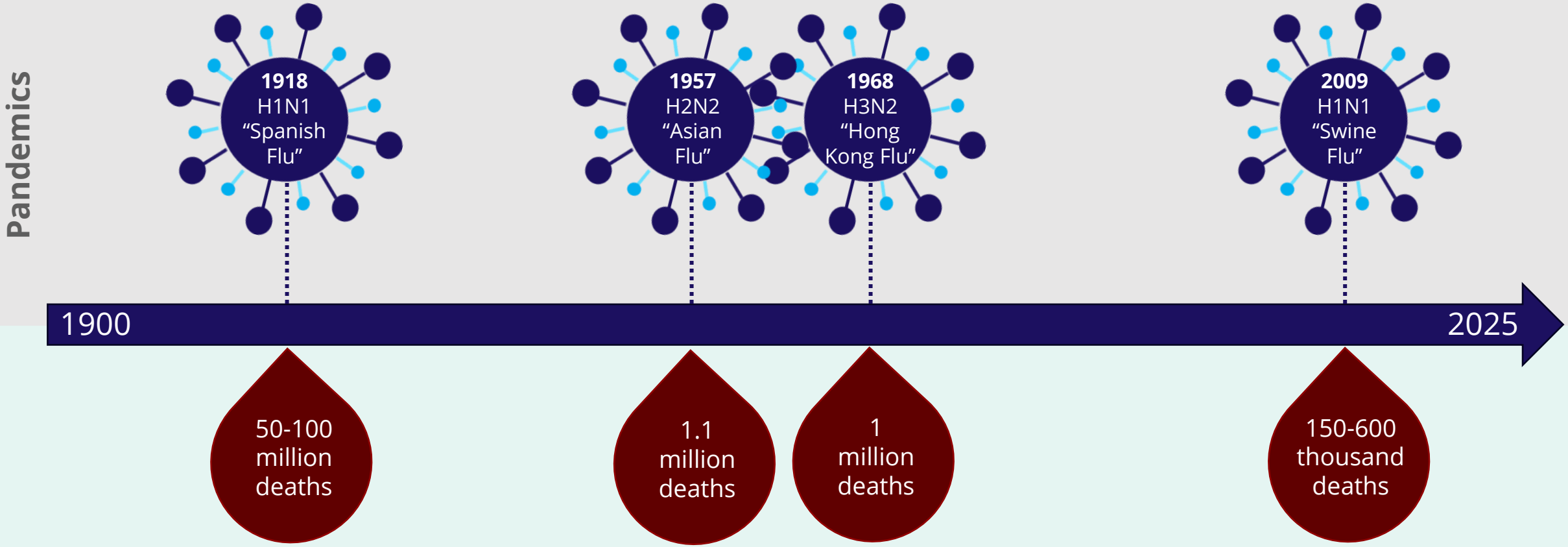
The “polybasic cleavage site” in hemagglutinin (HA) confers pathogenicity



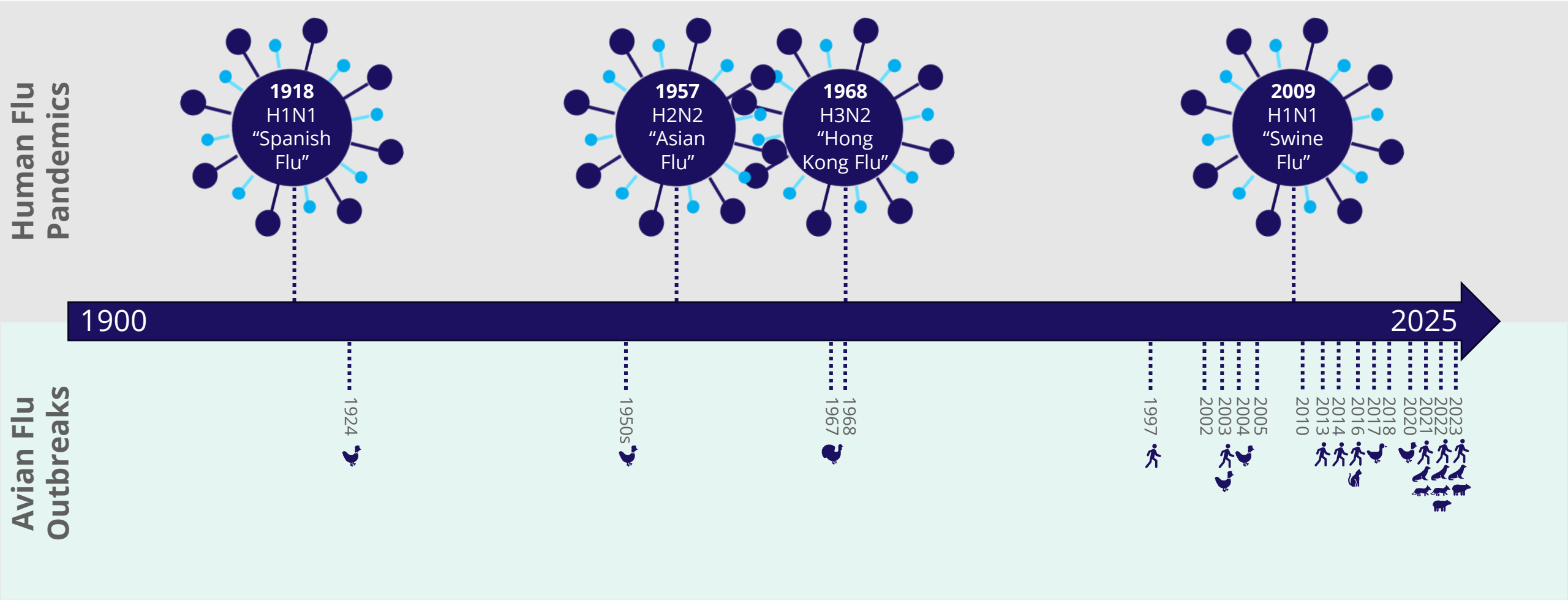
Pathogenicity		Protease accessibility
Low	R--ETR ↓ G	Cleaved by proteases in limited organs
Intermediate	R--KKR ↓ G	
High	RR-KKR ↓ G	Cleaved by ubiquitous proteases
High	RRRKKR ↓ G	
High	KRRKTR ↓ G	

Kida Y, et al. Pathogens 10(12): 1597, 2021. PubMed: 34959552
Steinhaue DA. Virology 258(1): 1–20, 1999. PubMed: 10329563

Human influenza A virus timeline

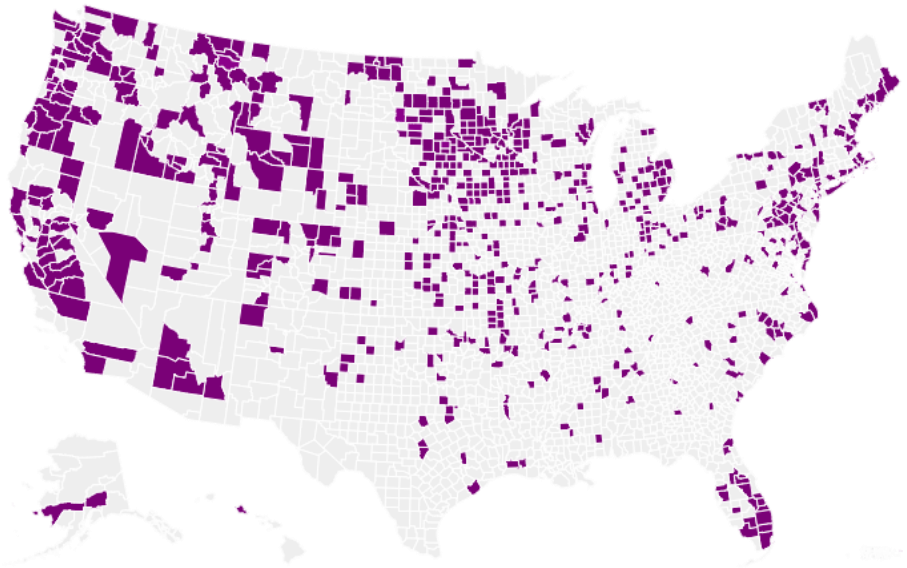


Influenza A virus timeline



HPAI outbreak situation in the US

2022 onwards

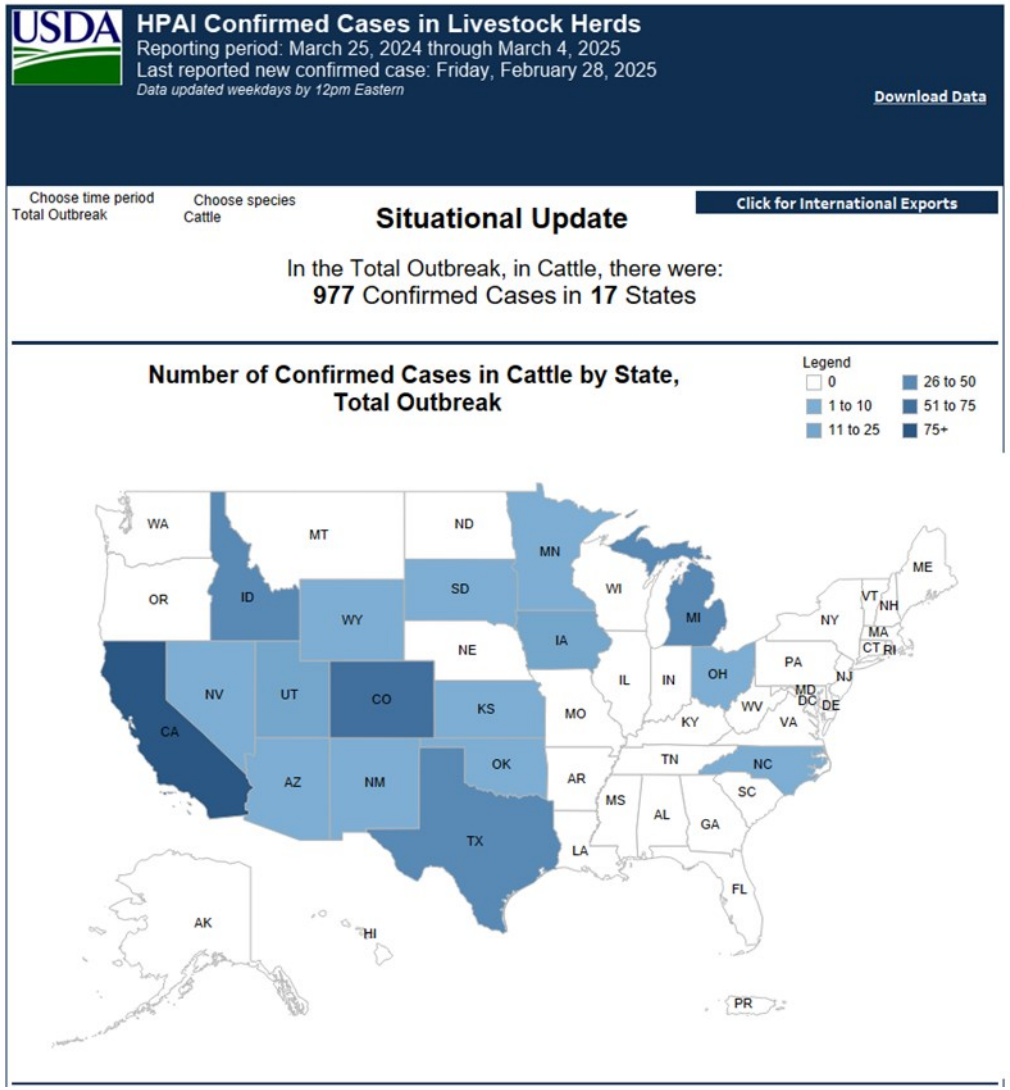


Birds Affected
166,191,381

Highly pathogenic avian influenza (HPAI) A(H5) viruses have been detected in U.S. wild aquatic birds, commercial poultry and backyard or hobbyist flocks beginning in January 2022. These are the first detections of HPAI A(H5) viruses in the U.S. since 2016. Preliminary genetic sequencing and RT-PCR testing on some virus specimens shows these viruses are HPAI A(H5N1) viruses from clade 2.3.4.4.

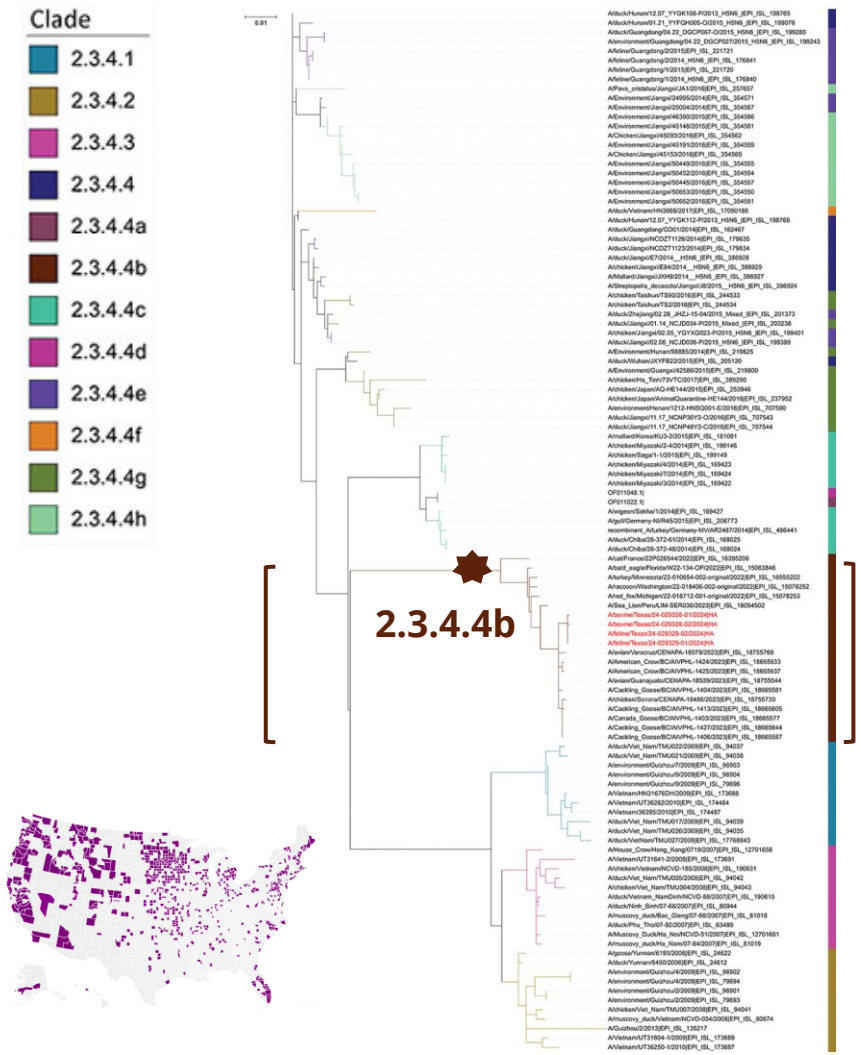
States Affected	Counties Affected	Reported Outbreaks
51	659	1,622

CDC. Avian influenza. https://www.cdc.gov/bird-flu/situation-summary/data-map-commercial.html

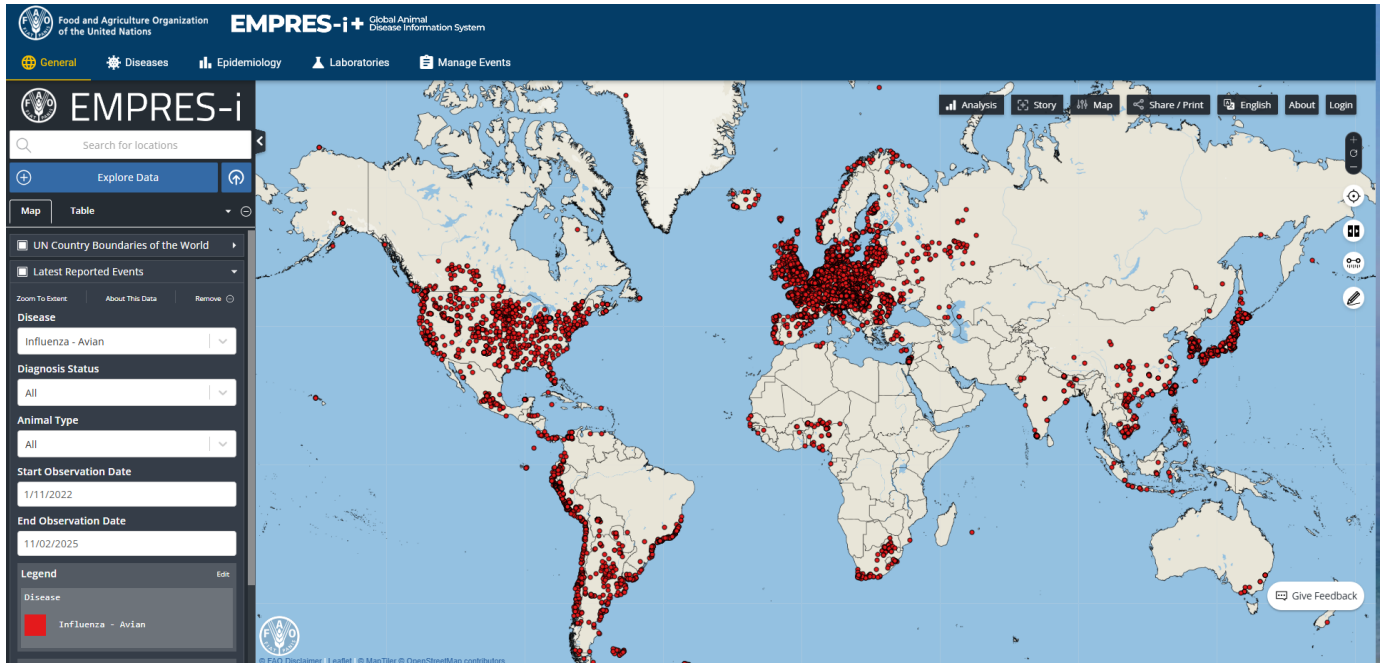


USDA. HPAI Confirmed Cases in Livestock. https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-detections/hpai-confirmed-cases-livestock

Outbreaks and the Emergence of HPAI H5N1 clade 2.3.4.4b



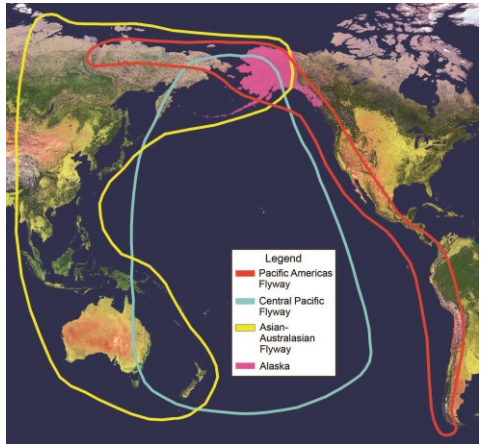
Avian Influenza Prevalence 2022-0225

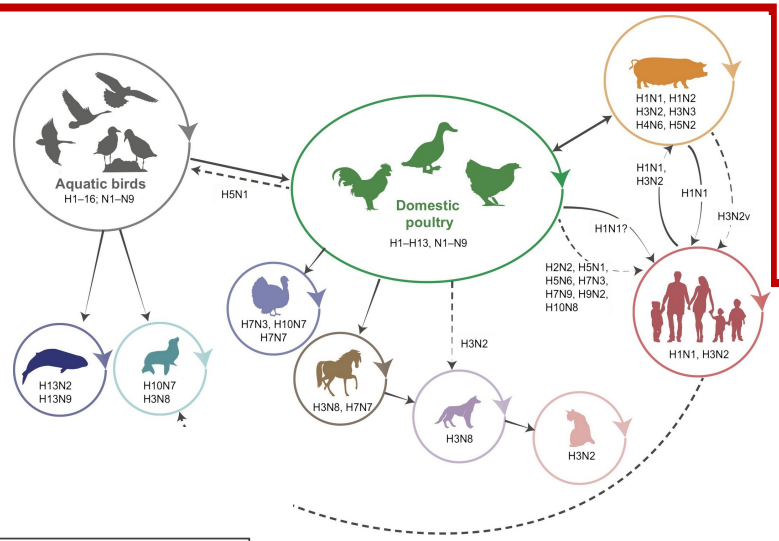


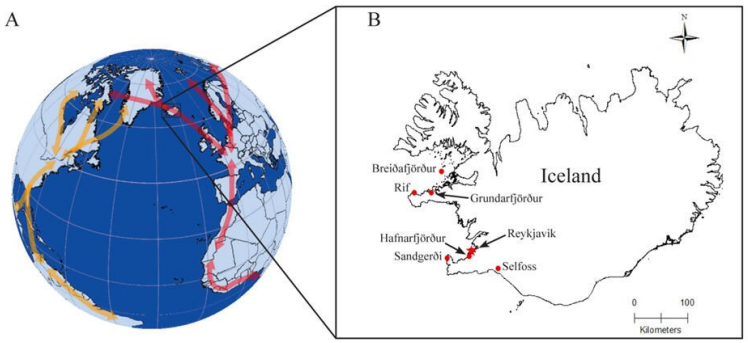
- https://data.apps.fao.org/empres-i/?lang=en&share=f-2038044a-e924-4cd3-8f47-01b0e5b28fe4
- Burrough ER, et al. Emerg Infect Dis 30(7): 1335-1343, 2024. PubMed: 38683888

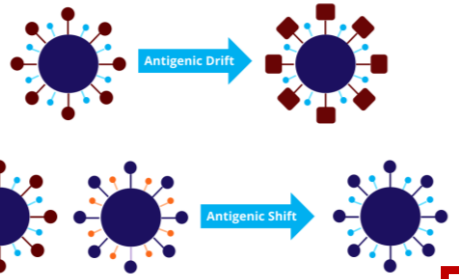
Surveillance is the key to assess and prevent outbreaks

Reliable surveillance depends on reliable diagnostics tools





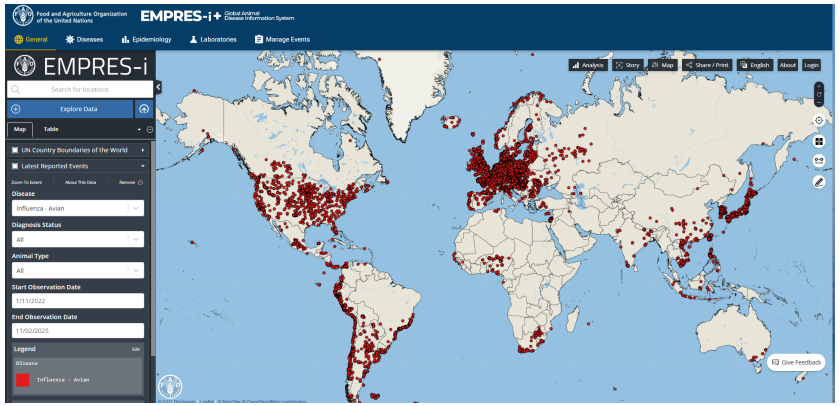




North Atlantic May Be a New Route for Spread of Avian Flu to North America

By Communications and Publishing March 19, 2014

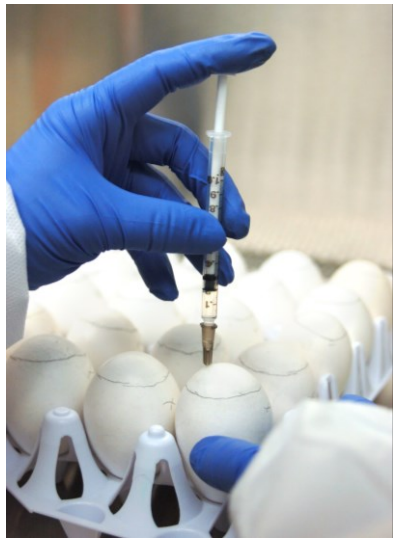
The North Atlantic region is a newly discovered important pathway for avian influenza to move between Europe and North America, according to a U.S. Geological Survey report published today.



Joseph U, et al. Influenza Other Respir Viruses 11(1): 74-84, 2016. PubMed: 27426214
https://www.usgs.gov/media/images/illustration-showing-migratory-bird-paths-across-pacific
https://www.usgs.gov/news/national-news-release/north-atlantic-may-be-new-route-spread-avian-flu-north-america
https://data.apps.fao.org/empres-i/?lang=en&share=f-2038044a-e924-4cd3-8f47-01b0e5b28fe4

Prophylactics & therapeutics

Vaccine production methods



Egg-based

- Most used method
- Process that has been around for >70 years
- Candidate viruses are grown in chicken eggs and the resulting viruses are then either inactivated or attenuated

Cell culture-based

- Approved by the FDA in 2012
- Candidate viruses are grown in cells such as:
  - MDCK (NBL-2) cells (ATCC® CCL-34™)
  - 293.STAT1 BAX KO cell line (ATCC® CRL-1573-VHG™)

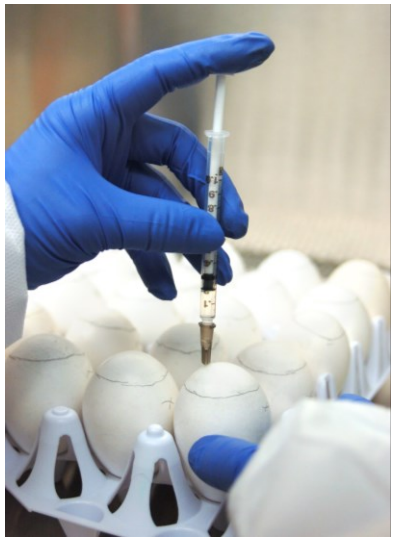
Recombinant technology

- Approved by the FDA in 2013
- Synthetic vaccines are produced with baculovirus vectors using HA genes from strains of interest
- These baculovirus vectors are propagated in Sf9 cells such as ATCC® CRL-1711™

mRNA

- Combined influenza and COVID-19 mRNA vaccines are in development as well as monovalent clade 2.3.4.4b H5N1 mRNA vaccines
- No flu vaccines using this method have yet been approved by the FDA

High-growth donor virus vaccine strains

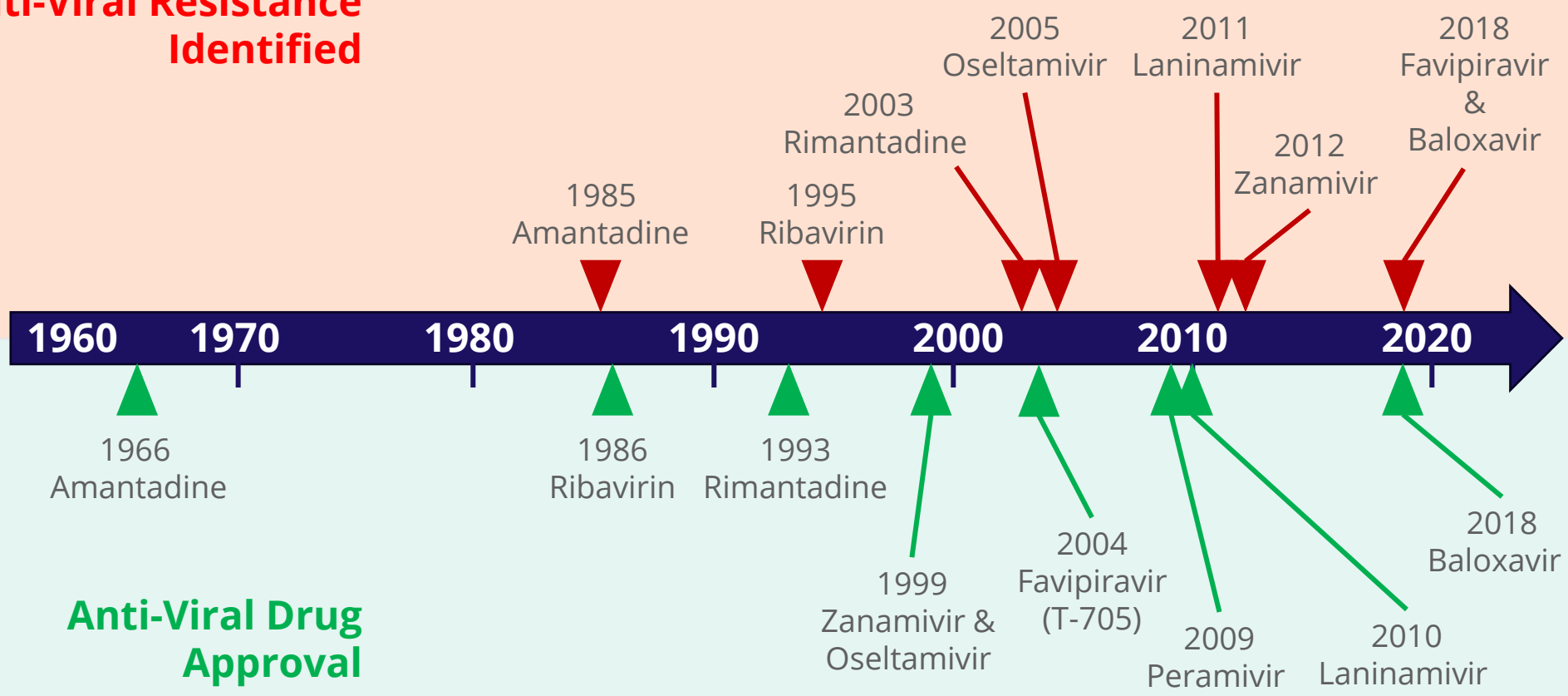


- Candidate vaccine viruses (CVVs) relevant to the upcoming flu season are recombined with a **“high-growth donor virus” strain such as A/Puerto Rico/8/34** (PR8), which usually grows well in embryonated chicken eggs.

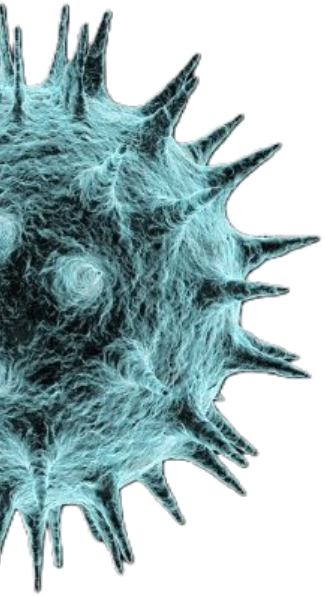
- **A/PR/8/34 uses**
  - Vaccines
    - Reassortment
    - Vaccine backbones
    - Chimeric genes
  - Respiratory disease research
  - Antiviral studies
  - Assay development

- **ATCC® A/PR/8/34 variants**
  - ATCC® VR-95™ (egg grown)
  - ATCC® VR-95PQ™ (high titer)
  - ATCC® VR-1469™ (cell culture grown)

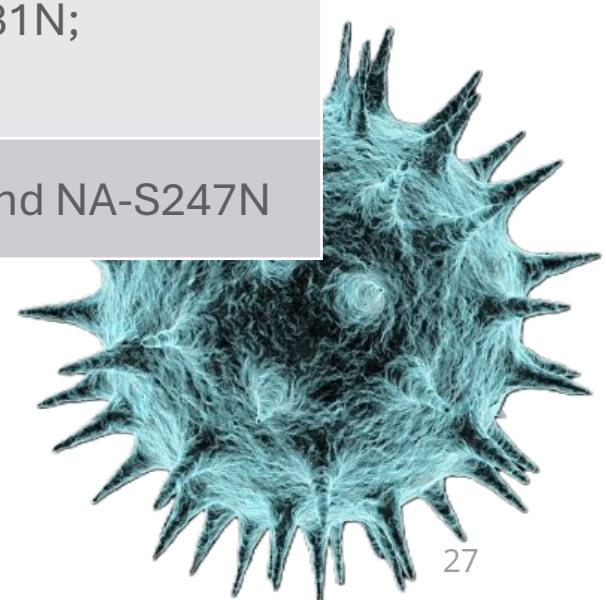
Antiviral drugs against influenza virus



Influenza drug-resistant strains at ATCC®



Catalog number	Strain designation	Antiviral Drug Resistance and Corresponding Mutation Profiles
VR-1987 ™	A/Washington/10/2008	Amantadine, rimantadine, M2: S31N
VR-1988 ™	A/Washington/29/2009	Amantadine, rimantadine, M2: S31N; Tamiflu (Oseltamivir), NA: H275Y
VR-3441™	A/Connecticut/11/2023	Tamiflu (Oseltamivir), NA-I223V and NA-S247N



Tamiflu is a registered trademark of Hoffmann-La Roche Inc.

Diagnostics and surveillance

FDA milk testing for H5N1 detection

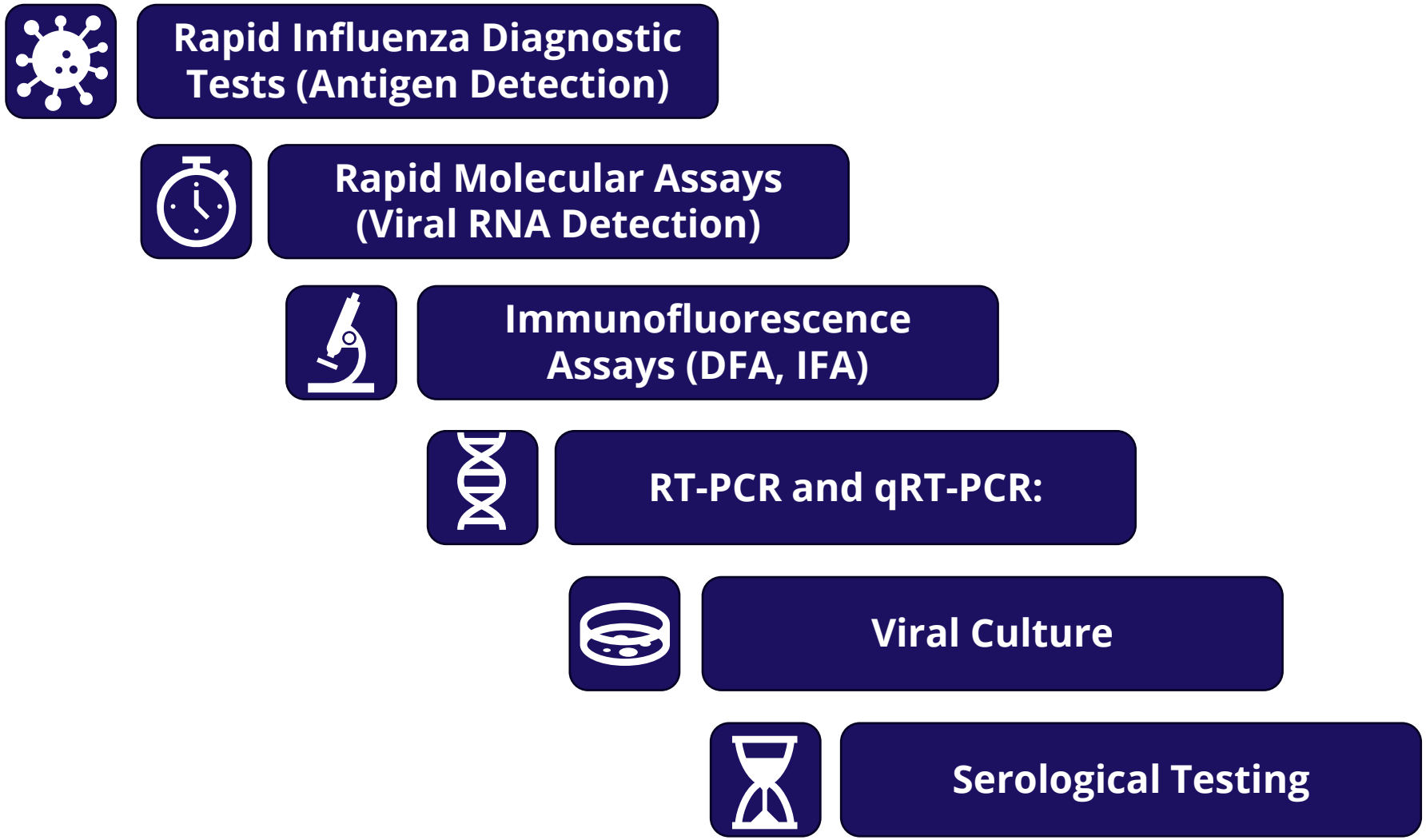
HPAI H5 Subtyping in Milk and Milk Products Using RT-qPCR

EXPIRATION 12/2025

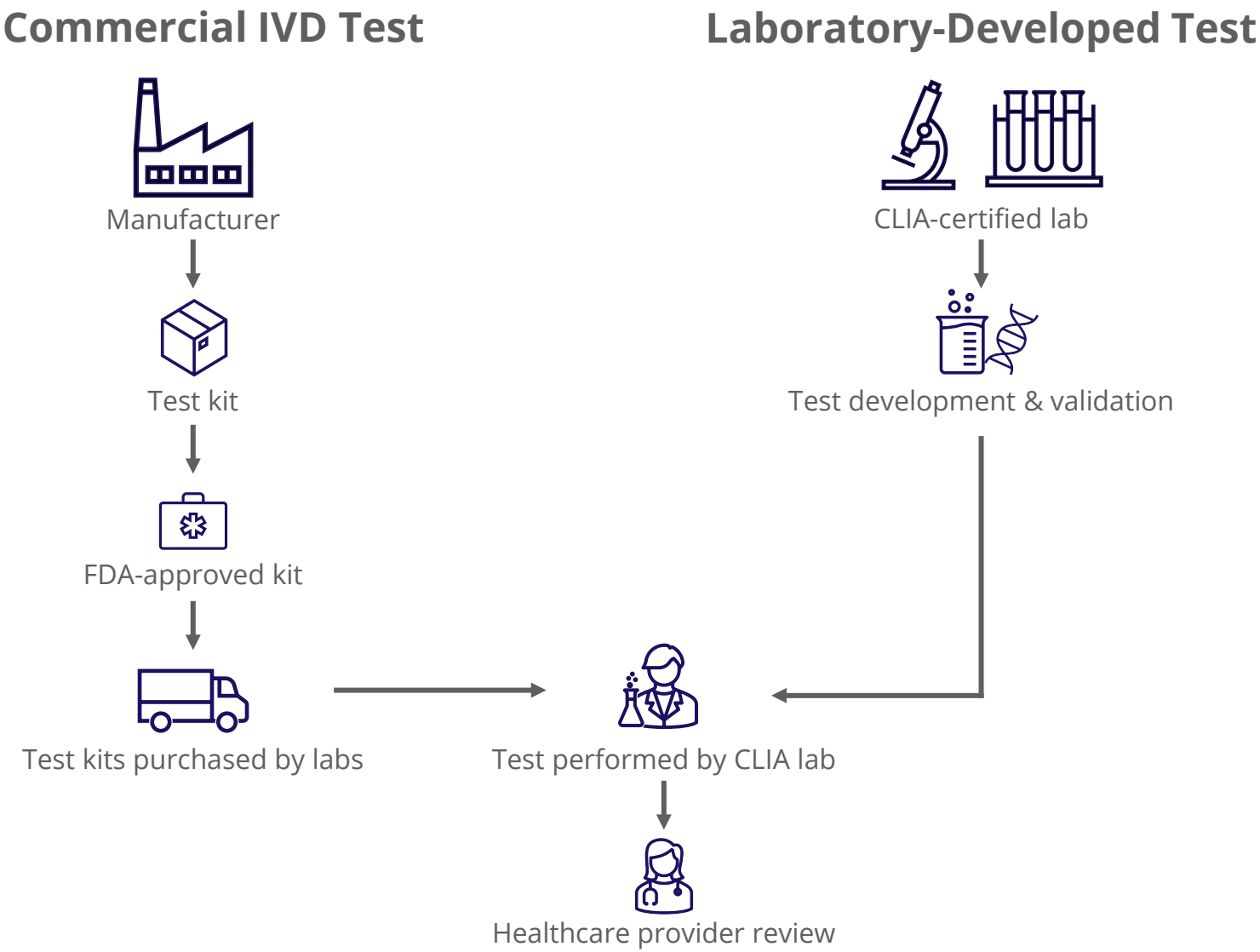
The recent emergence of the Highly Pathogenic Avian Influenza (HPAI) in cattle in the U.S has elevated concerns over whether the virus can result in contamination of the milk supply for humans and animals and has spurred the need for effective and efficient testing of milk for HPAI. Currently, molecular diagnostic tools are being used to screen milk for potential HPAI contamination, but confirmation must be accomplished through traditional propagation methods to determine if any viable virus is present in the milk. To support response for this event, FDA will screen milk and milk products for Influenza A virus (IAV) followed by H5 subtyping and submit positive samples to a diagnostic laboratory for confirmation testing.

From the FDA protocol for milk testing
https://www.fda.gov/media/180739/download

Influenza detection and diagnostic testing methods



Clinical diagnostic tests



ATCC® offerings for test validation and 510k clearance

Sensitivity, LOD, and specificity

Influenza

- Influenza products (viruses, native RNA, synthetic RNA, and antisera and monoclonal antibodies)
  - Influenza A
    - H1N1, H1N1, pdm2009, H3N2, H3N8, H5N1, H5N2, H5N6, H7N7, H7N9, H9N7, H9N2
  - Influenza B
    - Victoria and Yamagata
  - Strains used in 510k clearances
  - Strain recommended in FDA draft guidance documents

Non-Influenza Respiratory Pathogens

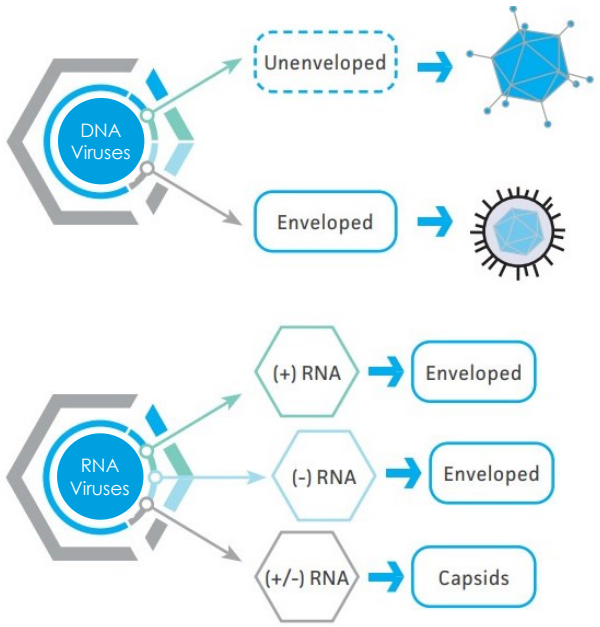
- Adenovirus (A-F)
- Seasonal CoV (229E, OC43, HKU1, NL63)
- SARS-CoV and CoV-2
- MERS-CoV
- Human Metapneumovirus
- Parainfluenza (1, 2, 3, 4a, 4b)
- Rhinovirus / Enterovirus (A-E)
- RSV (A, B)
- *Bordetella pertussis* and *B. parapertussis*
- *Chlamydophila pneumoniae*
- *Mycoplasma pneumoniae*
- *Mycobacterium tuberculosis*

Cross Reactivity Panel

- Commensals and other non respiratory pathogens

Browse our portfolio

Reference materials for viral metagenomics



Composition of Virome Analytical Refence Materials
Human herpesvirus 5 strain AD169 (ATCC® VR-538™)
Human mastadenovirus strain F (ATCC® VR-931™)
Influenza B virus strain B/Florida/4/2006 (ATCC® VR-1804™)
Zika virus strain MR 766 (ATCC® VR-1838™)
Reovirus 3 strain Dearing (ATCC® VR-824™)
Human respiratory syncytial virus strain A2 (ATCC® VR-1540™)

Intended Use	Content	ATCC® Catalog No.	Number of Organisms	Specification (ddPCR)	Applications
Virome Analysis	Virus Mix	MSA-2008™	6	2×10^3 genome copies/µL per virus	• Assay development, optimization, verification, and validation. • Reproducibility assessment (routine QC).
	Nucleic Acid Mix	MSA-1008™	6	2×10^4 genome copies/µL per virus	

ddPCR is a registered trademark of Bio-Rad Laboratories, Inc.

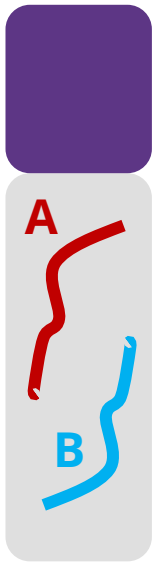
10 new influenza synthetic RNA products

Quantitative BSL-1 analytical reference materials

ATCC® Product	Influenza strain used for design (subtype/lineage)
VR-3384SD™	B/Malaysia/2506/2004 (Victoria lineage)
VR-3385SD™	B/Brisbane/60/2008 (Victoria lineage)
VR-3386SD™	A/Brisbane/59/07 (H1N1)
VR-3387SD™	A/Hiroshima/52/2005 (H3N2)
VR-3388SD™	A/Netherlands/2629/2009 (H1N1pdm2009)
VR-3436SD™	A/white-tailed eagle/Japan/OU-1/2022 (H5N1)
VR-3437SD™	A/Shanghai/4664T/2013 (H7N9)
VR-3438SD™	A/chicken/Wenzhou/334b/2013 (H7N7)
VR-3439SD™	A/goose/Guangdong/GS018/2015 (H5N6)
VR-3440SD™	A/ostrich/Yunnan/438/2014 (H9N2)

10 new influenza synthetic RNA products

Quantitative BSL-1 analytical reference materials



- Two transcript design covering 5 genome segments
  - Transcript A contains HA and NP
  - Transcript B contains M1/M2, NA, and NEP/NS1
- Quantified by ddPCR
  - 1×10^5 to 1×10^6 copies/µL
- Polybasic cleavage site removed in HPAI viral products

ddPCR is a registered trademark of Bio-Rad Laboratories, Inc.

Validation of influenza synthetic RNA products

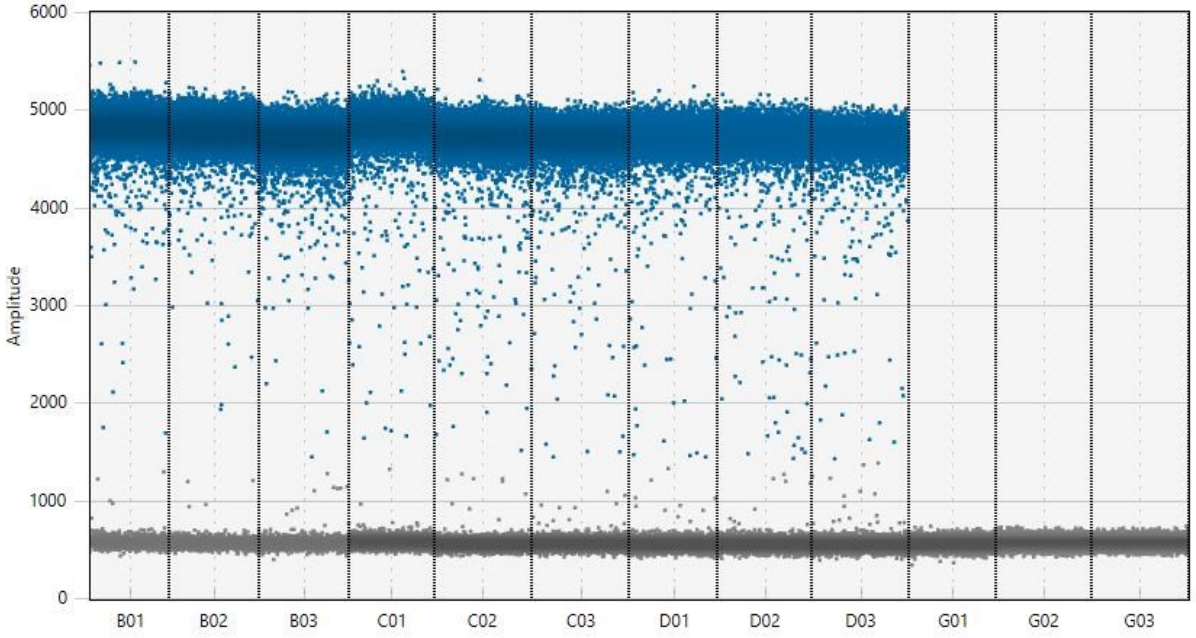
Quantification by ddPCR

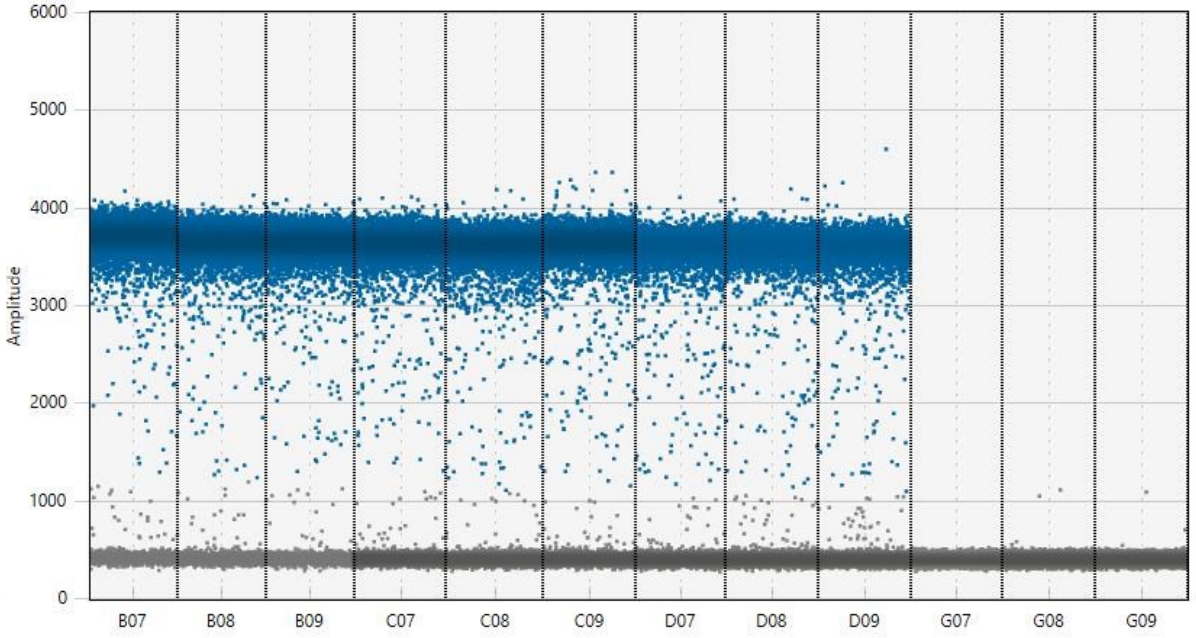
ATCC® Product	Influenza strain used for design (subtype/lineage)
VR-3436SD™	A/white-tailed eagle/Japan/OU-1/2022 (**H5N1**)

- Product specification range of 1 x 10^5 to 1 x 10^6 genome equivalent copies/µL.

- Quantification of transcript A, targeting the gene HA
- The assay used is from Hoffmann *et al.*, 2016, doi: 10.1038/srep27211 and has been cited by the WOAH (OIE)

- Quantification of transcript B, targeting the gene M
- The assay used is from the CDC Flu SC2 multiplex assay





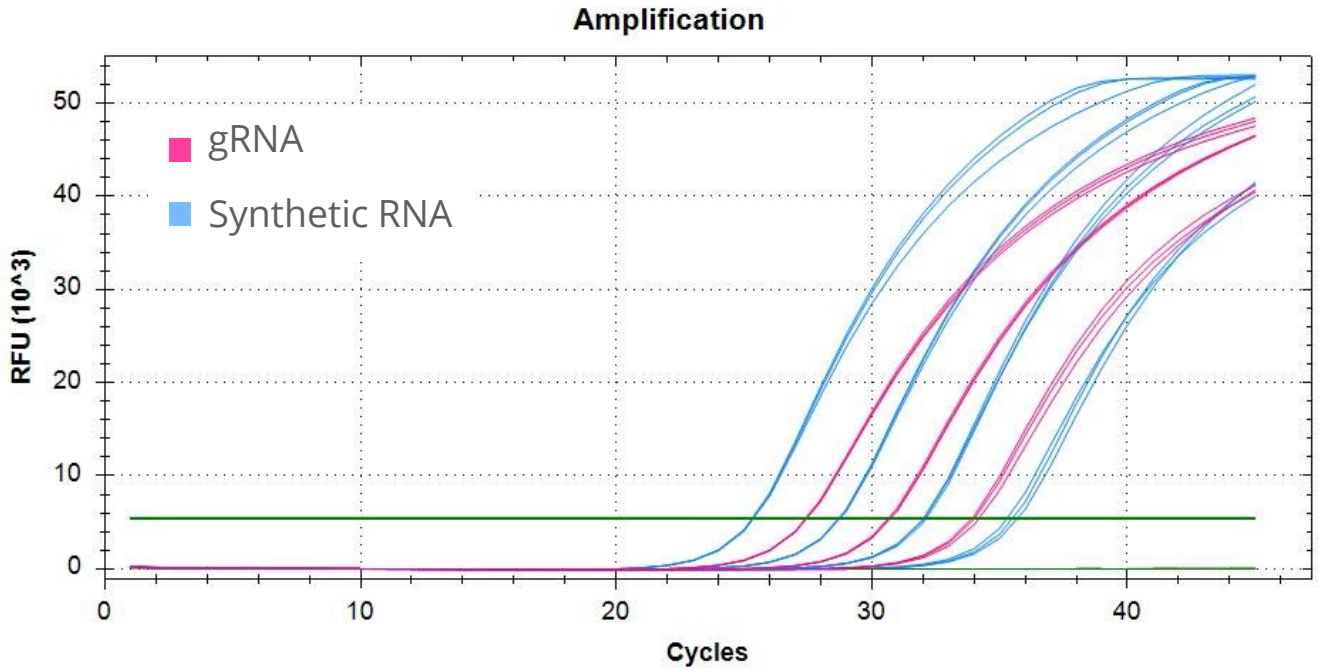
ddPCR is a registered trademark of Bio-Rad Laboratories, Inc.

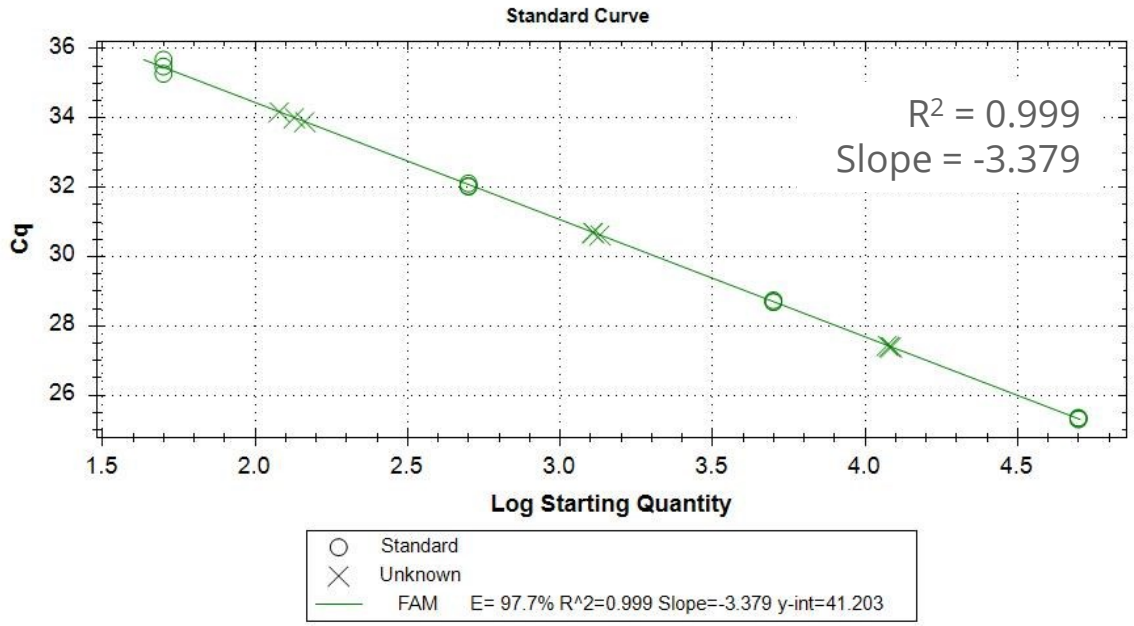
Performance Validation of Influenza Synthetic RNA

Comparative performance as analytical reference material or positive control

ATCC® Product	Influenza strain used for design (subtype/lineage)
VR-3436SD™	A/white-tailed eagle/Japan/OU-1/2022 (**H5N1**)

H5-specific assay cited by the WOAH (OIE)





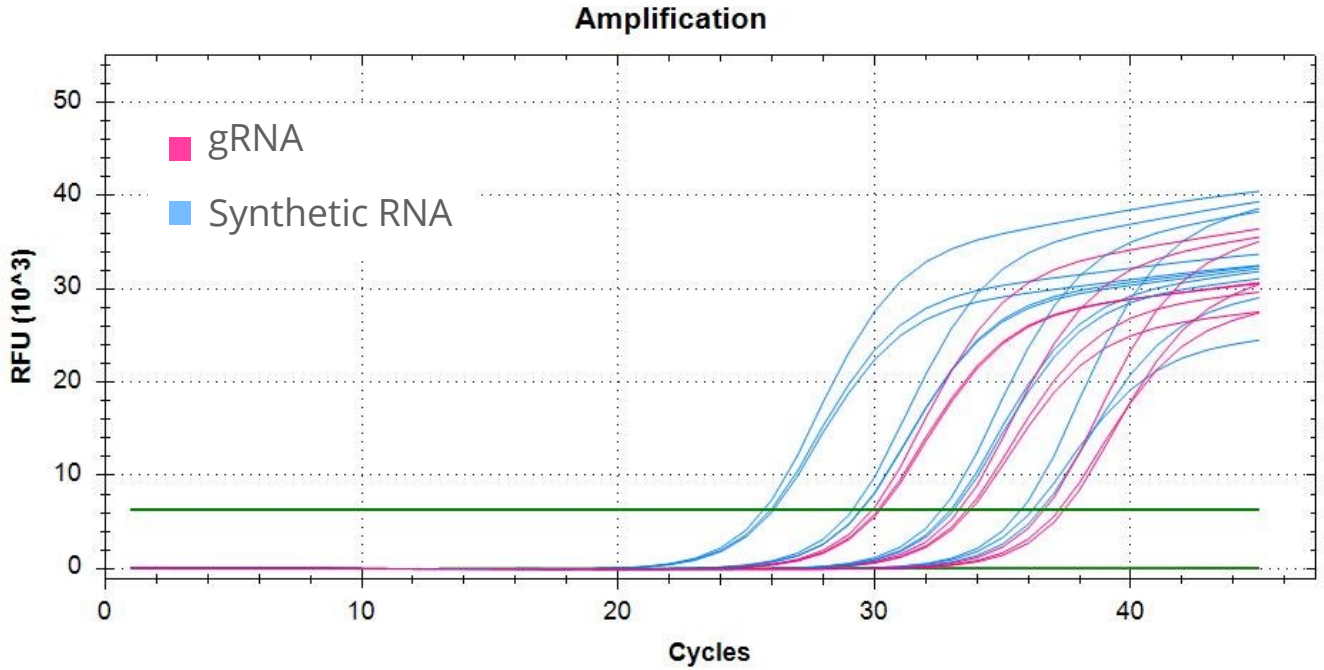
ATCC data using an assay by Hoffmann *et al.,* 2016, doi: 10.1038/srep27211

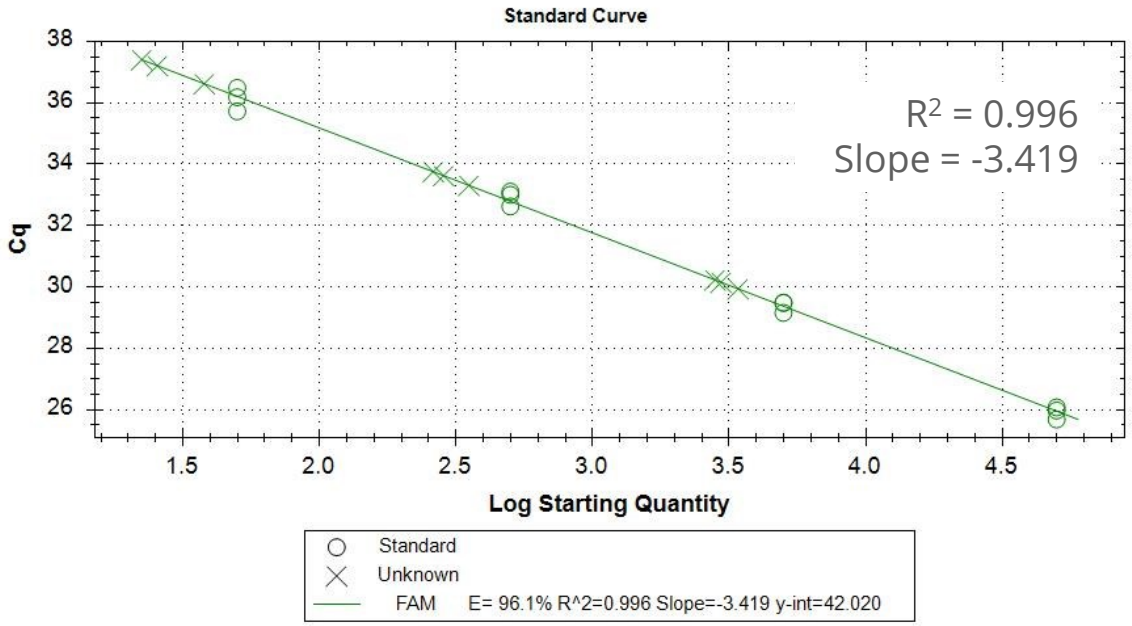
Validation of influenza synthetic RNA products

Product utility as an analytical reference material or positive control

ATCC® Product	Influenza strain used for design (subtype/lineage)
VR-3436SD™	A/white-tailed eagle/Japan/OU-1/2022 (**H5N1**)

CDC's universal influenza A assay targeting the M gene





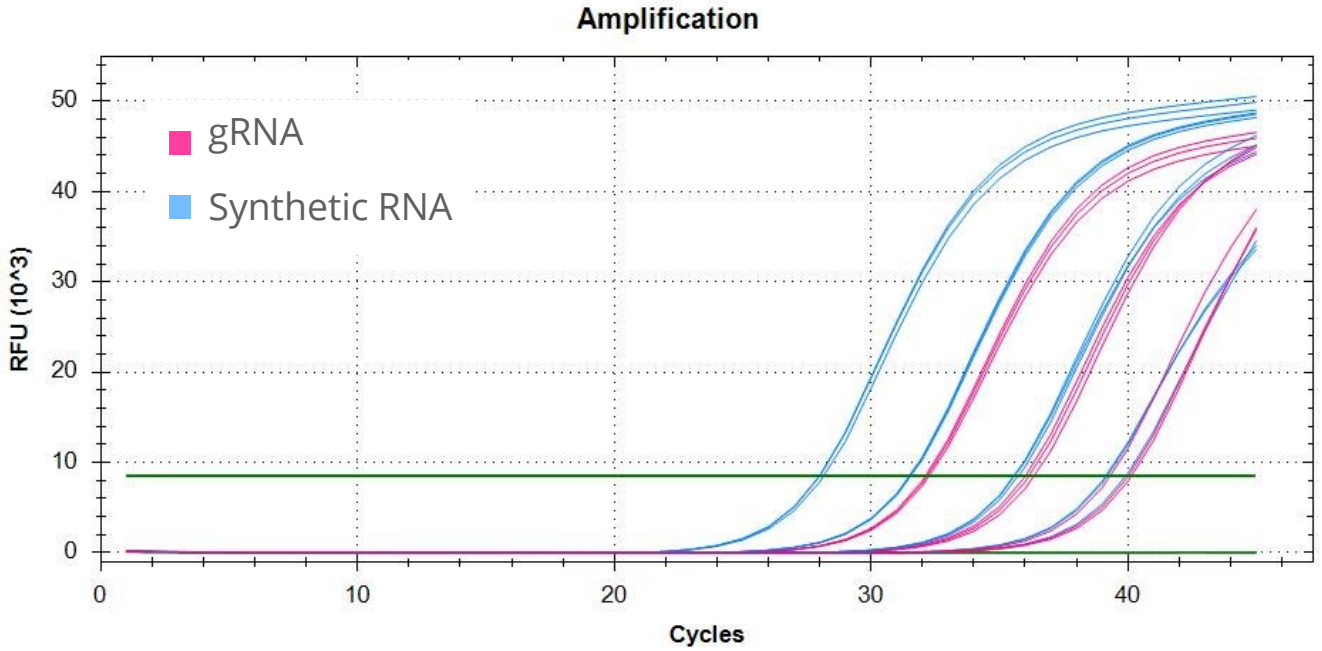
ATCC data using an assay from the CDC Flu SC2 multiplex assay

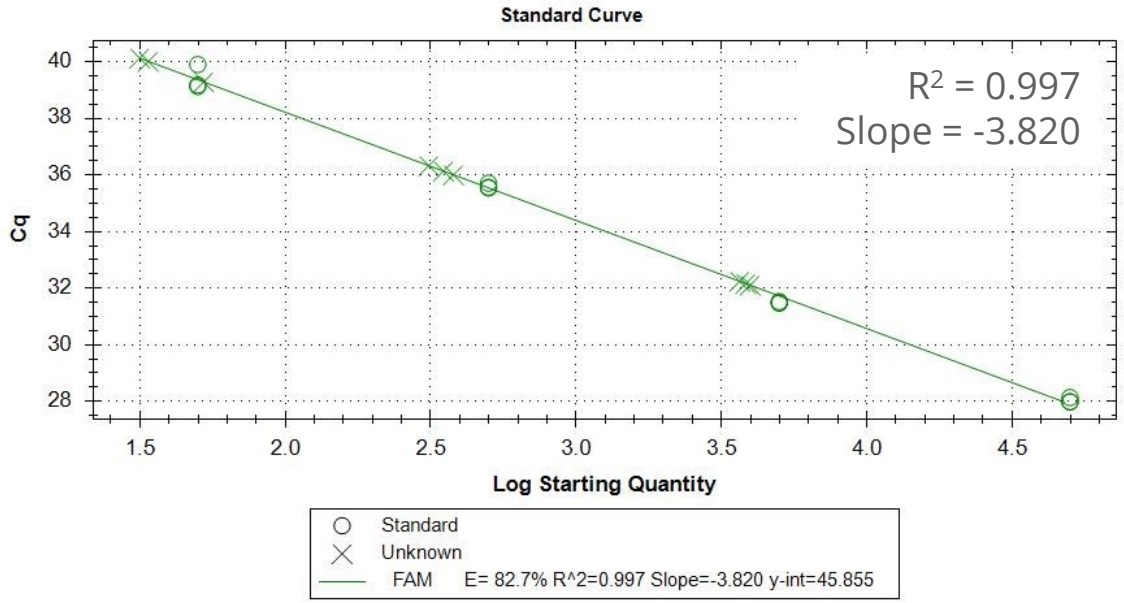
Validation of influenza synthetic RNA products

Product utility as an analytical reference material or positive control

ATCC® Product	Influenza strain used for design (subtype/lineage)
VR-3440SD™	A/ostrich/Yunnan/438/2014 (**H9N2**)

CDC's universal influenza A assay targeting the M gene





ATCC data using an assay from the CDC Flu SC2 multiplex assay

How does ATCC help?

The ATCC influenza portfolio

- **Influenza A** – **50** viral products from human, equine, swine, and avian sources representing subtypes H1N1, H1N1pdm09, H3N2, H3N8, H5N2, and H9N7.
- **Influenza B** – **27** viral products from human sources, including tissue-culture adapted and recombinant strains.
- **Genomic RNA** – **35** preparations of genomic RNA from Influenza A virus subtypes H1N1, H1N1pdm09, and H3N2, and influenza B virus lineages Victoria and Yamagata.
- **Synthetic RNA** – **10** new synthetic RNA products: 5 representing avian subtypes of global health concern, 3 representing human influenza A (H1N1, H1N1pdm09, and H3N2), and 2 representing influenza B (Victoria lineage).
- **Antisera and monoclonal antibodies** – **3** preparations of antisera against Influenza A strains, and **18** preparations of monoclonal antibodies against various subtypes including H1N1, H5N1, H7N7, and H9N2.
- **Propagation hosts** – **2** cell lines and associated media and reagents for the propagation of tissue culture-adapted viral strains.

Conclusions

- **ATCC® supports the scientific community with biological products and innovative solutions**, with a portfolio of over 80,000 microorganisms and 5,000 cell lines
- **The increasing health and economic risks posed by human and avian influenza** strains underscore the necessity of robust surveillance systems
- **Continuous surveillance,** reliable diagnostic testing, and control measures are essential for managing infectious diseases' risks, including influenza
- **Reliable diagnostics and surveillance tools depend on reliable analytical reference materials**
- **ATCC® continues to support the development and implementation of diagnostics and surveillance tests** with reliable authenticated reference materials

ATCC®

CREDIBLE LEADS TO INCREDIBLE

Questions

ATCC®

CREDIBLE LEADS TO INCREDIBLE

Thank You

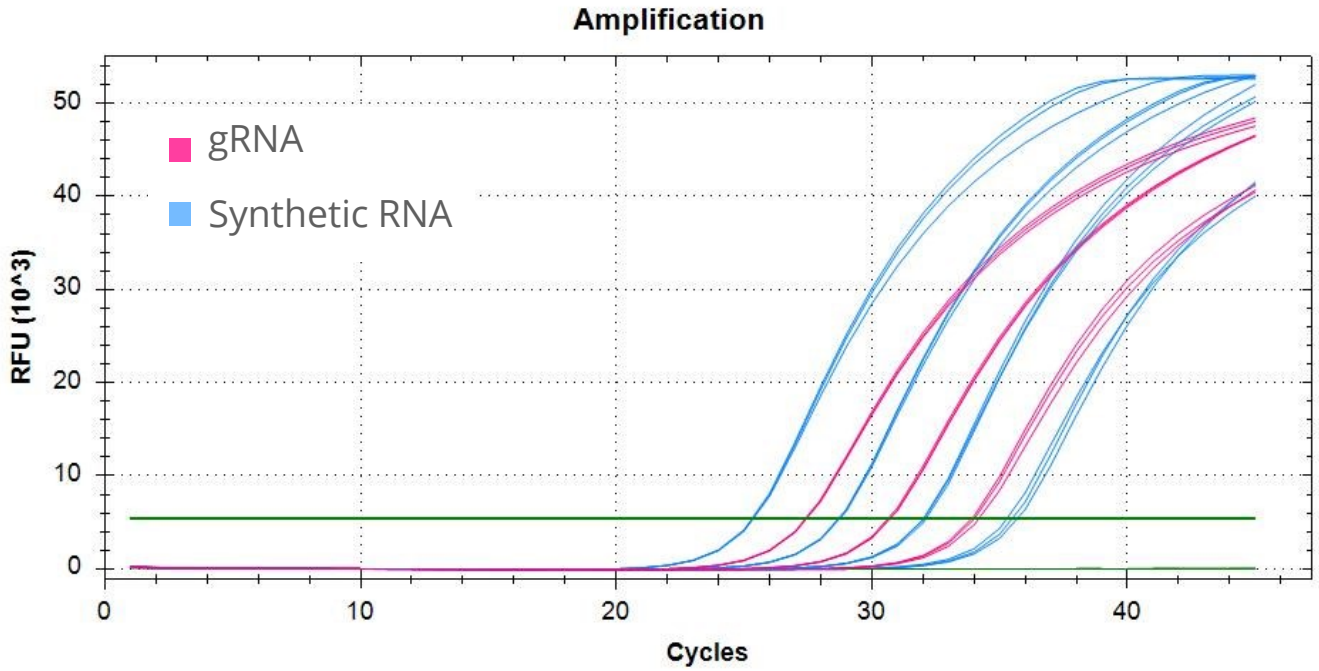
Supplementary Data

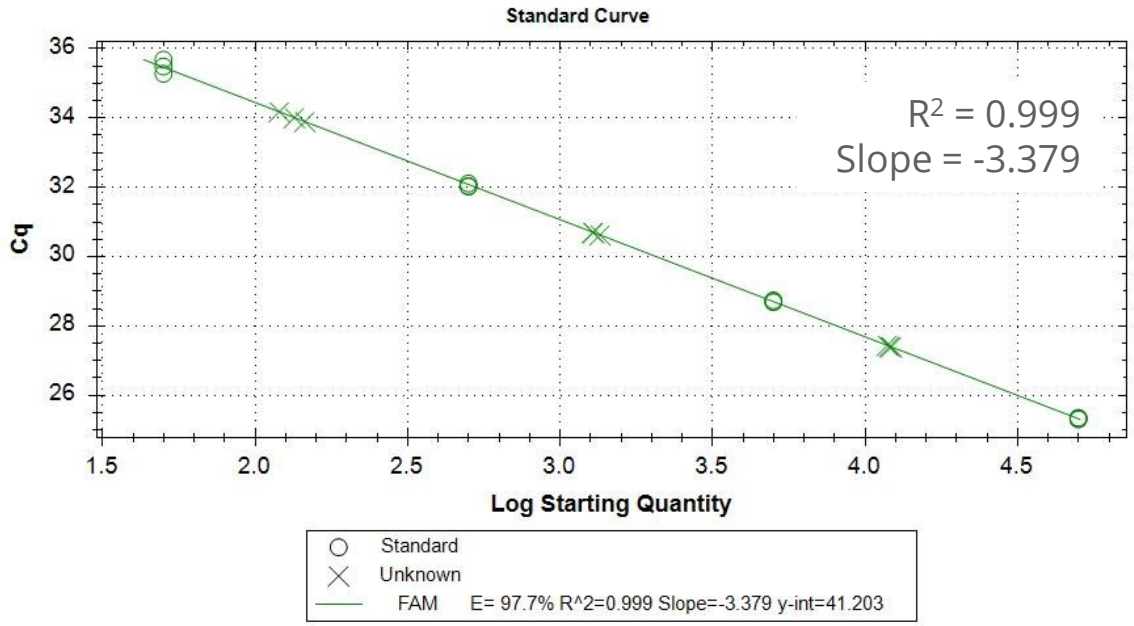
Validation of influenza synthetic RNA products

Product utility as an analytical reference material or positive control

ATCC® Product	Influenza strain used for design (subtype/lineage)
VR-3436SD™	A/white-tailed eagle/Japan/OU-1/2022 (**H5N1**)

H5-specific assay cited by the WOAH (OIE)





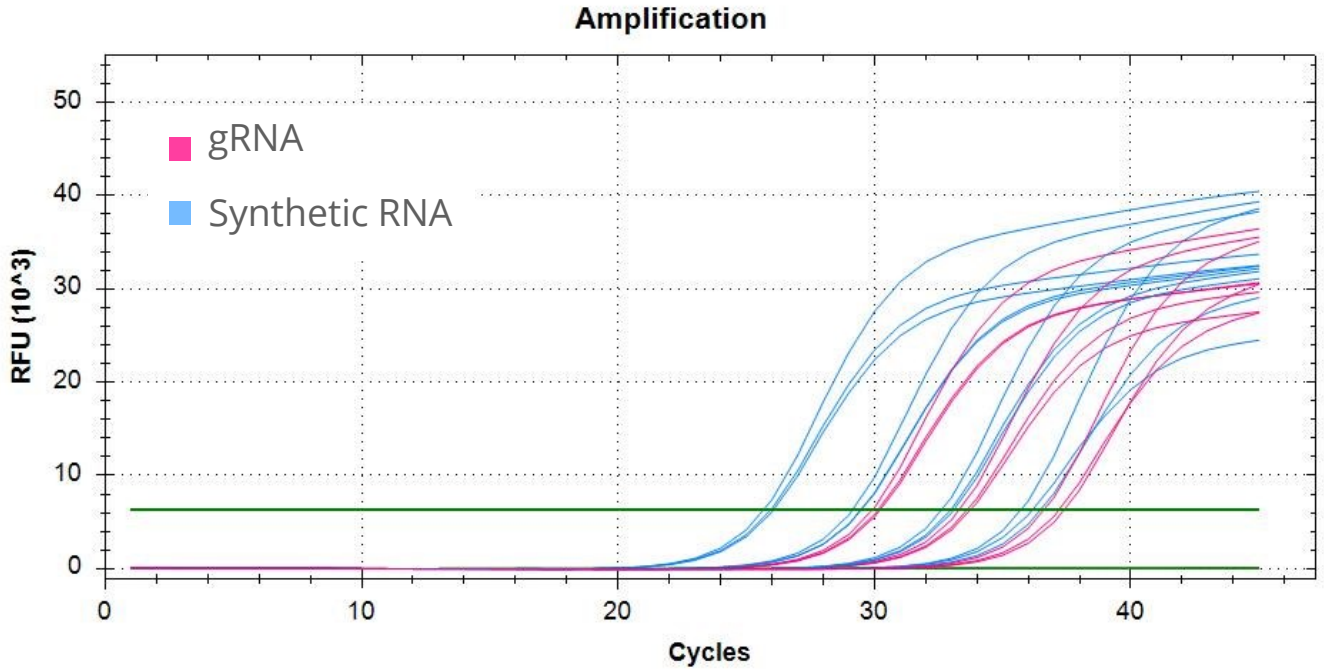
ATCC data using an assay by Hoffmann *et al.,* 2016, doi: 10.1038/srep27211

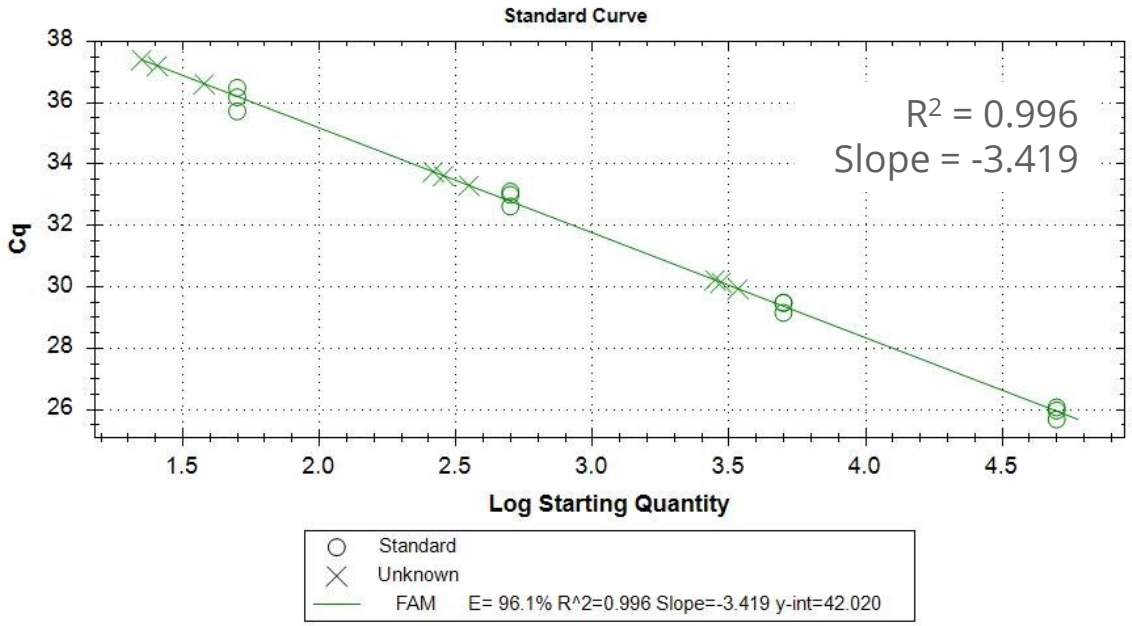
Validation of influenza synthetic RNA products

Product utility as an analytical reference material or positive control

ATCC® Product	Influenza strain used for design (subtype/lineage)
VR-3436SD™	A/white-tailed eagle/Japan/OU-1/2022 (**H5N1**)

CDC's universal influenza A assay targeting the M gene

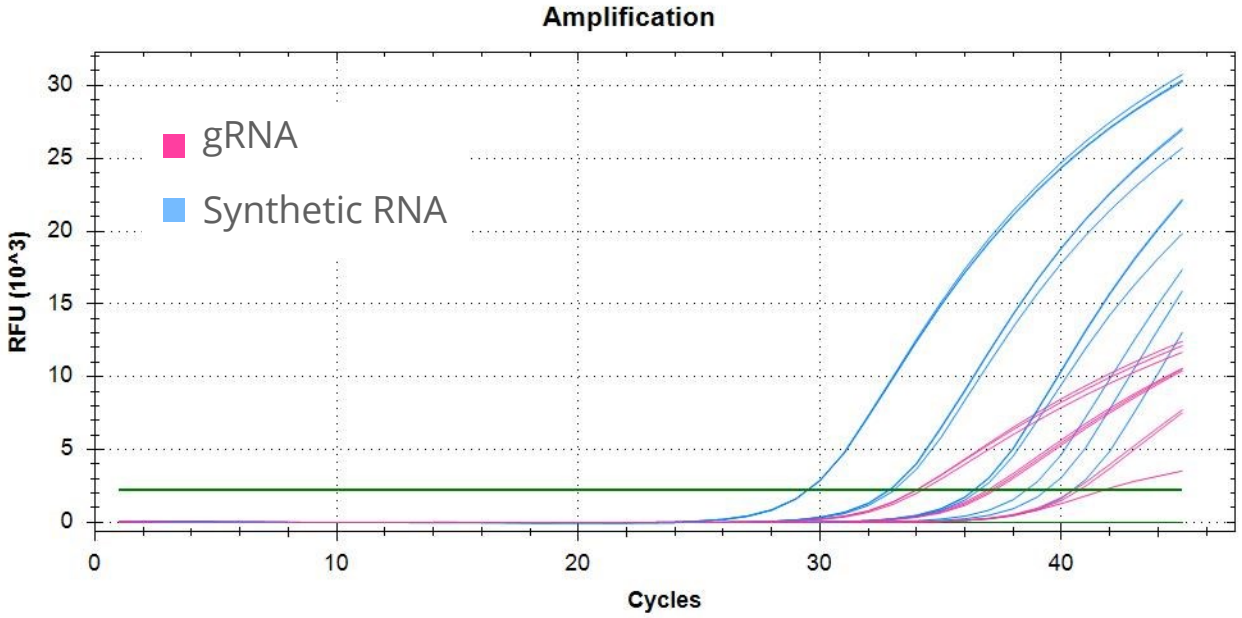


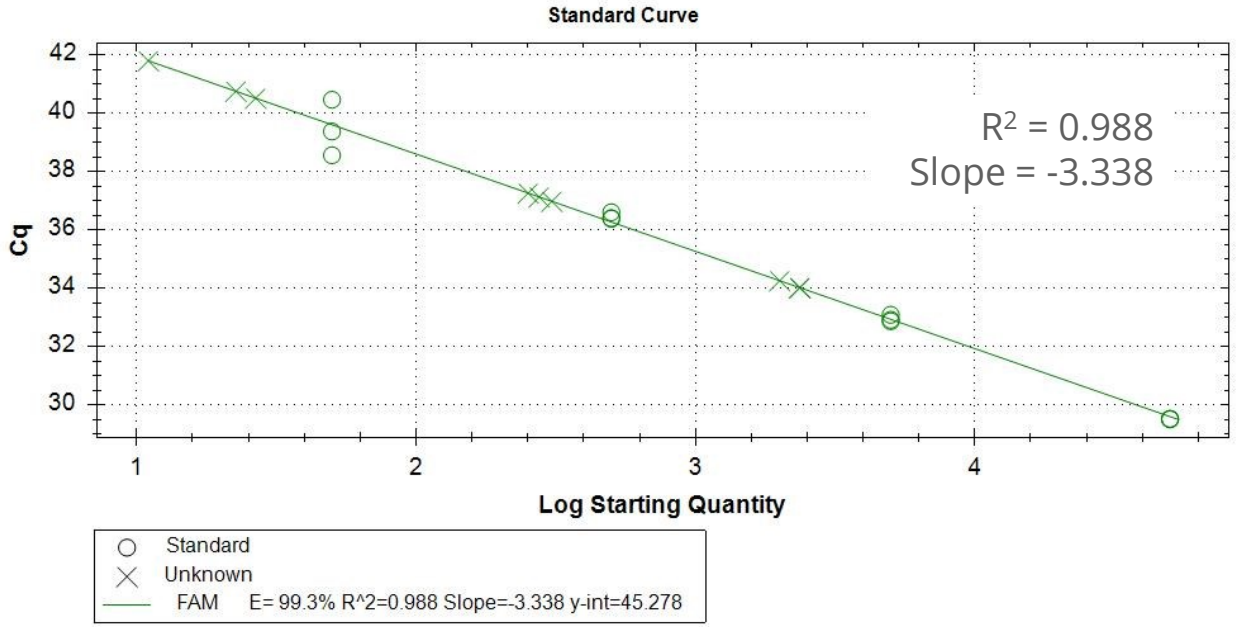


ATCC data using an assay from the CDC Flu SC2 multiplex assay

FDA Milk Assay

ATCC® Product	Influenza strain used for design (subtype/lineage)
VR-3436SD™	A/white-tailed eagle/Japan/OU-1/2022 (**H5N1**)



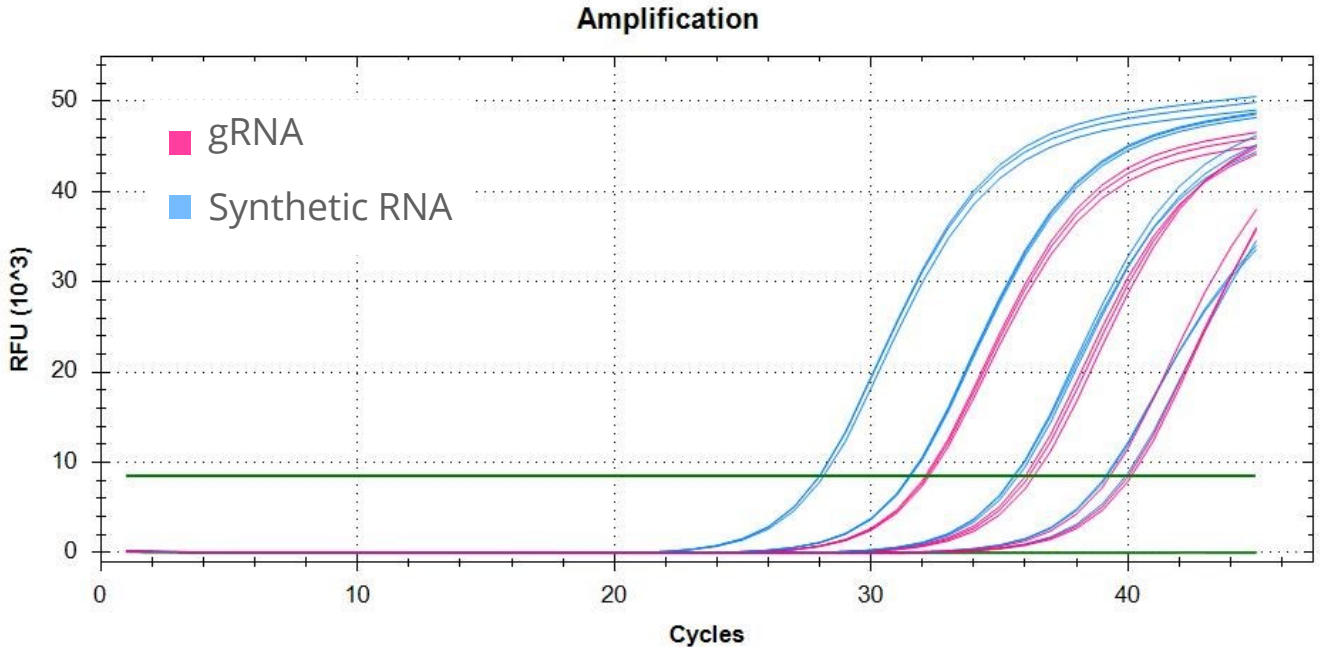


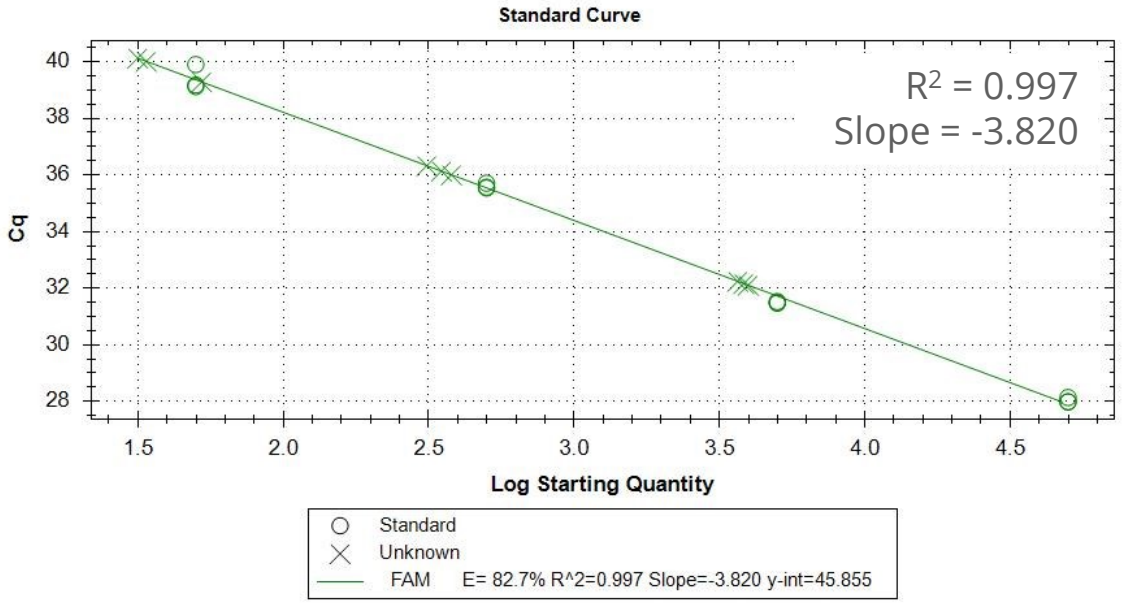
Validation of influenza synthetic RNA products

Product utility as an analytical reference material or positive control

ATCC® Product	Influenza strain used for design (subtype/lineage)
VR-3440SD™	A/ostrich/Yunnan/438/2014 (**H9N2**)

CDC's universal influenza A assay targeting the M gene





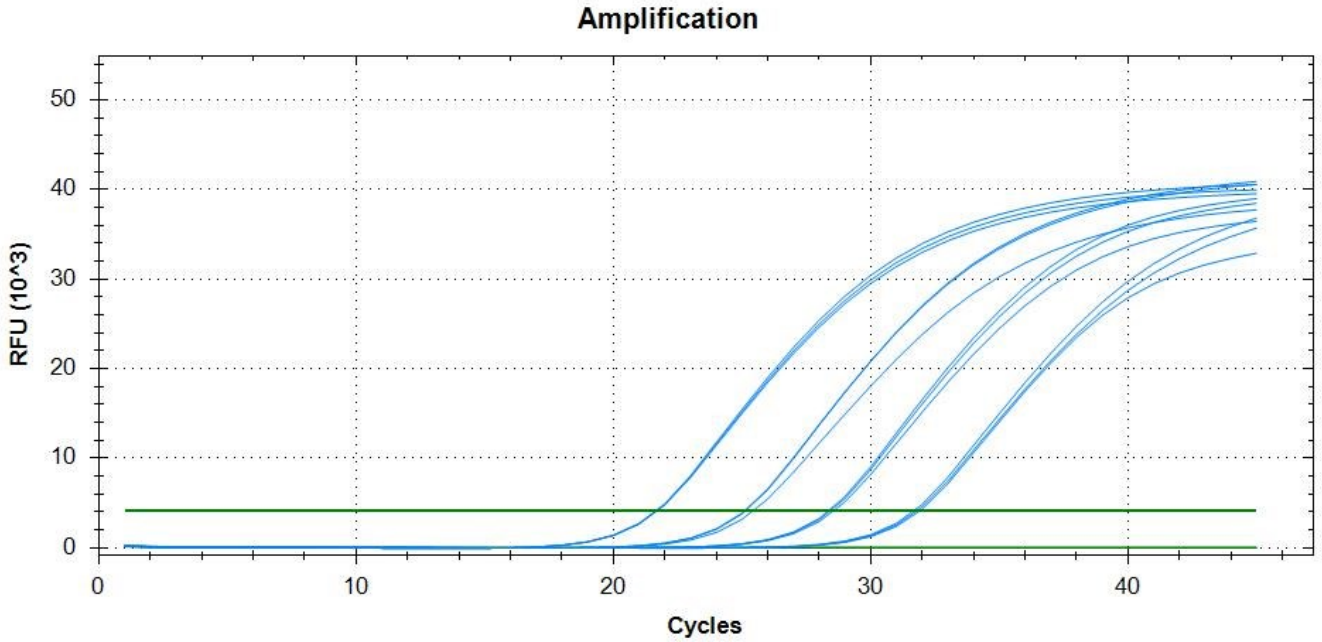
ATCC data using an assay from the CDC Flu SC2 multiplex assay

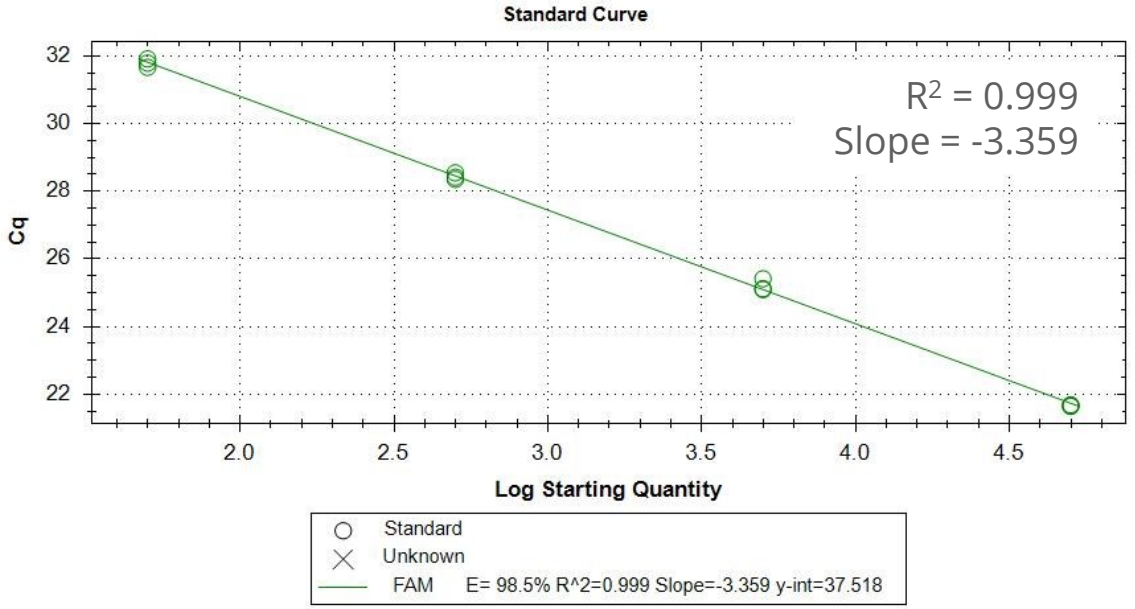
Validation of influenza synthetic RNA products

Product utility as an analytical reference material or positive control

ATCC® Product	Influenza strain used for design (subtype/lineage)
VR-3437SD™	A/Shanghai/4664T/2013 (**H7N9**)

H7-specific assay recommended by the WHO.





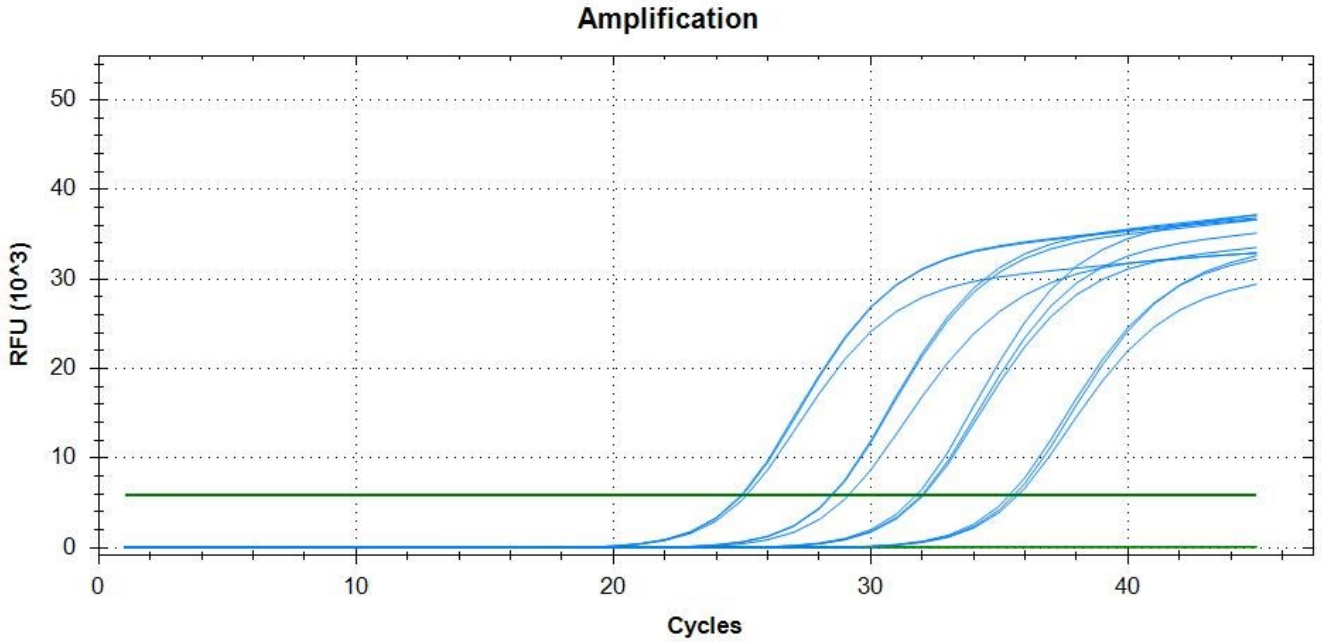
ATCC data using an assay from the WHO information for the molecular detection of influenza viruses document, 2021 update.

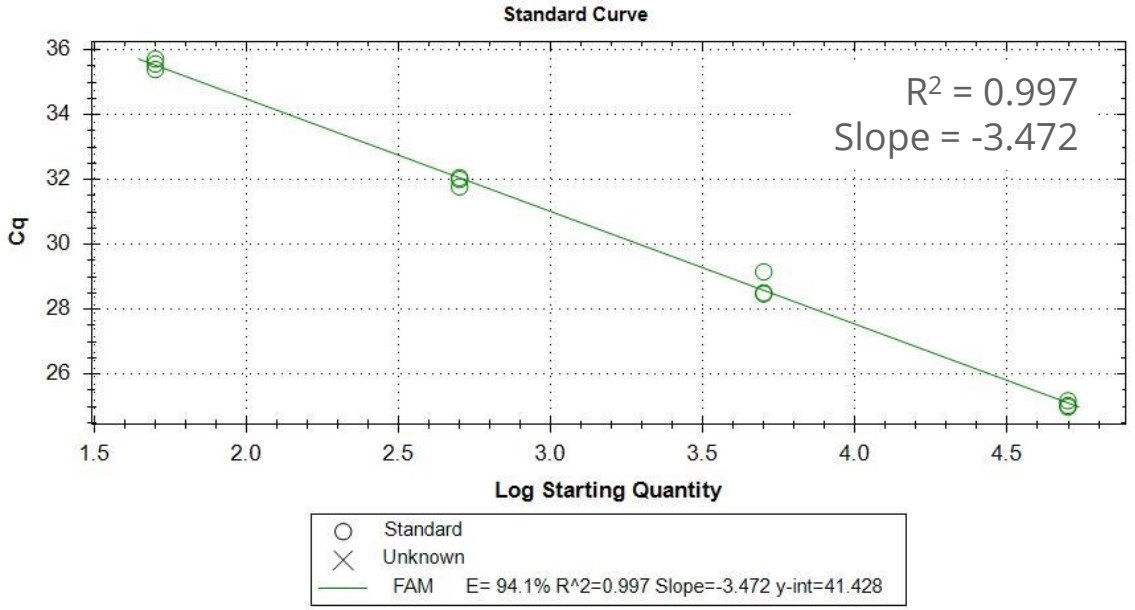
Validation of influenza synthetic RNA products

Product utility as an analytical reference material or positive control

ATCC® Product	Influenza strain used for design (subtype/lineage)
VR-3437SD™	A/Shanghai/4664T/2013 (**H7N9**)

CDC's universal influenza A assay targeting the M gene





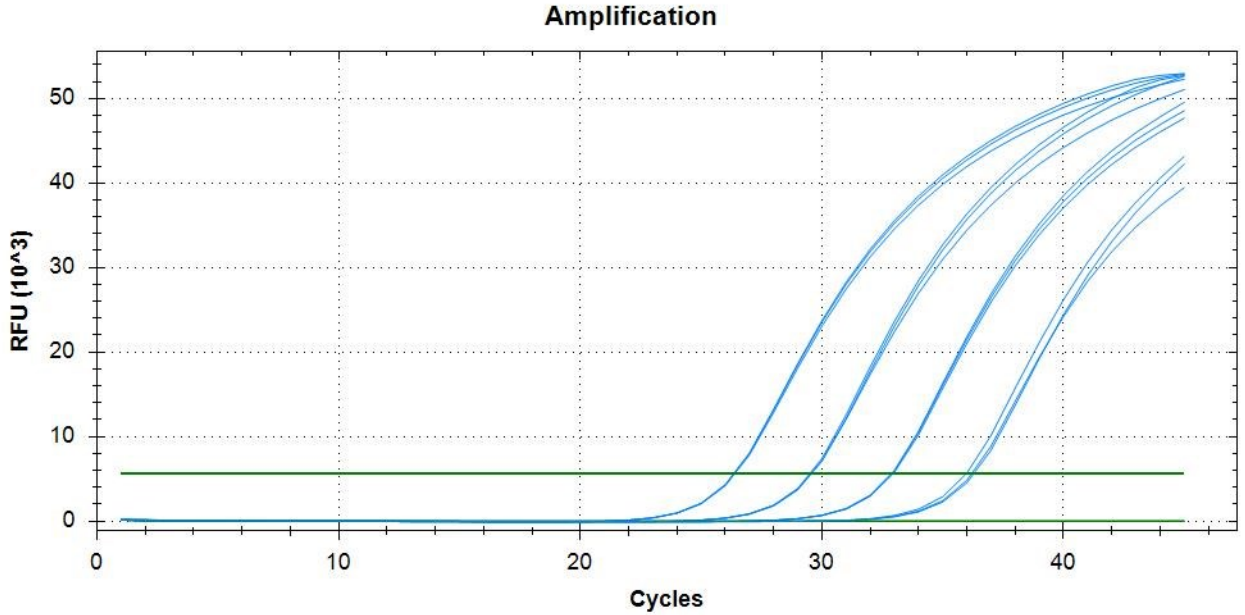
ATCC data using an assay from the CDC Flu SC2 multiplex assay

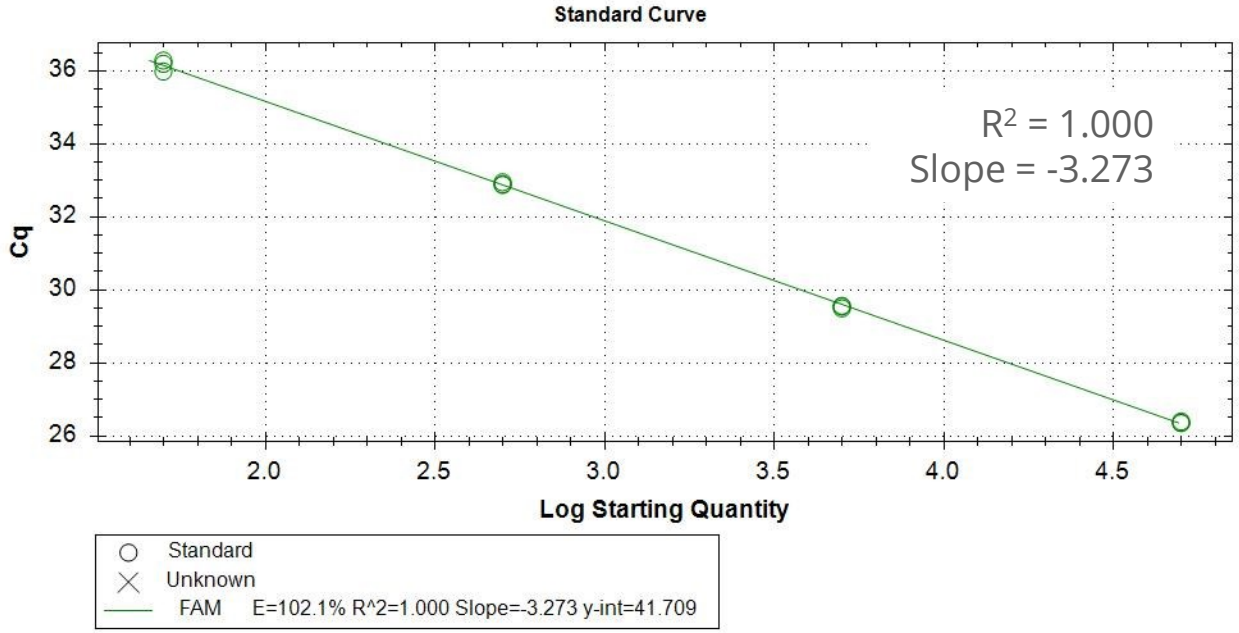
Validation of influenza synthetic RNA products

Product utility as an analytical reference material or positive control

ATCC® Product	Influenza strain used for design (subtype/lineage)
VR-3439SD™	A/goose/Guangdong/GS018/2015 (**H5N6**)

H5-specific assay cited by the WOAH (OIE)





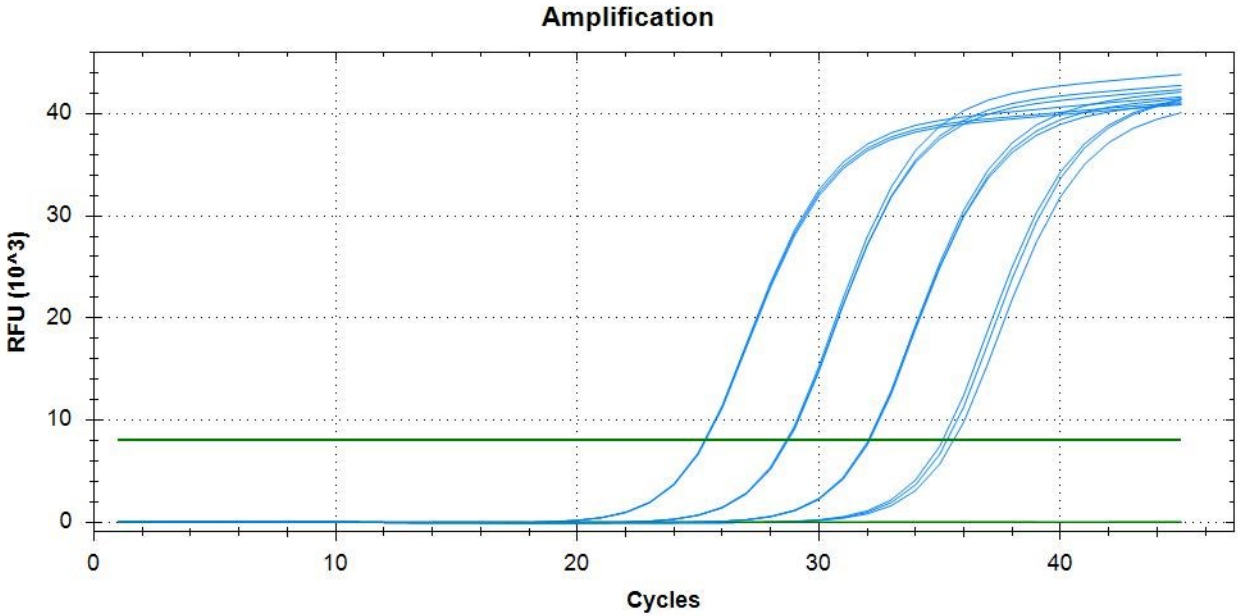
ATCC data using an assay by Hoffmann *et al.,* 2016, doi: 10.1038/srep27211

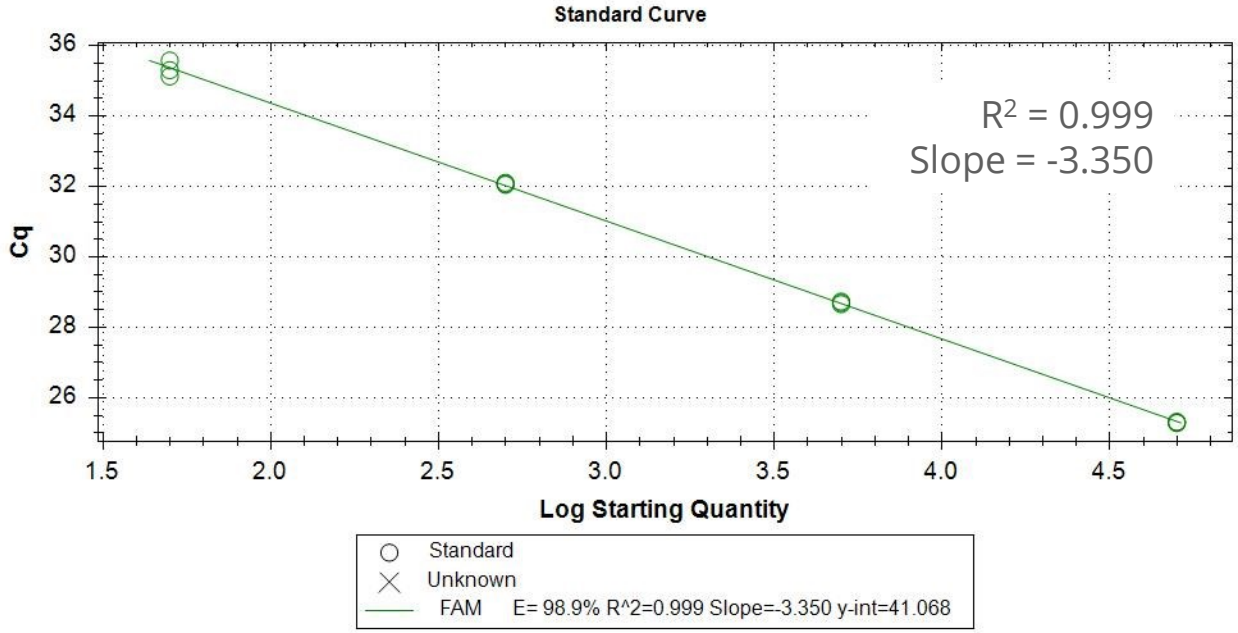
Validation of influenza synthetic RNA products

Product utility as an analytical reference material or positive control

ATCC® Product	Influenza strain used for design (subtype/lineage)
VR-3439SD™	A/goose/Guangdong/GS018/2015 (**H5N6**)

CDC's universal influenza A assay targeting the M gene





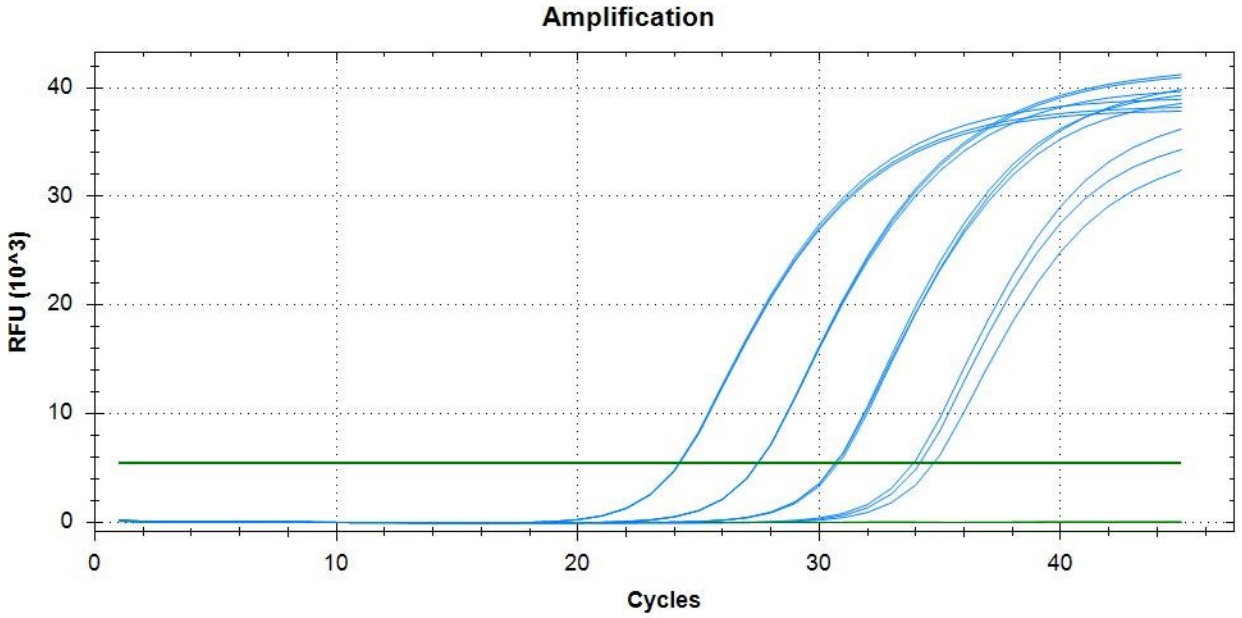
ATCC data using an assay from the CDC Flu SC2 multiplex assay

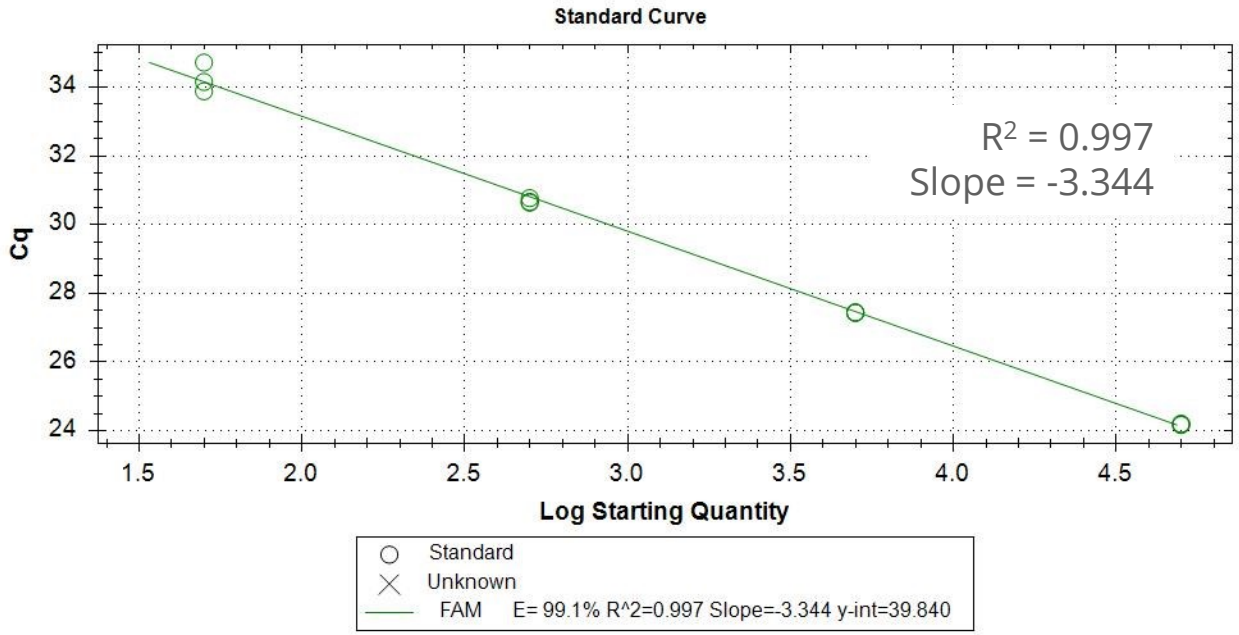
Validation of influenza synthetic RNA products

Product utility as an analytical reference material or positive control

ATCC® Product	Influenza strain used for design (subtype/lineage)
VR-3438SD™	A/chicken/Wenzhou/334b/2013 (**H7N7**)

H7-specific assay recommended by the WHO.





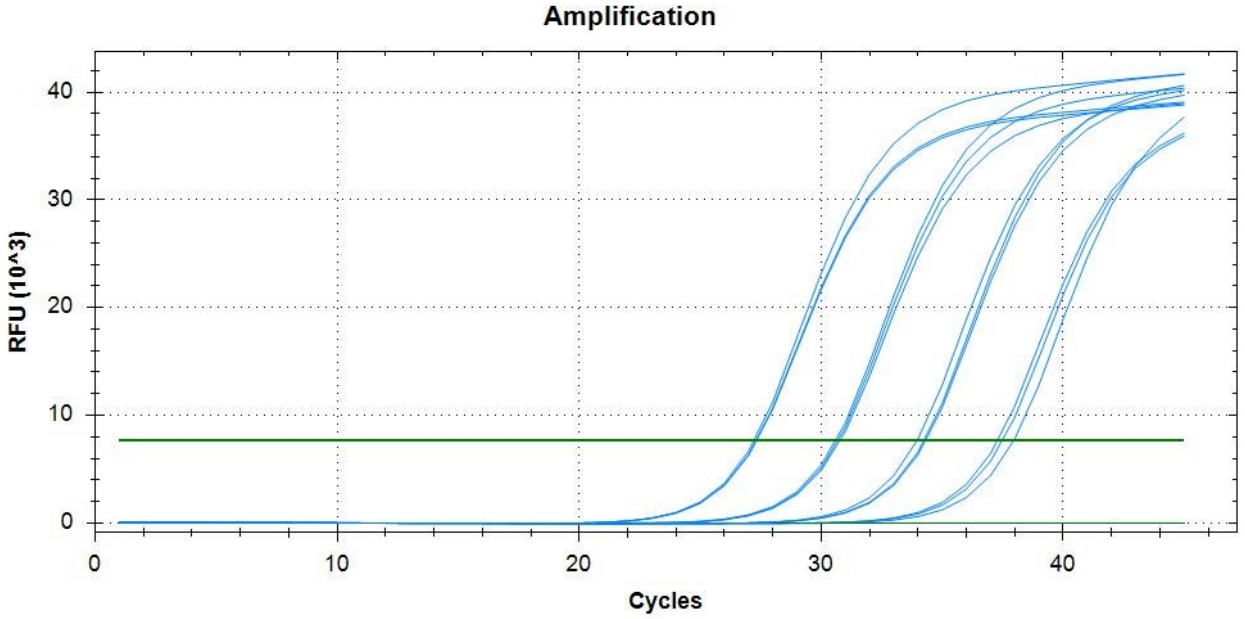
ATCC data using an assay from the WHO information for the molecular detection of influenza viruses document, 2021 update.

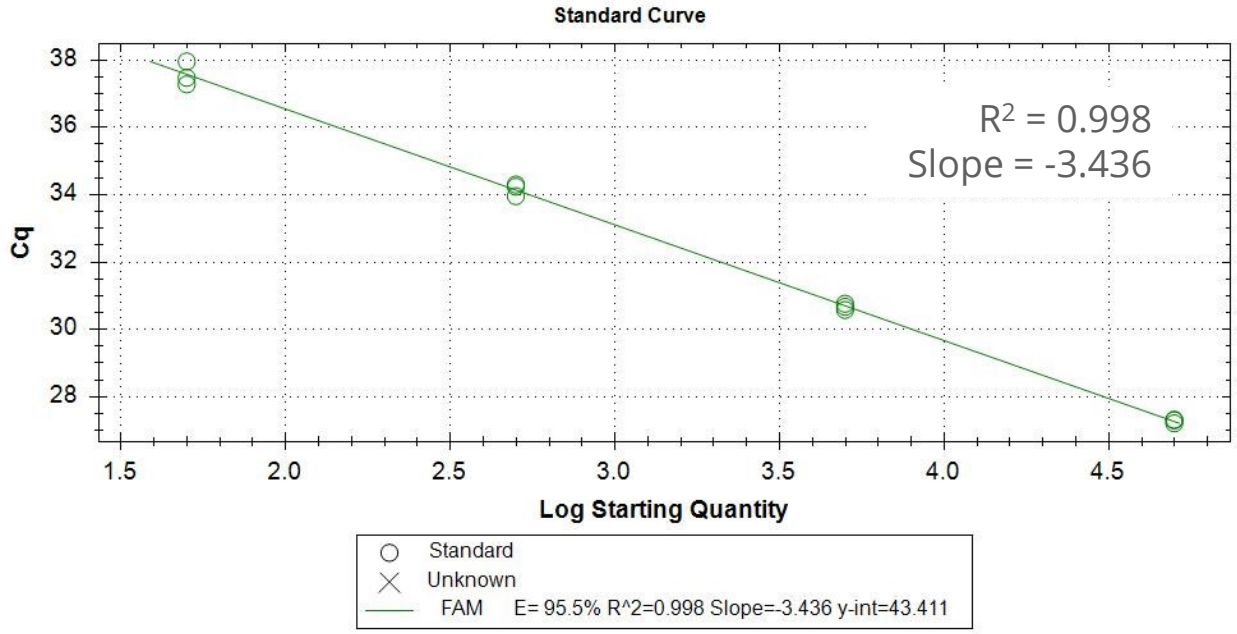
Validation of influenza synthetic RNA products

Product utility as an analytical reference material or positive control

ATCC® Product	Influenza strain used for design (subtype/lineage)
VR-3438SD™	A/chicken/Wenzhou/334b/2013 (**H7N7**)

CDC's universal influenza A assay targeting the M gene





ATCC data using an assay from the CDC Flu SC2 multiplex assay

ATCC Influenza Resources

Metagenomic Recourses

- Metagenomic Recourses
  - https://www.atcc.org/products/msa-2008
  - https://www.atcc.org/microbe-products/applications/microbiome-research#t=productTab&numberOfResults=24
  - https://www.atcc.org/applications/molecular-diagnostics-development/ngs-standards
-

ATCC Influenza Resources

Cells

- Cells
  - MDCK (NBL-2) cells (ATCC® CCL-34™) https://www.atcc.org/products/ccl-34-vhg
  - CCL-81-VHG™ https://www.atcc.org/products/ccl-81-vhg
  - 293.STAT1 BAX KO cell line (ATCC® CRL-1573-VHG™) https://www.atcc.org/products/crl-1573-vhg
  - Sf9 CRL-1711 ™ https://www.atcc.org/products/crl-1711