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

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

Common respiratory viruses and their associated disease

- **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.
Influenza viruses circulating in the human population

- **Influenza B**
- **Influenza A group 1**
- **Influenza A group 2**

Timeline:
- **H1N1 (1918)**
- **H2N2 (1957)**
- **H3N2 (1968)**
- **H1N1 (1977)**
- **Influenza B (1940)**
- **H1N1 (2009)**

Years:
- 1918
- 1940
- 1960
- 1980
- 2000
Influenza pandemics

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

<table>
<thead>
<tr>
<th>Flu Pandemic</th>
<th>Year</th>
<th>Subtype</th>
<th>Estimated Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1918 (Spanish flu)</td>
<td>1918-1920</td>
<td>H1N1</td>
<td>20-100 million</td>
</tr>
<tr>
<td>Asian flu</td>
<td>1957-1958</td>
<td>H2N2</td>
<td>&gt;1 million</td>
</tr>
<tr>
<td>Hong Kong flu</td>
<td>1968-1969</td>
<td>H3N2</td>
<td>0.5-1 million</td>
</tr>
<tr>
<td>2009 flu pandemic</td>
<td>2009-2010</td>
<td>H1N1</td>
<td>18-284 thousand</td>
</tr>
</tbody>
</table>

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

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.

CDC estimated influenza disease burden since 2010

Deaths: 12,000 – 56,000
Hospitalizations: 140,000 – 710,000
Cases: 9,200,000 – 35,600,000
Worldwide distribution of influenza

Human influenza A—seasonal H1N1
Human influenza A—pdm09/H1N1
Human influenza A—seasonal H3N2
Human influenza B

Number of sequences

1000
100
10

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.

<table>
<thead>
<tr>
<th>Common Respiratory Viruses</th>
<th>URT</th>
<th>LRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza virus (A, B)</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Respiratory syncytial virus (A, B)</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Parainfluenza virus (1-4)</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Human metapneumovirus</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Rhinovirus (A, B, C)</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Coronavirus (HKU1, NL63, 229E, OC43)</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>
Global distribution of common non-influenza respiratory viruses

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

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

- 2001: hMPV
- 2002: NL63/HKU1-CoV
- 2003: WU/KI Polyomavirus
- 2004: SARS-CoV
- 2005: Bovavirus, Influenza H5N1
- 2006: Pandemic H1N1 (Swine Flu)
- 2007: Influenza H3N2v
- 2008: MERS-CoV
- 2009: Influenza H7N9
- 2010: Enterovirus D68
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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

<table>
<thead>
<tr>
<th></th>
<th>Influenza-syncytial virus</th>
<th>Human parainfluenza virus</th>
<th>Human metapneumovirus</th>
<th>Coronavirus</th>
<th>Rhinovirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>1561 vaccine trials;</td>
<td>13 vaccine trials;</td>
<td>3 vaccine trials;</td>
<td>4 vaccine trials;</td>
<td>12 vaccine trials;</td>
</tr>
<tr>
<td></td>
<td>190 antiviral drug trials</td>
<td>0 antiviral drug trials</td>
<td>0 antiviral drug trials</td>
<td>4 antiviral drug trials</td>
<td>3 antiviral drug trials</td>
</tr>
<tr>
<td>European Union</td>
<td>357 vaccine trials;</td>
<td>1 vaccine trial;</td>
<td>0 vaccine trials;</td>
<td>0 vaccine trials;</td>
<td>1 vaccine trial;</td>
</tr>
<tr>
<td></td>
<td>11 antiviral drug trials</td>
<td>0 antiviral drug trials</td>
<td>0 antiviral drug trials</td>
<td>0 antiviral drug trials</td>
<td>0 antiviral drug trials</td>
</tr>
</tbody>
</table>

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

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
A popular approach: To elicit antibodies to the conserved “stem” of the viral HA protein, as opposed to the globular head domain.
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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Control materials are critical for validating diagnostic tests, evaluating the efficacy of antiviral compounds, and developing vaccines

<table>
<thead>
<tr>
<th>Reference Material</th>
<th>Benefit</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic DNA/RNA</td>
<td>• Easy to design and synthesize; access to genes from unculturable viruses</td>
<td>Do not resemble the complexity of the whole genome</td>
</tr>
<tr>
<td></td>
<td>• Reference material from BSL-3 organisms</td>
<td></td>
</tr>
<tr>
<td>Genomic DNA/RNA</td>
<td>• Mimics complexity of the whole genome</td>
<td>Genetic stability/rare mutations are difficult to obtain</td>
</tr>
<tr>
<td>Whole virus</td>
<td>• Mimics complexity of the whole genome</td>
<td>Genetic stability/rare mutations are difficult to obtain</td>
</tr>
<tr>
<td>Specimens (e.g., stool &amp; blood)</td>
<td>• Representative</td>
<td>Not a sustainable source</td>
</tr>
</tbody>
</table>
## ATCC® Molecular Standards: genomic and synthetic nucleic acids

<table>
<thead>
<tr>
<th></th>
<th>Quantitative Synthetic Molecular Standards</th>
<th>Quantitative Genomic Molecular Standards</th>
<th>Genomic Nucleic Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Synthetic DNA or RNA</strong></td>
<td>Synthetic DNA or RNA preparations quantified via digital PCR</td>
<td>Whole genome preparations quantified via digital PCR</td>
<td>Whole genome preparations extracted from ATCC® Genuine Cultures</td>
</tr>
</tbody>
</table>

- **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.
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
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

<table>
<thead>
<tr>
<th>Test / Method</th>
<th>Specification</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viability (Infectivity)*</td>
<td>CEID&lt;sub&gt;50&lt;/sub&gt; ≥ 5 x 10&lt;sup&gt;3&lt;/sup&gt; per mL</td>
<td>1.4 x 10&lt;sup&gt;6&lt;/sup&gt; CEID&lt;sub&gt;50&lt;/sub&gt; per mL</td>
</tr>
<tr>
<td>Authentication**</td>
<td>Virus identity verified by hemagglutinin gene sequencing (≥ 99% homology)</td>
<td>Pass</td>
</tr>
<tr>
<td>Hemagglutination Titer Using 0.5% Turkey Red Blood Cells</td>
<td>≥ 16</td>
<td>1024</td>
</tr>
<tr>
<td>Type/Subtype Identification and Purity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza A virus, RT-PCR of RNA</td>
<td>Detected</td>
<td>Detected</td>
</tr>
<tr>
<td>Influenza A virus, pandemic, RT-PCR of RNA</td>
<td>None detected</td>
<td>None detected</td>
</tr>
<tr>
<td>Influenza A virus, subtype H1 seasonal, RT-PCR of RNA</td>
<td>None detected</td>
<td>None detected</td>
</tr>
<tr>
<td>Influenza A virus, subtype H1 pandemic, RT-PCR of RNA</td>
<td>Detected</td>
<td>Detected</td>
</tr>
<tr>
<td>Influenza A virus, subtype H3, RT-PCR of RNA</td>
<td>None detected</td>
<td>None detected</td>
</tr>
<tr>
<td>Influenza A virus, subtype H5, RT-PCR of RNA</td>
<td>None detected</td>
<td>None detected</td>
</tr>
<tr>
<td>Influenza B virus, RT-PCR of RNA</td>
<td>None detected</td>
<td>None detected</td>
</tr>
<tr>
<td>Test for Mycoplasma Contamination</td>
<td>DNA detection by PCR of extracted Test Article nucleic acid</td>
<td>None detected</td>
</tr>
<tr>
<td>Sterility Test (21-day incubation)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harpe's HTYE broth, 37°C and 26°C, aerobic</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>Tryplicase soy broth, 37°C and 26°C, aerobic</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>Sabouraud broth, 37°C and 26°C, aerobic</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>Sabouraud agar, Emmons modified, 37°C, aerobic</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>Blood agar, 37°C, aerobic</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>Brucella agar, 37°C, anaerobic</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>Thiglycollate broth, 37°C, anaerobic</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>DMEM with 10% FBS, 37°C</td>
<td>No growth</td>
<td>No growth</td>
</tr>
</tbody>
</table>

*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 EP190054.
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** – Preparations 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](http://www.atcc.org/influenza)
Purified, high-titer viruses

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

**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

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

ATCC Collection

- Enteric viruses
- Respiratory viruses
- Tumor-causing viruses
- Emerging viruses
- Vector-borne viruses
**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 world's most diverse microbial collections
ATCC virology resources

Resources for ATCC virology products and services:

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

- Endemic and emerging respiratory virus infections represent a significant global health 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