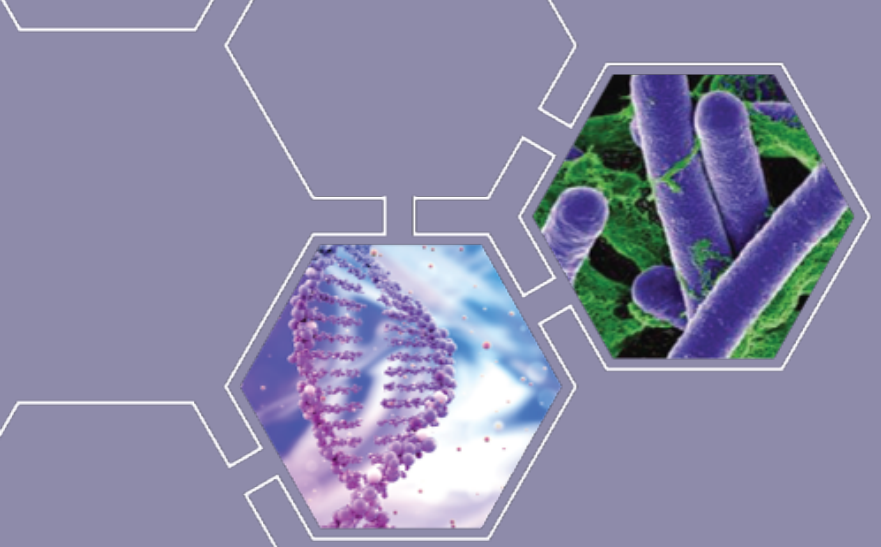
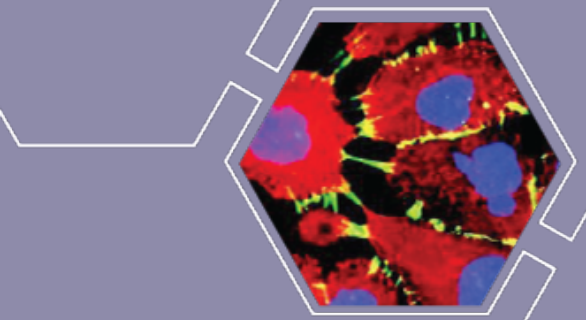




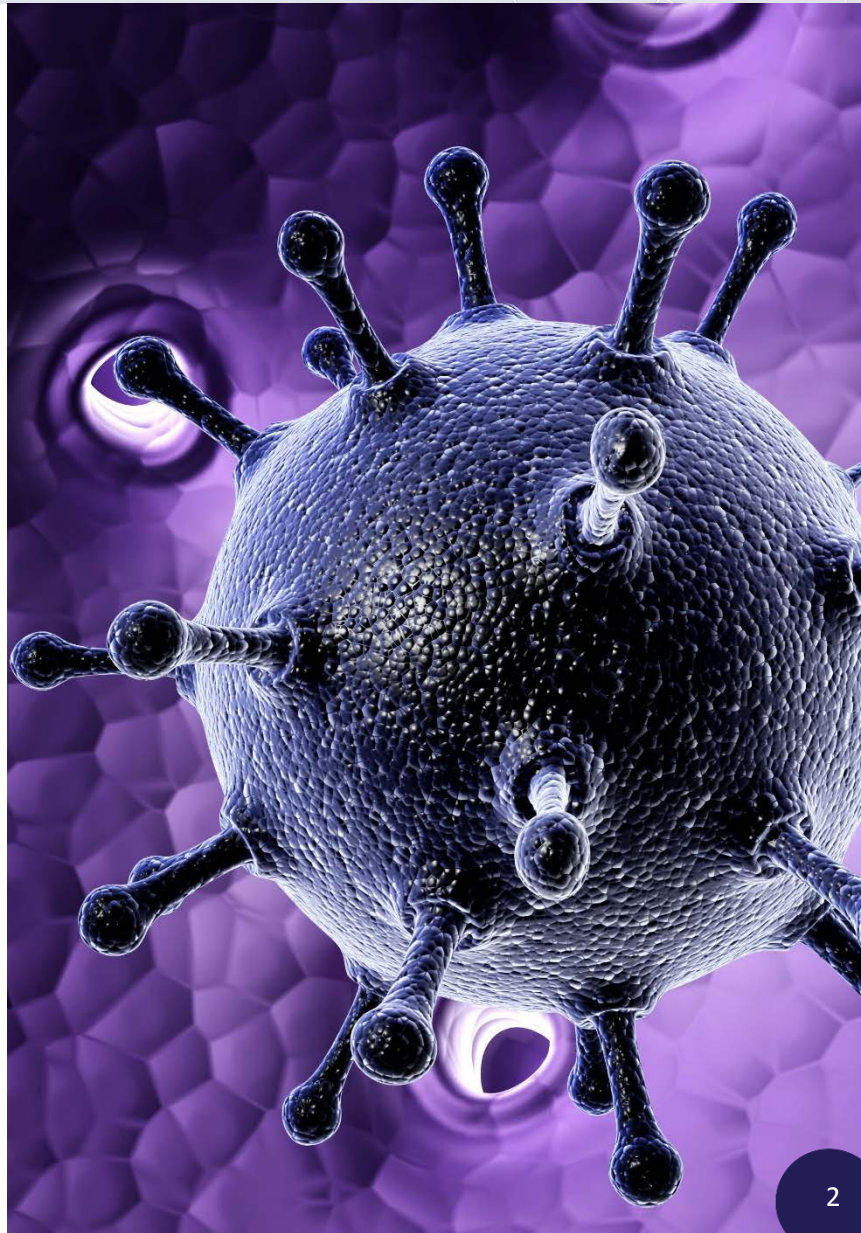
The Importance of Authenticated Viral Standards in Respiratory Disease Research and Therapeutic Development

Reed Shabman, Ph.D.
Lead Scientist, Virology
ATCC Standards Resource Center

ATCC®

Agenda

- **ATCC overview**
- Worldwide significance of viral respiratory diseases
- Respiratory virus countermeasures
  - Diagnostics, small molecules, and vaccines
  - Case study: the universal influenza vaccine
- Importance of controls
  - Authenticated synthetics, strains, and formats
- Efforts to expand the collection
- Conclusions



ATCC overview

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D & Services center in Gaithersburg, MD
- Worldwide brand name and quality recognition
- World's premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard and innovative biological solutions
  - Growing portfolio of products and services
  - Sales and distribution in over 140 countries, 15 International distributors
- Talented team of 475+ employees; > one third with advanced degrees
- Multiple accreditations including ISO 9001 and ISO 13485

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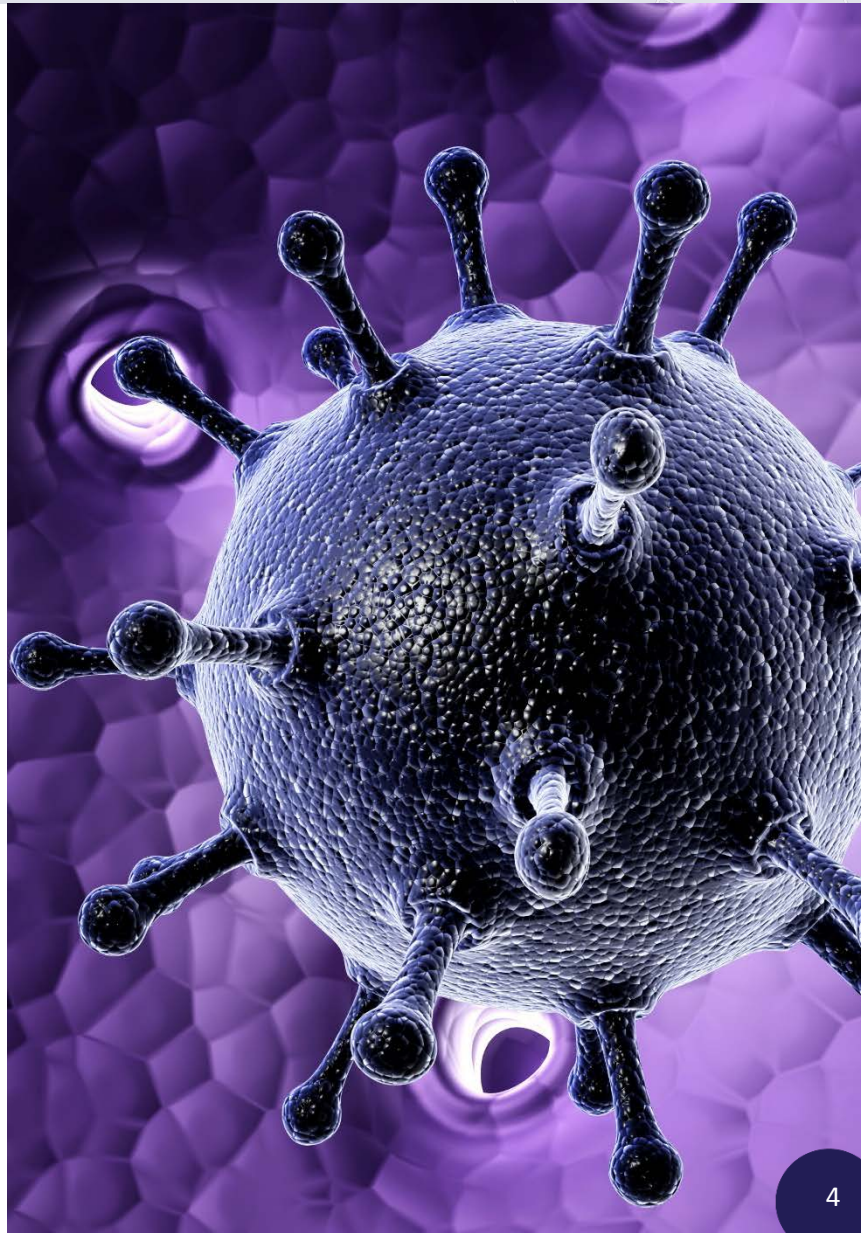
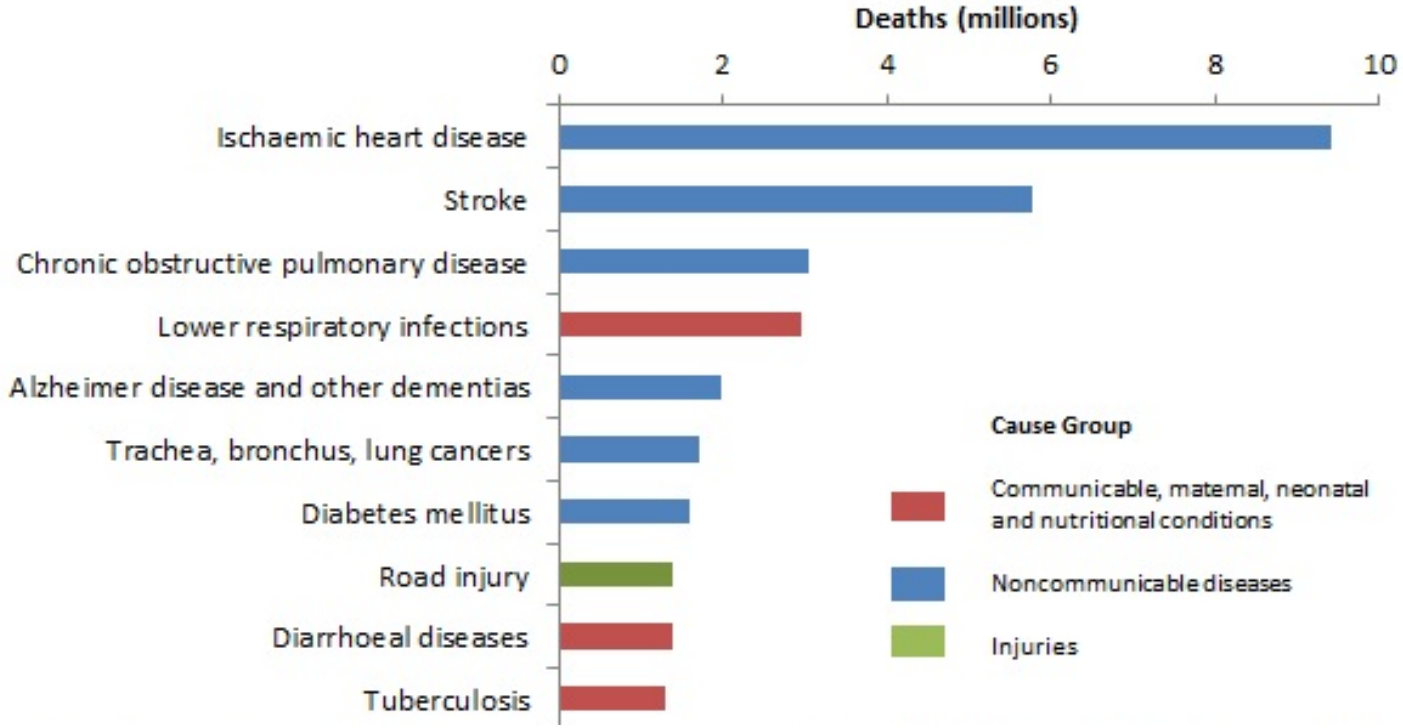


Respiratory infections and human health

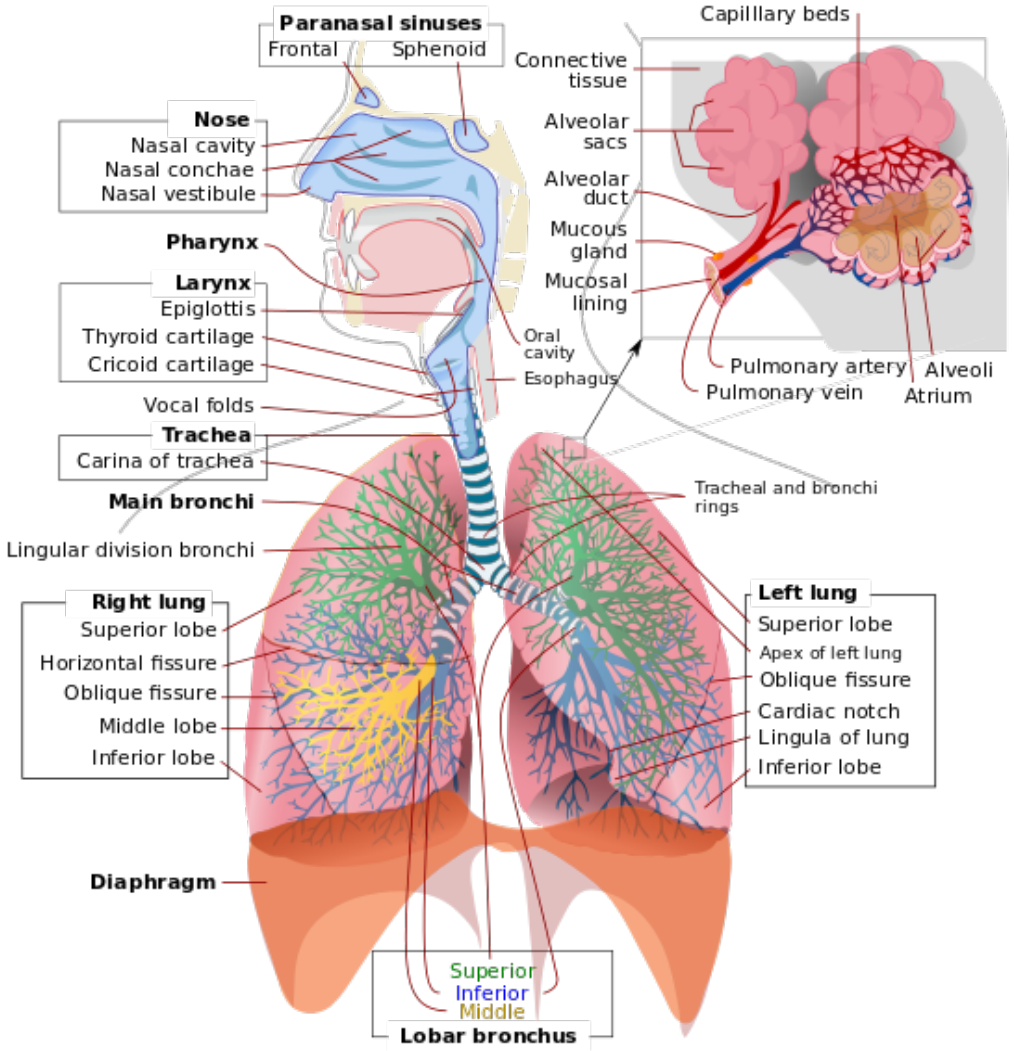
Top 10 global causes of deaths, 2016



- Lower respiratory tract infections are the 4th leading cause of death globally and the leading cause of death due to infection
- Even when not fatal, respiratory infections are often severe

Global Health Estimates 2016: Deaths by Cause, Age, Sexy, by Country and Region, 2000-2016. Geneva, World Health Organization; 2018.

Common respiratory viruses and their associated disease



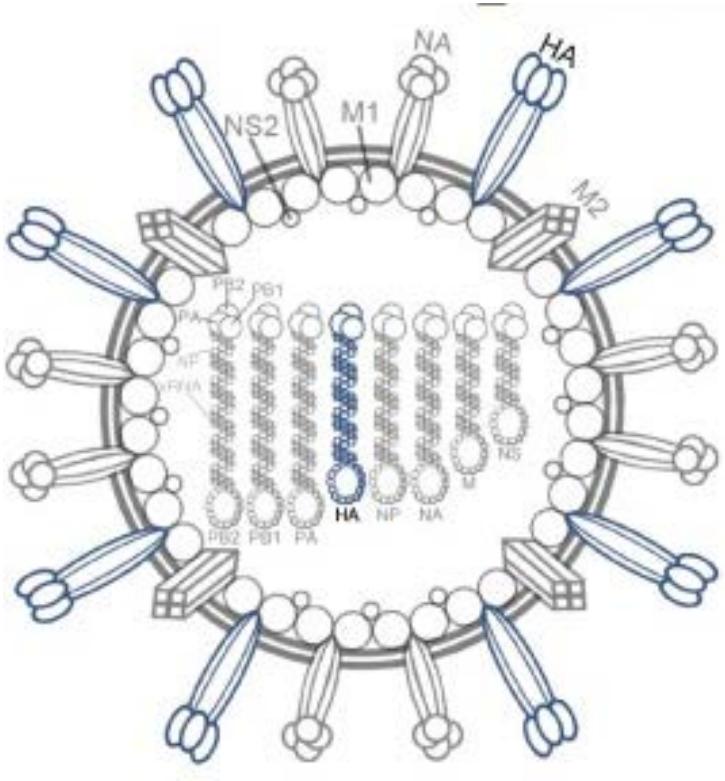
Common Respiratory Viruses	URT	LRT
Influenza virus (A, B)	✓	✓
Respiratory syncytial virus (A, B)	✓	✓
Parainfluenza virus (1-4)	✓	✓
Human metapneumovirus		✓
Rhinovirus (A, B, C)	✓	
Coronavirus (HKU1, NL63, 229E, OC43)	✓	✓
Adenovirus	✓	

Infections result in:

Upper respiratory tract (URT) infection: common cold, tonsillitis, and pharyngitis

Lower respiratory tract (LRT) infection: croup, bronchitis, bronchiolitis, and pneumonia

Influenza viruses

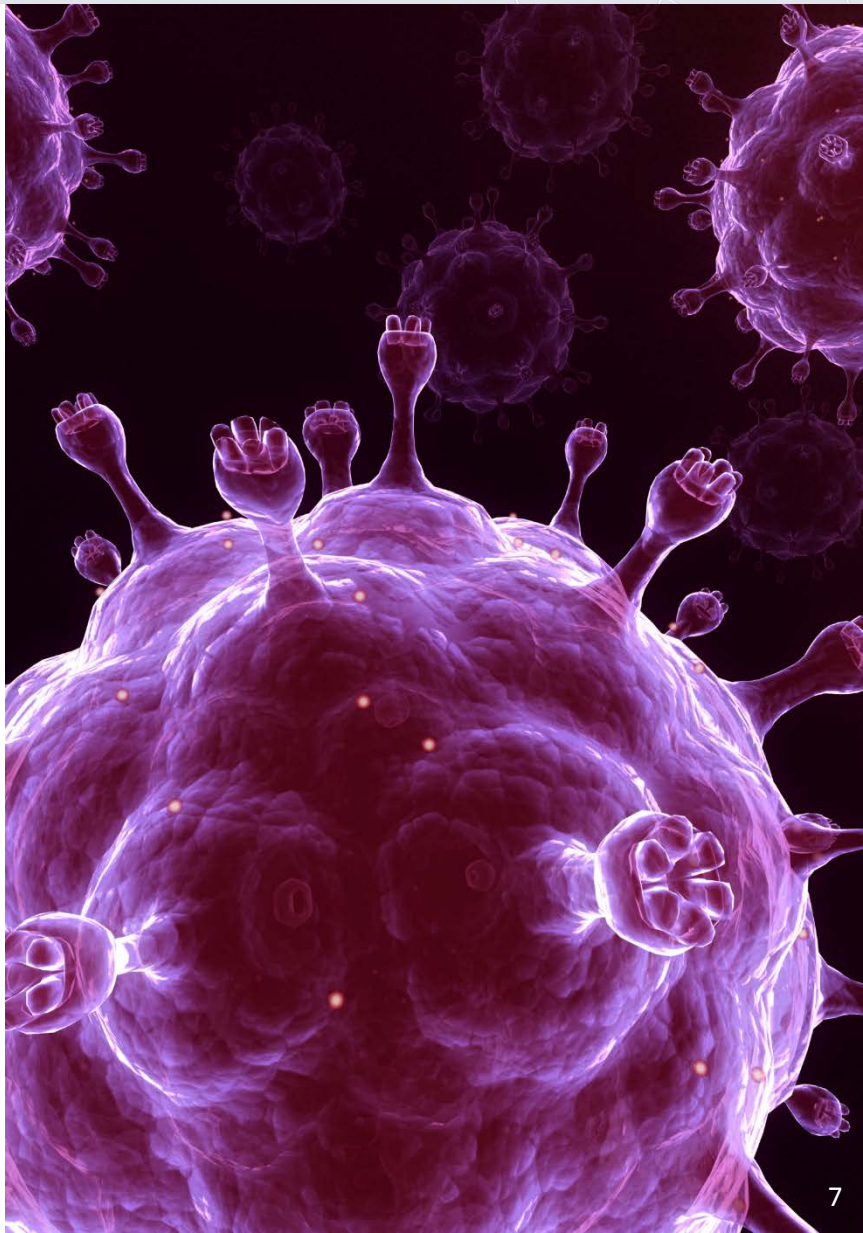


Eight ssRNA segments encode 12 proteins

Nine structural proteins:
Segment 1: PB2
Segment 2: PB1
Segment 3: PA
Segment 4: HA
Segment 5: NP
Segment 6: NA
Segment 7: M1 and M2
Segment 8: NS2 (NEP)

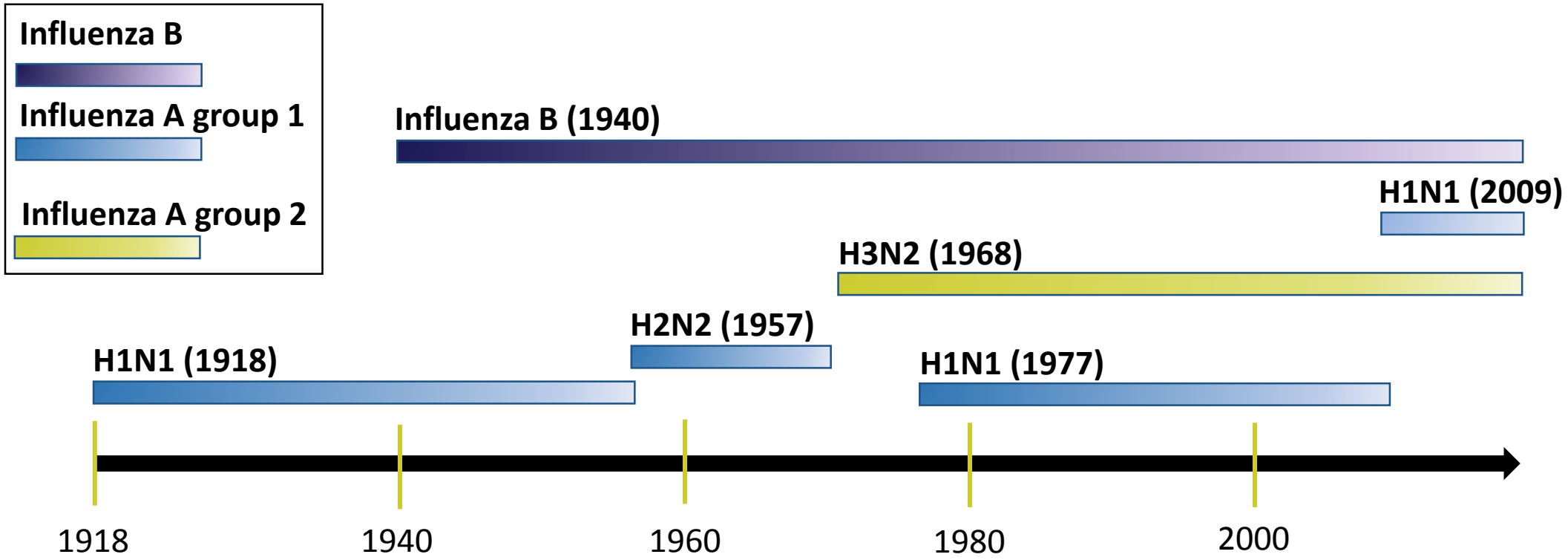
Three non-structural proteins:
Segment 2: PB1-F2 and N40
Segment 8: NS1

Note: N40 is a newly discovered protein that is still not completely understood.



Sriwilaijaroen N, Suzuki Y. Proc Jpn Acad Ser B Phys Biol Sci 88(6): 226-249, 2012.

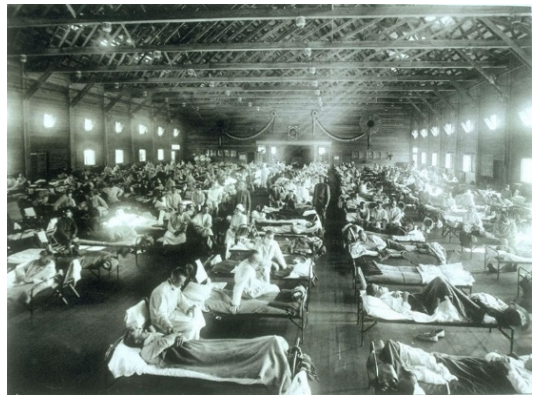
Influenza viruses circulating in the human population



Influenza pandemics

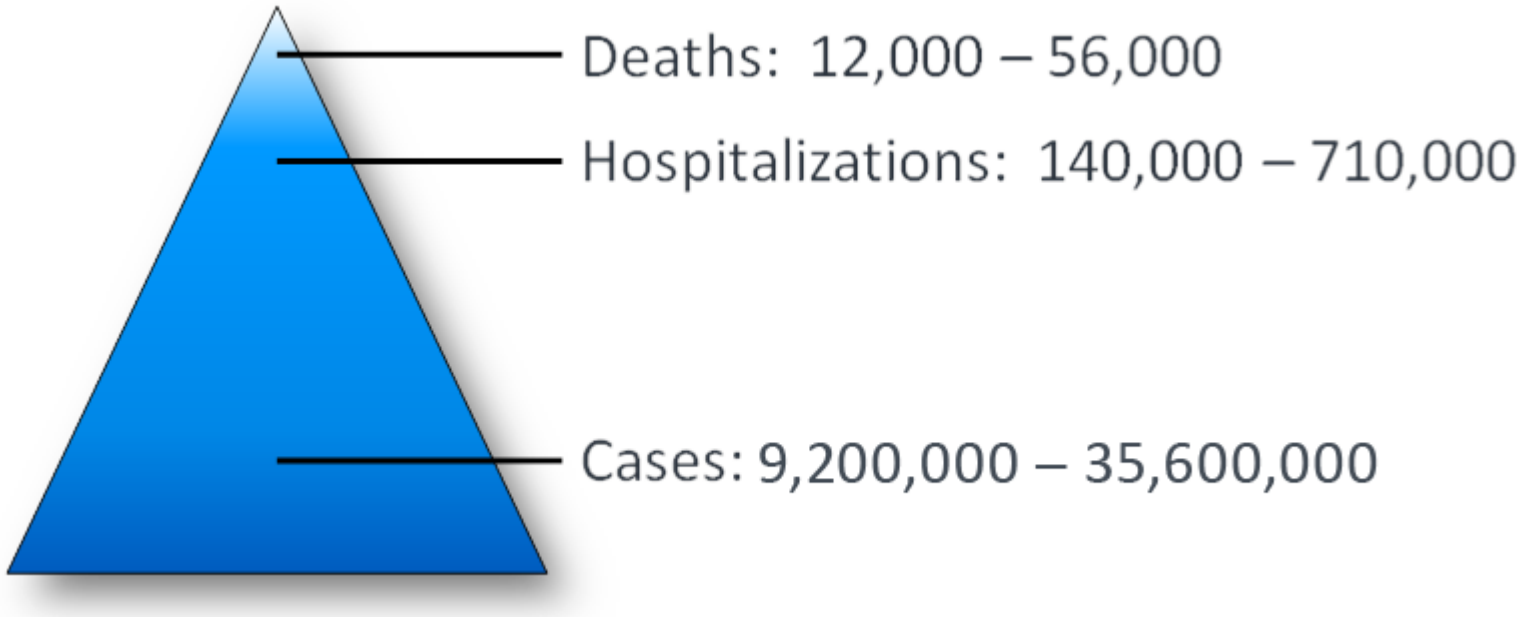
Historical and future Influenza Pandemics remain a significant threat to human health

Flu Pandemic	Year	Subtype	Estimated Deaths
1918 (Spanish flu)	1918-1920	H1N1	20-100 million
Asian flu	1957-1958	H2N2	>1 million
Hong Kong flu	1968-1969	H3N2	0.5-1 million
2009 flu pandemic	2009-2010	H1N1	18-284 thousand



Potential pandemic threat:
Influenza A virus subtypes H5N1 and H7N9 are highly infectious strains that continue to infect poultry and people, resulting in severe respiratory illness and potential death.

Influenza in the United States

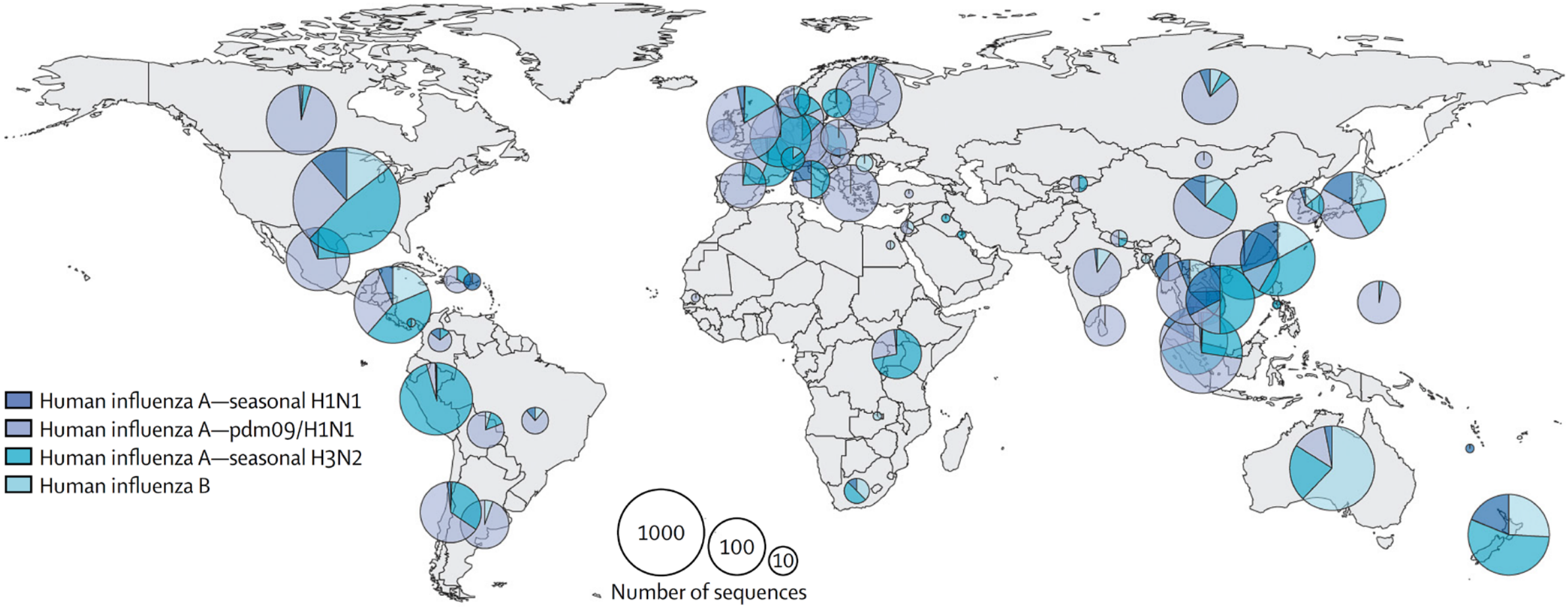


CDC estimated influenza disease burden since 2010

Global surveillance of influenza viruses is essential to ensure the efficacy of seasonal influenza vaccines, and to monitor circulating strains for pandemic potential or resistance against antiviral drugs.

Centers for Disease Control and Prevention. Estimated Influenza Illnesses, Medical Visits, Hospitalizations, and Deaths Averted by Vaccination in the United States

Worldwide distribution of influenza



Tang JW, *et al.* Lancet Infect Dis 17: e320-326, 2017 License Number: 4425481327840

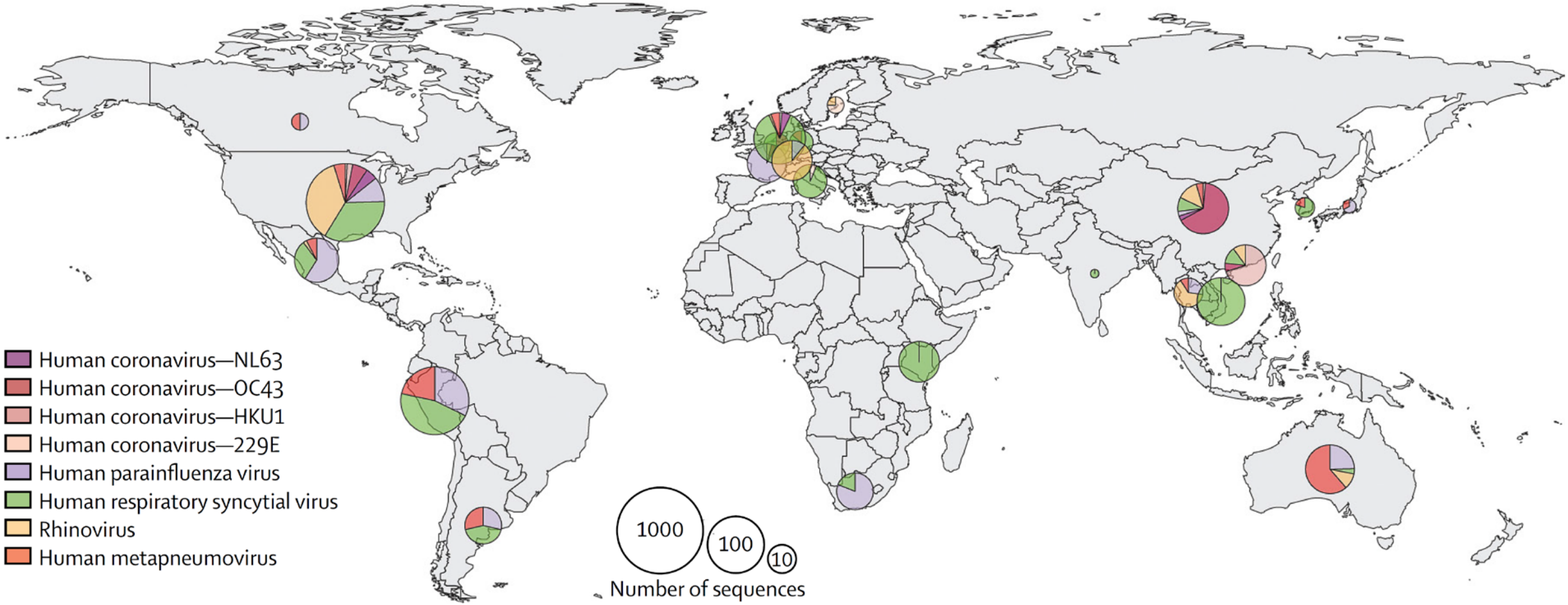
Viral respiratory infections beyond influenza

Less well-established is the surveillance and treatment for non-influenza respiratory viruses

- Many are RNA viruses, which have relatively high genomic mutation rates.
- Compared to influenza viruses, these viruses combined are responsible for a greater annual morbidity and mortality rate across all age groups.

Common Respiratory Viruses	URT	LRT
Influenza virus (A, B)	✓	✓
Respiratory syncytial virus (A, B)	✓	✓
Parainfluenza virus (1-4)	✓	✓
Human metapneumovirus		✓
Rhinovirus (A, B, C)	✓	
Coronavirus (HKU1, NL63, 229E, OC43)	✓	✓
Adenovirus	✓	

Global distribution of common non-influenza respiratory viruses

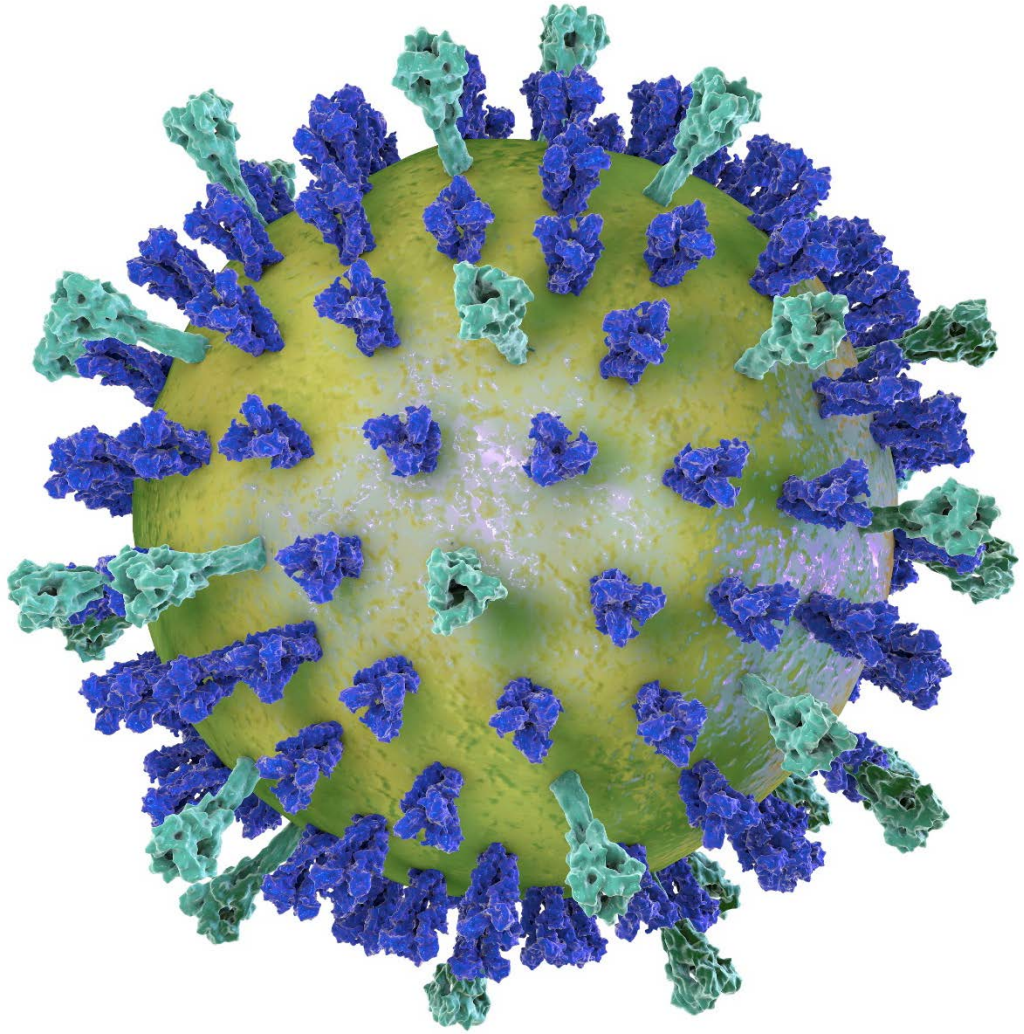


Tang JW, *et al.* Lancet Infect Dis 17: e320-326, 2017 License Number: 4425481327840

Respiratory syncytial virus

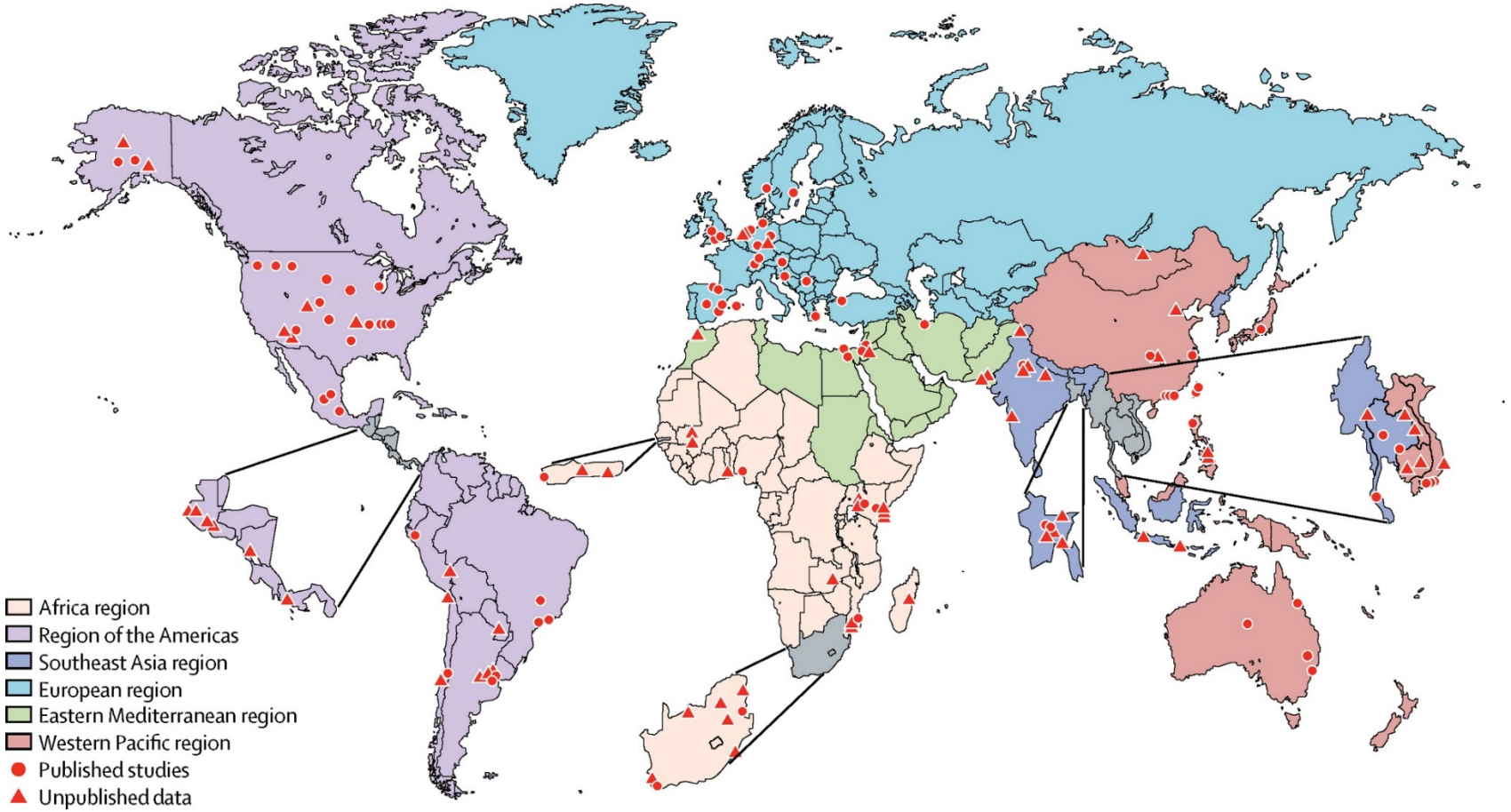
RSV global disease burden

- Acute lower respiratory infection is one of the leading causes of morbidity and mortality in children under 5
- In 2005:
  - ~34 million new episodes of RSV occurred worldwide in young children
  - 10% of RSV infections were severe enough to necessitate hospital admission
  - 55,000-200,000 deaths among children could be attributed to RSV
- The WHO's Battle against Respiratory Viruses (BRaVe) initiative has highlighted the need for enhanced clinical and epidemiological surveillance for respiratory viruses with a focus on the development of a vaccine for RSV



RSV is a global concern

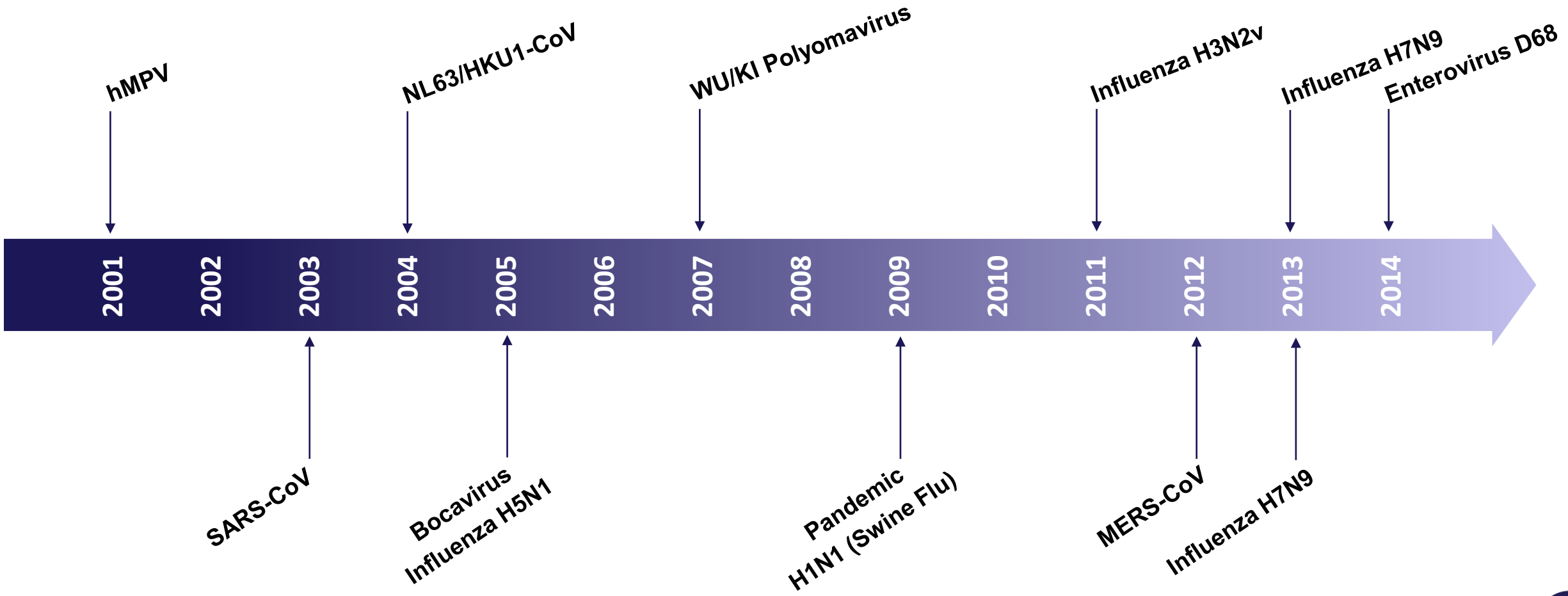
Locations of studies reporting incidence, hospital admission, and in-hospital case fatality in children with acute lower respiratory infections caused by RSV



Shi T, et al. Lancet 390: 10.1016/S0140-6736(17)30938-8, 2017

Emerging respiratory viruses

Emerging respiratory viruses are linked to multiple outbreaks and potential pandemic spread



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  - Authenticated synthetics, strains, and formats
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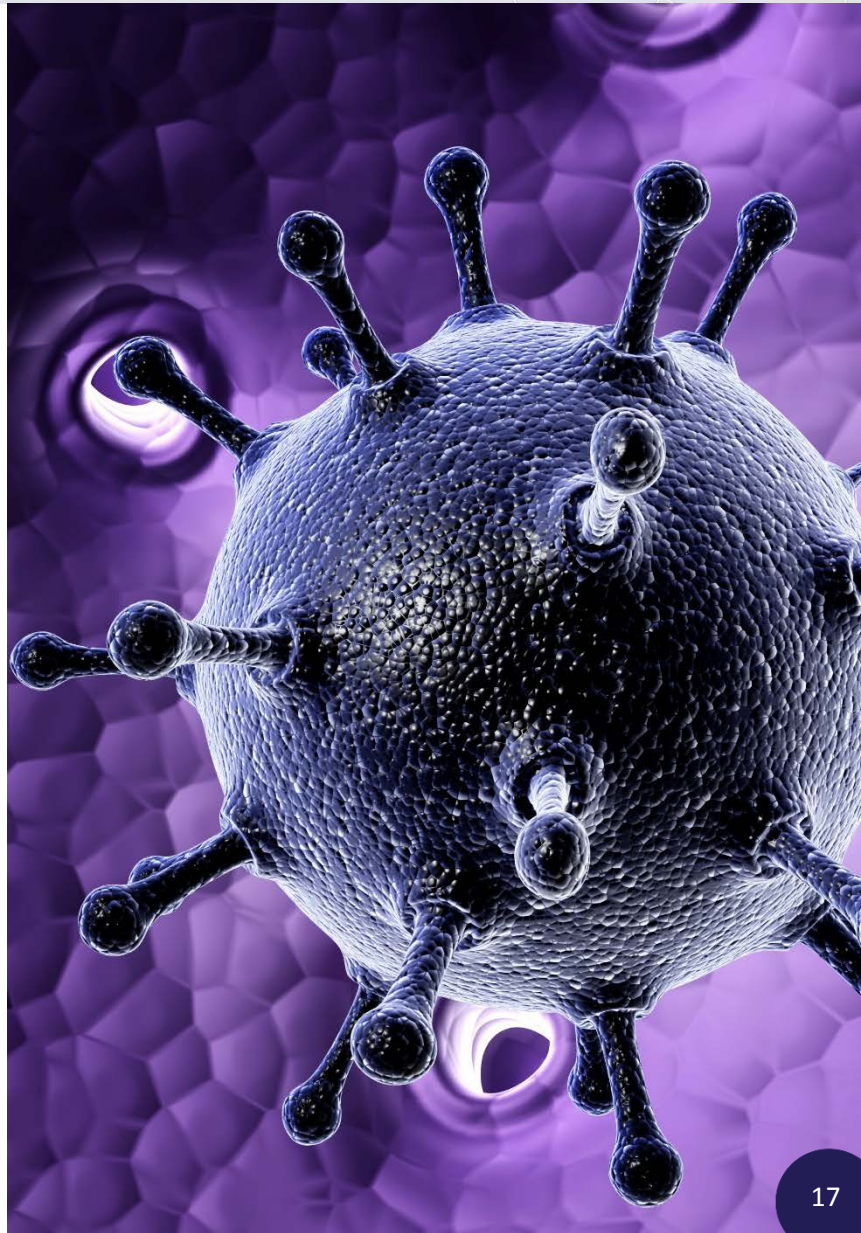


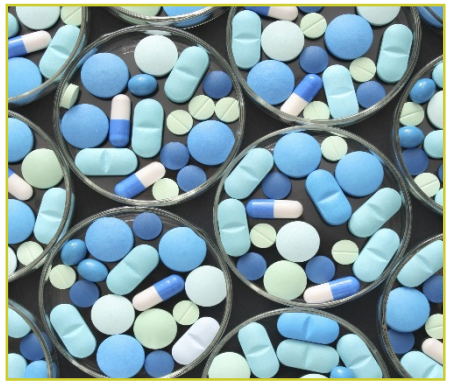
Respiratory virus countermeasures

Rapid detection (diagnostics), drug treatment, and vaccines are essential approaches for controlling respiratory virus infections

Diagnostics

Direct detection from a clinical sample, indirect detection (virus isolation), and serology

Antiviral Drugs



Viral clearance during acute and persistent infections

Vaccines

Critical for the prevention of respiratory diseases

Respiratory virus countermeasures

Influenza vaccine and antiviral clinical trials represent the majority of respiratory virus studies, with RSV as a close second

	Influenza	Respiratory syncytial virus	Human parainfluenze virus	Human metapneumovirus	Coronavirus	Rhinovirus
USA	1561 vaccine trials; 190 antiviral drug trials	49 vaccine trials; 33 antiviral drug trials	13 vaccine trials; 0 antiviral drug trials	3 vaccine trials; 0 antiviral drug trials	4 vaccine trials; 4 antiviral drug trials	12 vaccine trials; 3 antiviral drug trials
European Union	357 vaccine trials; 11 antiviral drug trials	4 vaccine trials; 13 antiviral drug trials	1 vaccine trial; 0 antiviral drug trials	0 vaccine trials; 0 antiviral drug trials	0 vaccine trials; 0 antiviral drug trials	1 vaccine trial; 0 antiviral drug trials

***Table*: Ongoing clinical trials associated with vaccine and antiviral drug development for the different respiratory viruses**

Tang JW, *et al.* Lancet Infect Dis 17: e320-326, 2017 License Number: 4425481327840

Influenza vaccines

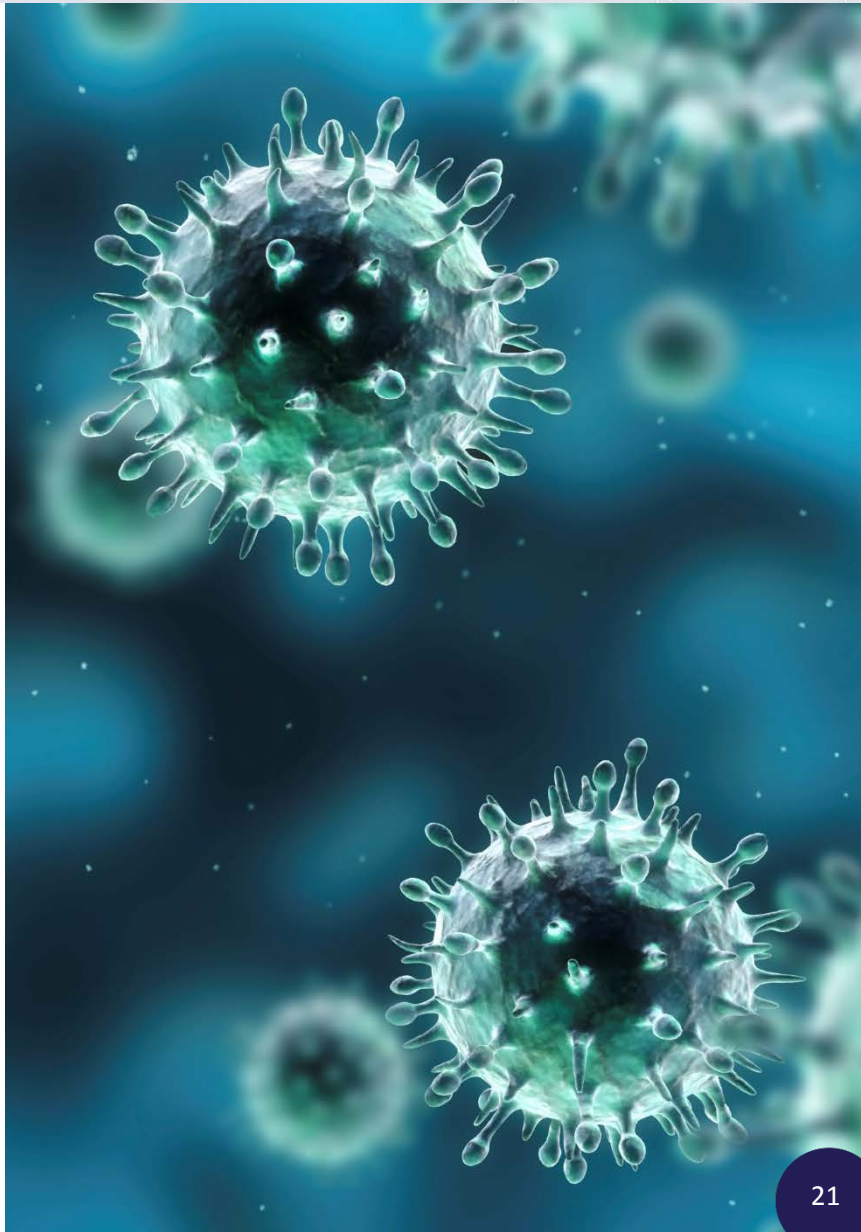
Vaccine viral strains are selected on the basis of surveillance data from the WHO Global Influenza Surveillance and Response System (GISRS)

- Vaccine virus propagation
  - Embryonated chicken eggs
  - Propagated in the allantoic fluid
  - Associated with mutations in hemagglutinin
  - Relies on the availability of eggs
- Cell culture
  - MDCK, Vero, or PER.C6 cells
  - Maintenance of hemagglutinin phenotype
  - Ease in expansion

Current flu vaccine composition

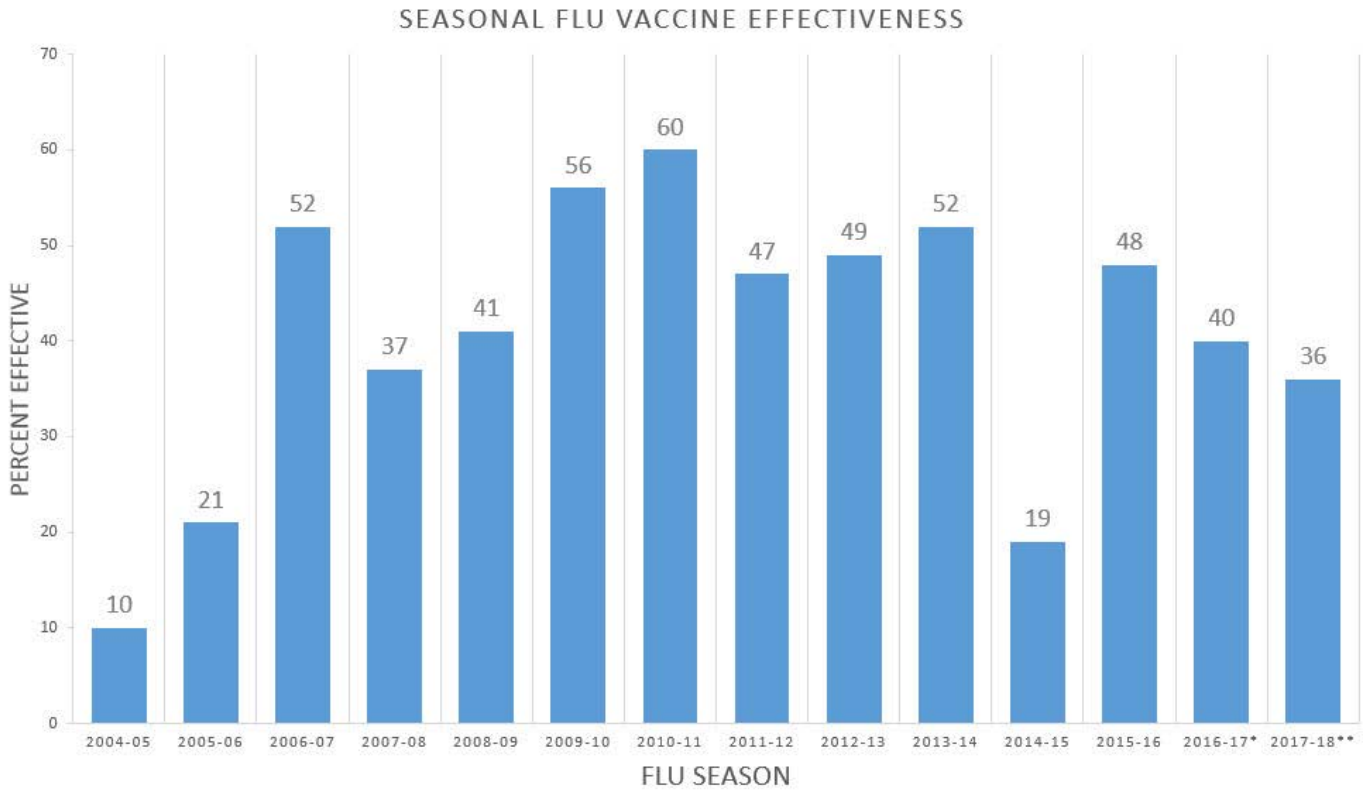
Current challenge: The potential for limited antigenic match between selected vaccine strains and circulating strains

- 2018-2019 Influenza vaccine composition
  - an A/Michigan/45/2015 (H1N1)pdm09-like virus
  - an A/Singapore/INFIMH-16-0019/2016 (H3N2)-like virus
  - a B/Colorado/06/2017-like virus (B/Victoria/2/87 lineage)
  - a B/Phuket/3073/2013-like virus (B/Yamagata/16/88 lineage)



Selected Influenza vaccine strains don't always protect against circulating strains

- Seasonal flu vaccine effectiveness studies indicate that since 2004, only 10% to 60% of individuals immunized are protected.
- During the 2017/2018 season, the effectiveness was only 36%.



Centers for Disease Control and Prevention. Seasonal Influenza Vaccine Effectiveness, 2004-2018.

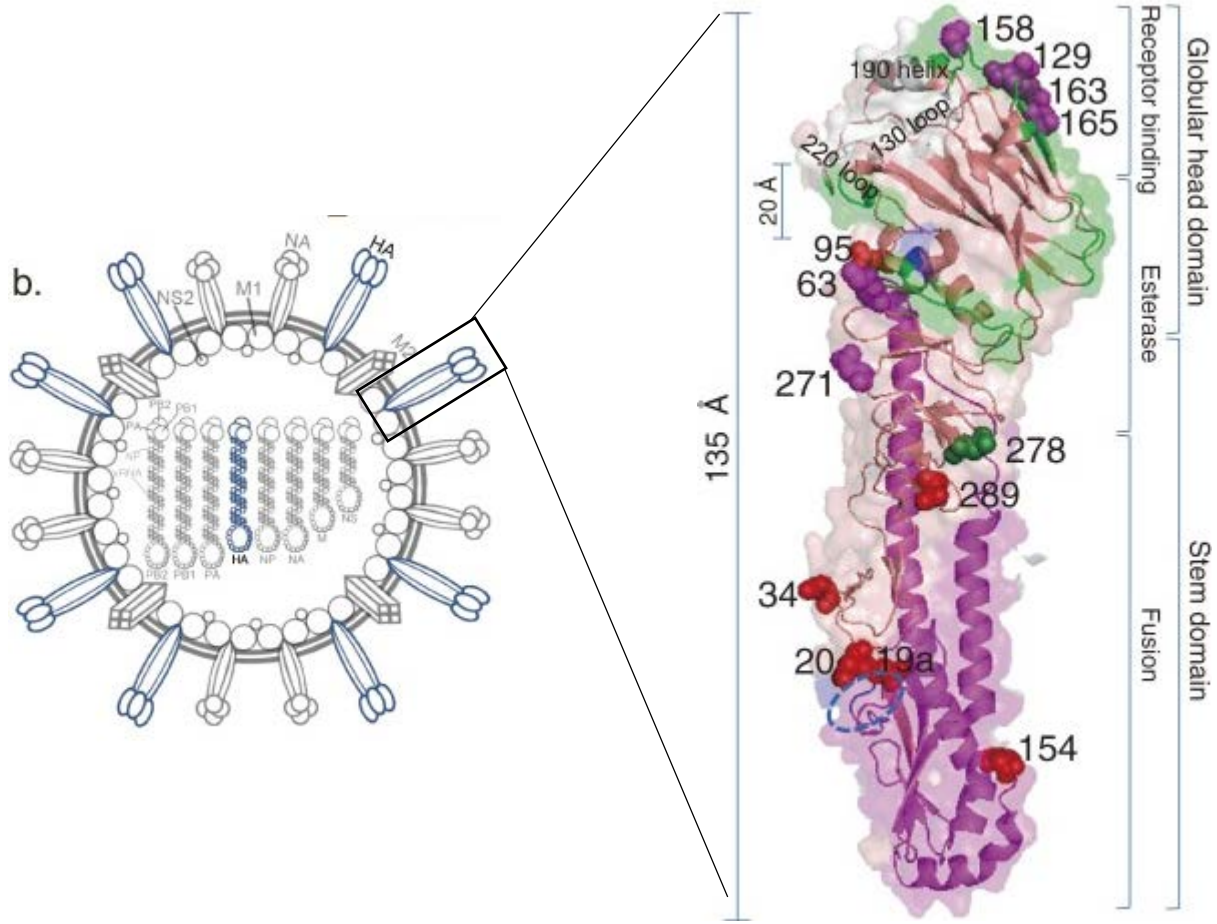
Addressing the flu mismatch problem: The development of a Universal Influenza Vaccine

Overall Goal

One immunization cocktail that can protect against multiple flu strains and subtypes

Toward a universal influenza vaccine

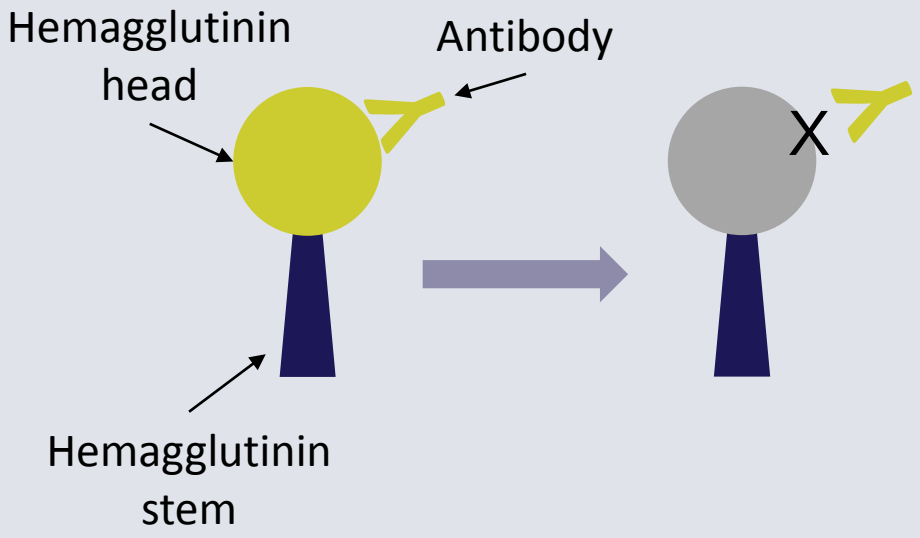
A popular approach: To elicit antibodies to the conserved “stem” of the viral HA protein, as opposed to the globular head domain



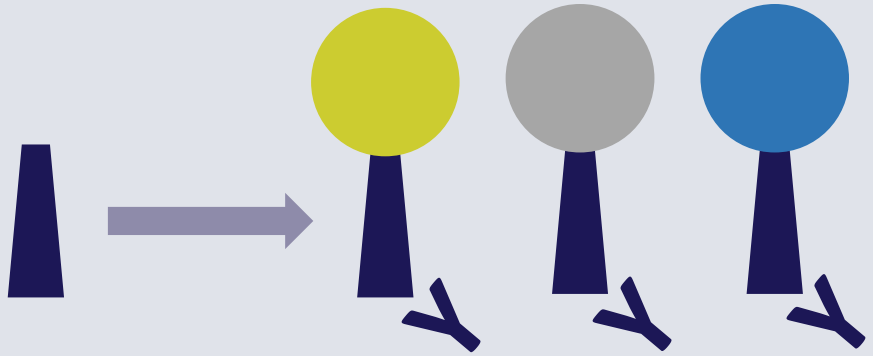
Sriwilaijaroen N, Suzuki Y. Proc Jpn Acad Ser B Phys Biol Sci 88(6): 226-249, 2012.

Toward a universal influenza vaccine

Most traditional vaccines generate antibodies against the hemagglutinin head. If the head changes, the vaccine no longer works.



One new vaccine approach prompts the body to make antibodies to the more stable stem. If the head changes, the vaccine still works.



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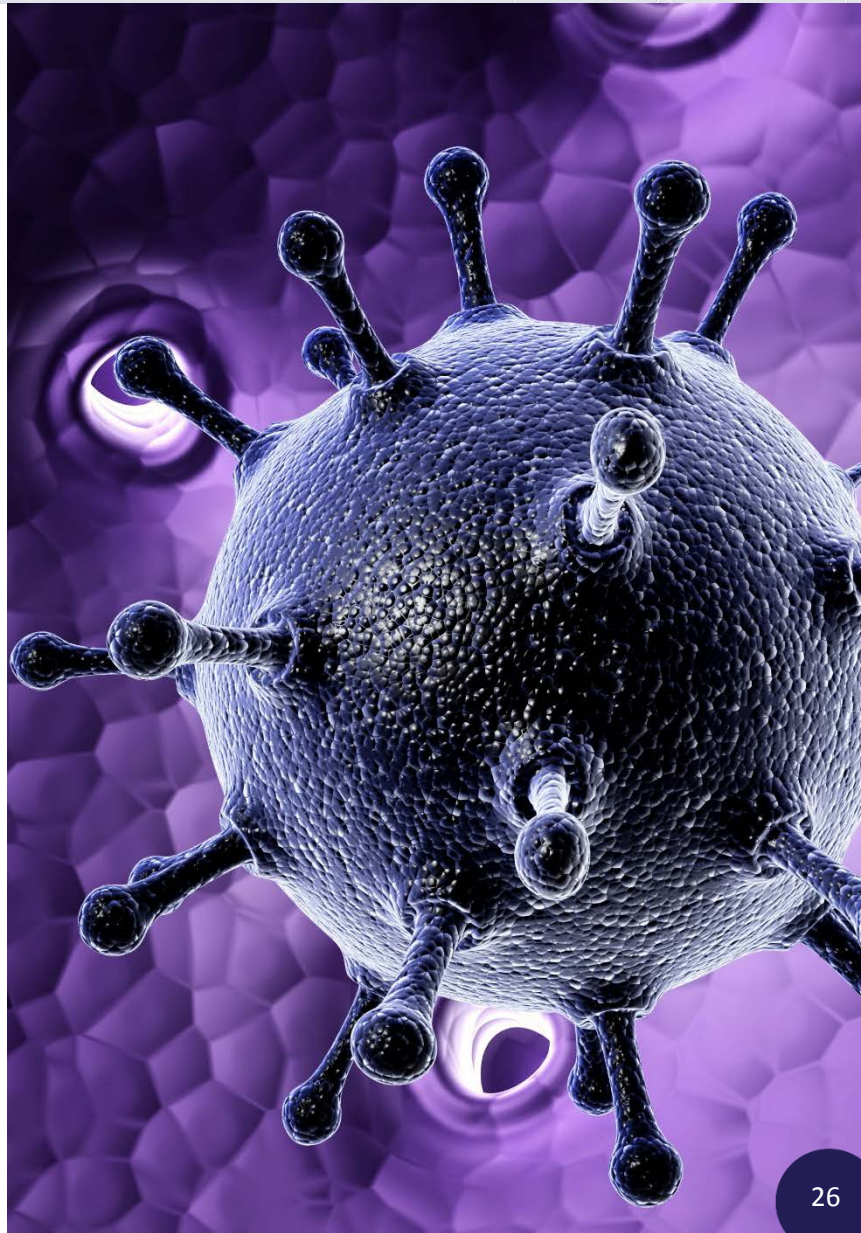


Importance of controls

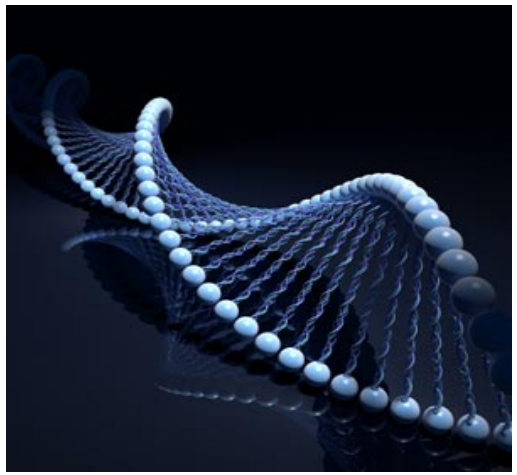
Control materials are critical for validating diagnostic tests, evaluating the efficacy of antiviral compounds, and developing vaccines

Reference Material	Benefit	Disadvantage
Synthetic DNA/RNA	• Easy to design and synthesize; access to genes from unculturable viruses • Reference material from BSL-3 organisms	Do not resemble the complexity of the whole genome
Genomic DNA/RNA	• Mimics complexity of the whole genome	Genetic stability/rare mutations are difficult to obtain
Whole virus	• Mimics complexity of the whole genome	Genetic stability/rare mutations are difficult to obtain
Specimens (*e.g.*, stool & blood)	• Representative	Not a sustainable source

ATCC® Molecular Standards: genomic and synthetic nucleic acids

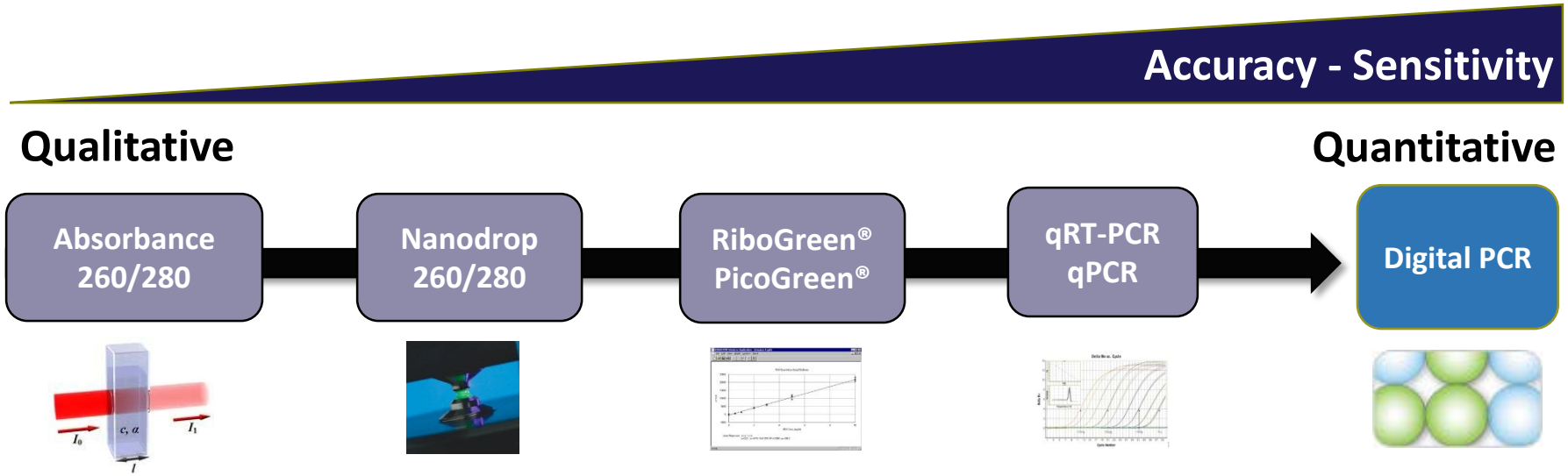
Quantitative Synthetic Molecular Standards
Synthetic DNA or RNA preparations quantified via digital PCR

Quantitative Genomic Molecular Standards
Whole genome preparations quantified via digital PCR



Genomic Nucleic Acids
Whole genome preparations extracted from ATCC® Genuine Cultures

Quantitative molecular standards



Advantages of digital PCR

High precision and accuracy

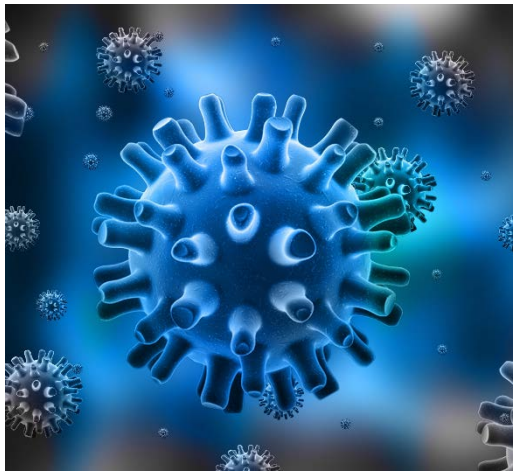
Target-specific quantification

Copy number of individual genes

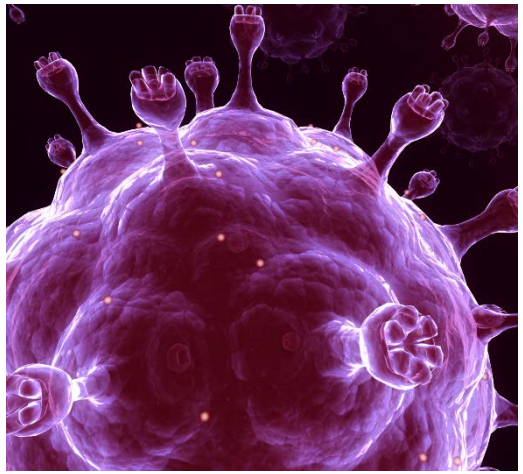
No need to generate cloned standards for standard curve

ATCC viral standards

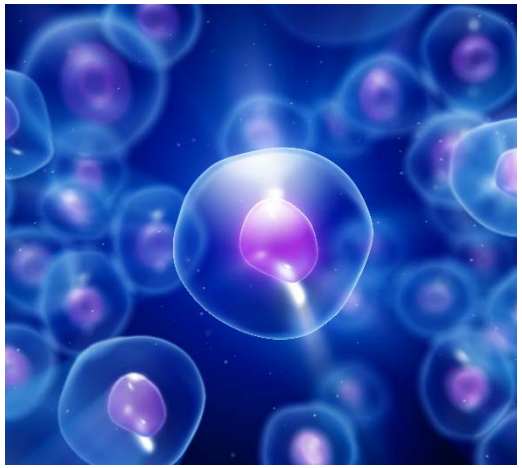
Whole Virus Stocks
Over 350 human and animal respiratory viral strains



Purified Viruses
High-titer, purified virus preparations representing several respiratory viruses



Related Reagents
Cell lines (*e.g.*, MDCK, Hep2, HeLa) and cell culture media



Strain authentication and viability testing

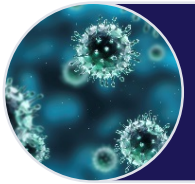
Example Certificate of Analysis for a new Influenza ATCC Accession (ATCC® VR-1884™)

Influenza A virus (H1N1pdm) A/California/07/2009 NYMC X-179A

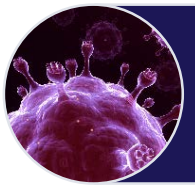
Test / Method	Specification	Result
Viability (Infectivity)*	$CEID_{50} \geq 5 \times 10^3$ per mL	1.4×10^9 CEID50 per mL
Authentication**	Virus identity verified by hemagglutinin gene sequencing (≥ 99% homology)	Pass
Hemagglutination Titer Using 0.5% Turkey Red Blood Cells	≥ 16	1024
Type/Subtype Identification and Purity		
Influenza A virus, RT-PCR of RNA	Detected	Detected
Influenza A virus, pandemic, RT-PCR of RNA	None detected	None detected
Influenza A virus, subtype H1 seasonal , RT-PCR of RNA	None detected	None detected
Influenza A virus, subtype H1 pandemic, RT-PCR of RNA	Detected	Detected
Influenza A virus, subtype H3, RT-PCR of RNA	None detected	None detected
Influenza A virus, subtype H5, RT-PCR of RNA	None detected	None detected
Influenza B virus, RT-PCR of RNA	None detected	None detected
Test for Mycoplasma Contamination		
Agar and broth culture (direct method; 14-day incubation at 37°C)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected
Sterility Test (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud agar, Emmons modified, 37°C, aerobic	No growth	No growth
Blood agar, 37°C, aerobic	No growth	No growth
Brucella agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
DMEM with 10% FBS, 37°C	No growth	No growth

***Titer notes:** 2 days on 9-day-old specific pathogen free chicken embryos (intra-allantoic inoculation) at 35 ± 2°C with humidity.
****Authentication notes:** Molecular authentication was performed by RT-PCR. An amplicon of approximately 1700 base pairs was generated. The amplicon was sequenced and shown to have at least 99% homology to EpiFlu Isolate ID EPI_ISL_30485 and EpiFlu Accession number EPI180554.

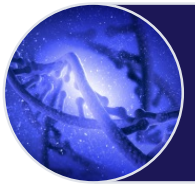
Example: ATCC influenza research materials



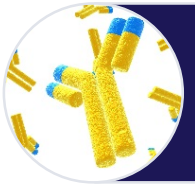
Influenza A – 50+ strains from human, equine, and swine sources



Influenza B – 15+ strains from human sources, including tissue-culture adapted strains



Genomic RNA –**P**reparations of genomic RNA from Influenza subtypes



Antisera and monoclonal antibodies – Antisera and monoclonal antibodies against Influenza

Propagation host – MDCK cell culture (ATCC® CCL-34™) and associated media and reagents for the propagation of tissue culture-adapted viral strains

www.atcc.org/influenza

Purified, high-titer viruses

ATCC® No.	Product Description	Propagation Host (ATCC® No.)
VR-1492PQ™	Purified Human gammaherpesvirus 4	B95-8
VR-283PQ™	Purified Human rhinovirus 16	H1HeLa (CRL-1958™)
VR-284PQ™	Purified Human rhinovirus 14	H1HeLa (CRL-1958™)
VR-1645PQ™	Purified Human rhinovirus 1B	H1HeLa (CRL-1958™)
VR-1804PQ™	Purified Influenza B virus	SPF embryonated CE, 9 days
VR-95PQ™	Purified Influenza A virus (H1N1)	SPF embryonated CE, 10-11 days
VR-544PQ™	Purified Influenza A virus (H3N2)	SPF embryonated CE, 10-11 days

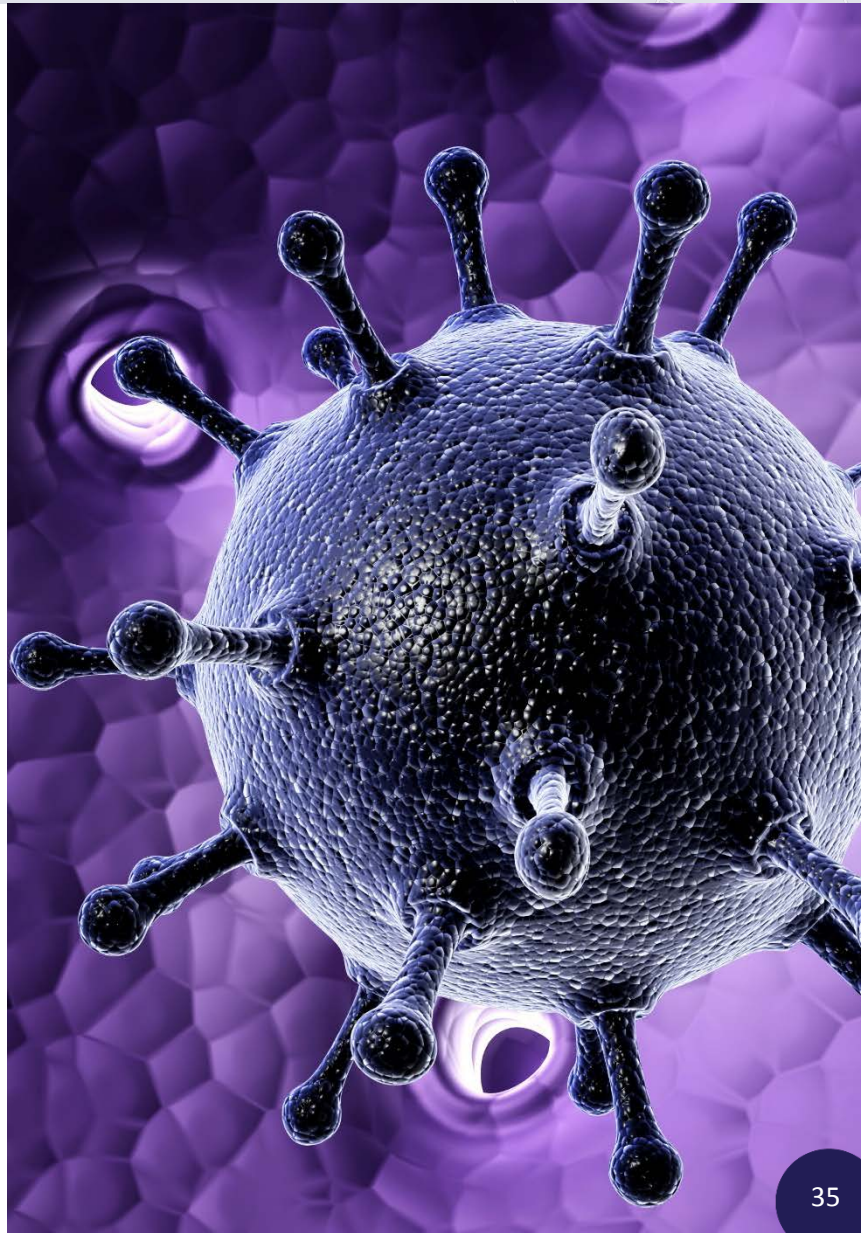
High titer – Infectious titer of $>10^7$ $TCID_{50}$/mL, $CEID_{50}$/mL, or PFU/mL
Quantified genome copy number – Evaluated by Droplet Digital™ PCR
Purity – Ultracentrifugation through sucrose
Authenticity – Verified identity and viability

New accessions available to the community from ATCC

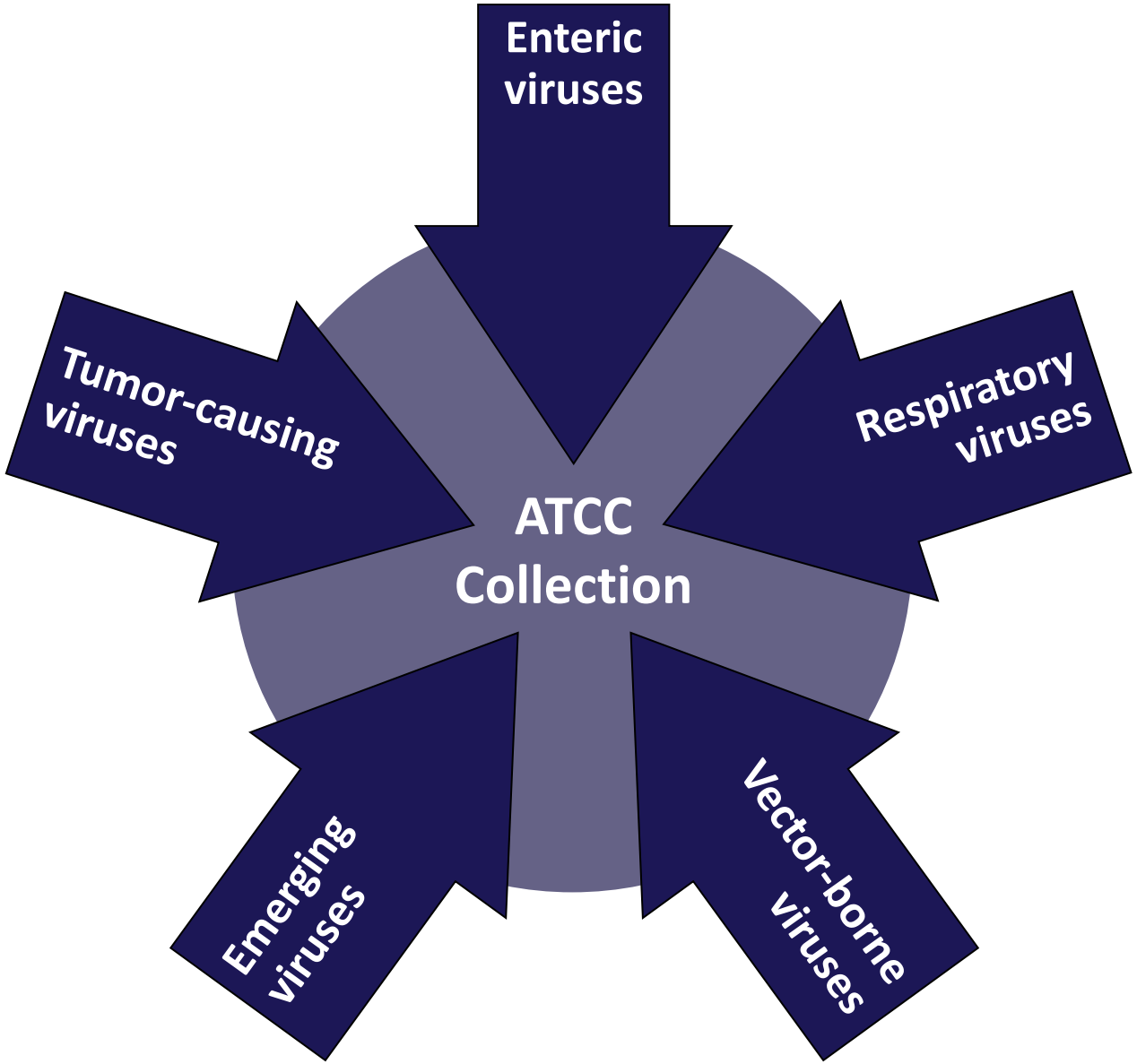
ATCC® No.	Strain Name	Availability
VR-1881™	Influenza A virus (H3N2); A/Wisconsin/67/2005	Coming soon
VR-1882™	Influenza A virus (H3N2); A/Wisconsin/15/2009	Available
VR-1883™	Influenza B virus; B/Wisconsin/1/2010	Available
VR-1884™	Influenza A virus (H1N1pdm); A/California/07/2009NYMC X-179A	Available
VR-1885™	Influenza B virus (BY); B/Wisconsin/1/2010BX-41A	Available
VR-1886™	Parechovirus A, type 3; US/MO-KC/2012/006	Available
VR-1887™	Parechovirus A, type 3; US/MO-KC/2014/001	Available
VR-1888™	Echovirus 9; Strain: Vispo	Coming soon
VR-1891™	Usutu virus; Strain: SAAR 1776	Coming soon
VR-1892™	Usutu virus; Strain: ENT MP 1626	Coming soon
VR-1893™	Influenza A virus; A/Florida/3/2006 (H1N1)	Available
VR-1894™	Influenza A virus; A/California/07/2009 pdm09 (H1N1)	Available
VR-1895™	Influenza A virus; A/California/08/2009 pdm09 (H1N1)	Available
VR-1899™	Mumps rubulavirus; Genotype G; MuV/Iowa.US/2006	Coming soon
VR-1900™	Macaca mulatta polyomavirus 1; Strain: Baylor (SVB2E -WT)	Coming soon

Agenda

- ATCC overview
- Worldwide significance of viral respiratory diseases
- Respiratory virus countermeasures
  - Diagnostics, small molecules, and vaccines
  - Case study: the universal influenza vaccine
- Importance of controls
  - Authenticated synthetics, strains, and formats
- **Efforts to expand the collection**
- Conclusions



ATCC virus outreach efforts

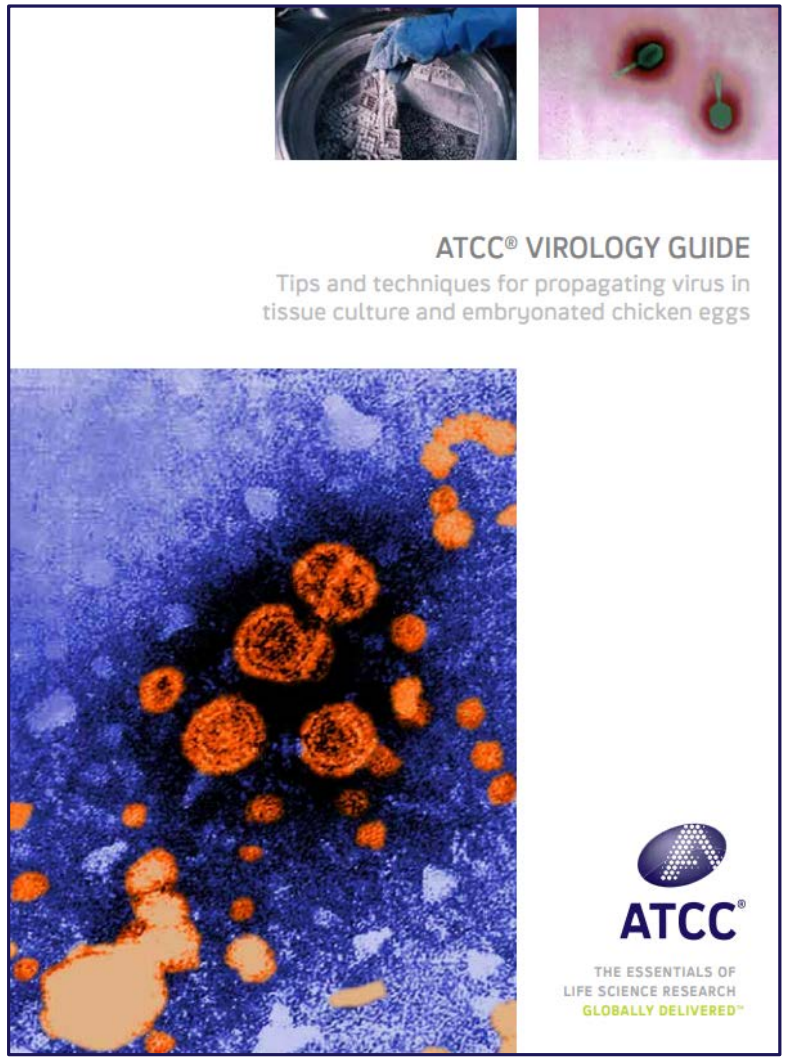


ATCC viral strain outreach

Challenge: Modernize the ATCC collection with contemporary strains and isolates to support the scientific needs of the industrial and academic community

Opportunity: To directly work with ATCC scientists and improve one of the worlds most diverse microbial collections

ATCC virology resources



Resources for ATCC virology products and services:

- General deposit inquiry page: www.atcc.org/Deposits
- Virus portfolio: www.atcc.org/Viruses
- Respiratory virus resources: www.atcc.org/Respiratory
- Purified viruses: www.atcc.org/PurifiedViruses
- Custom services: www.atcc.org/CustomServices
- Tech service support: www.atcc.org/Support
- Culture guides: www.atcc.org/Guides

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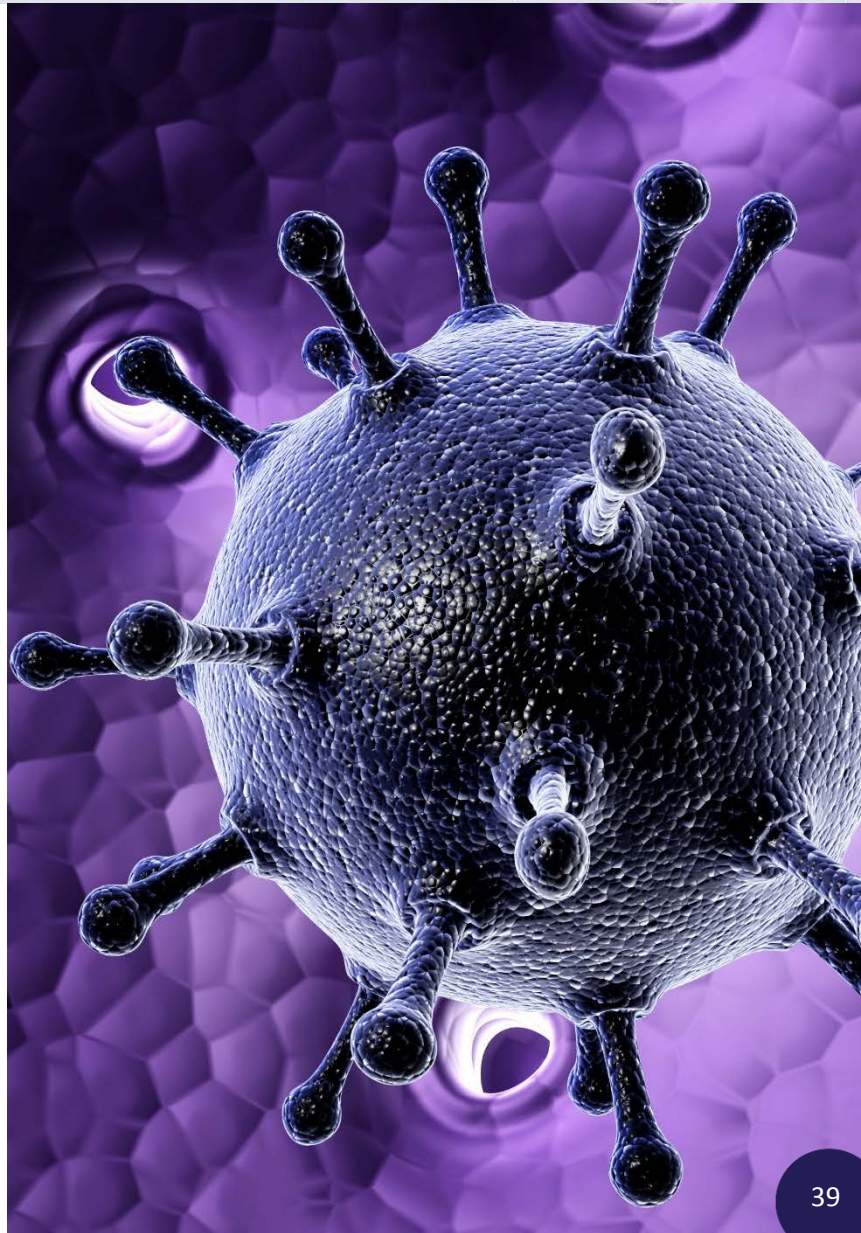
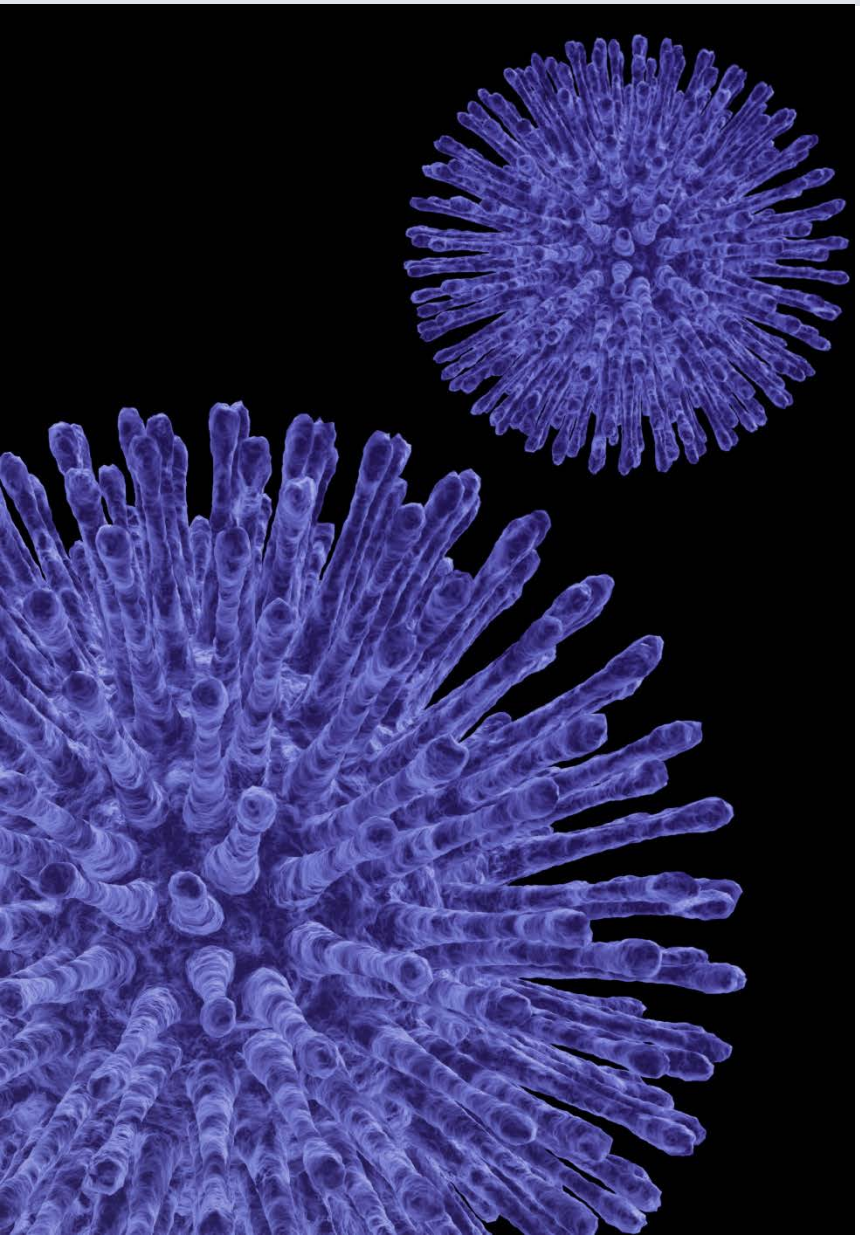


Conclusions



- Endemic and emerging respiratory virus infections represent a significant global heath burden
- Understanding respiratory virus epidemiology and pathogenesis is critical for the development of antiviral therapies
- Authenticated viral standards and derivatives are essential tools for the development and validation of novel preventative and therapeutic techniques
- ATCC respiratory virus resources provide valuable tools for viral research and product development
- ATCC is actively working with the scientific community to modernize both our virology and microbiology collection

Questions & Answers

Q&A