ATCC INFLUENZA RESEARCH MATERIALS

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About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World’s premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard products and innovative solutions
- Broad range of biomaterials
  - Cell lines
  - Microorganisms
  - Native & synthetic nucleic acids
  - Reagents
Outline

- Introduction and clinical significance
- Prevention and treatment
- ATCC influenza research materials
Influenza virus

- Family Orthomyxoviridae
- ssRNA - segmented genome
- Enveloped virus
- Surface proteins:
  - Hemagglutinin (HA)
  - Neuraminidase (NA)
- Matrix proteins (M1 & M2)
- Ribonucleoprotein complex:
  - PB1, PB2, PA, and NP
- NS

Every year, seasonal influenza infections result in:
- 3-5 million hospitalizations worldwide
- 250-500 thousand deaths worldwide
- $26.8-87.1 billion in healthcare costs in the United States alone

Photo credit: Dan Higgins.
Life cycle

Photo credit: Nicolle Rager Fuller, National Science Foundation
Subtypes and strains

Influenza A
- Classified by subtype & strain
- 18 HA & 11 NA subtypes
- Hosts: Human, birds, poultry, swine, dogs, horses
- Major cause of epidemics

Influenza B
- Classified by strain
- Host: Human
- Associated with less severe outbreaks than Influenza A
- Have not caused pandemics

Influenza C
- Host: Human
- Associated with mild illness in humans
- Do not cause epidemics or pandemics

Influenza
Antigenic drift and shift

Antigenic drift

Antigenic shift
## Influenza pandemics

<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.
Symptoms and severity

Symptoms

• Fever
• Sore throat
• Muscle pains
• Severe headache
• Coughing
• Weakness
• Fatigue

Individuals at high risk:

• Young children
• Pregnant women
• People 65 years and older
• People with chronic medical conditions
Symptoms and severity

Severity

- Influenza viral strain
- Vaccine availability
- How many people are vaccinated
- Vaccine efficacy

Annual influenza-associated deaths:
CDC estimates that the number of influenza-associated deaths in the United States from 1976-2007 ranged from 3,000-49,000 individuals per season. Approximately 90% of deaths each influenza season occur in people 65 years and older.
Diagnosis

• Virus culture
  – Requires 3-10 days

• Reverse transcription polymerase chain reaction
  – Sensitive, specific, fast, multiplex format

• Rapid antigen testing
  – Immunoassays

• Serologic testing
  – Hemagglutination inhibition
  – Microneutralization

Direct Hemagglutination

Positive Reaction:
- Hemagglutinating virus + Red blood cells = Agglutination

Negative Reaction:
- Non-hemagglutinating virus + Red blood cells = No agglutination
Prevention

Social Distancing

Personal Hygiene

Vaccination
Prevention

- Travel less
- Work from home
- Close schools

Social Distancing
Prevention

- Travel less
- Work from home
- Close schools

- Cover your mouth when you cough or sneeze
- Wash your hands
- Use of surgical masks in healthcare settings

Personal Hygiene
Prevention

- Travel less
- Work from home
- Close schools
- Cover your mouth when you cough or sneeze
- Wash your hands
- Use of surgical masks in healthcare settings
- Vaccination against seasonal influenza virus strains

Vaccination
Influenza vaccines

Vaccine virus selection

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

• Vaccines:
  – **Trivalent**: Two Influenza A strains and one Influenza B strain
  – **Quadrivalent**: Four strains
  – **Monovalent**: H1N1 pdm09

• Potential for limited antigenic match between selected vaccine strains and circulating strains
Influenza vaccines

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
Influenza vaccines

Vaccine virus types

• Inactivated vaccine
  – Vaccine is prepared as whole inactivated, split virion, or subunit
  – Reassortant master donor virus:
    • A/Puerto Rico/8/34 (H1N1) (ATCC® VR-95™)

• Live attenuated vaccine
  – Vaccine is prepared as a live, cold-adapted, temperature-sensitive variant
  – Reassortant master donor viruses:
    • A/Ann Arbor/6/60 (H2N2)
    • B/Ann Arbor/1/66

Reperant et al. F1000Prime Rep 6: eCollection, 2014
Current flu vaccine composition

2014-2015 Influenza trivalent vaccine
- A/California/7/2009 (H1N1)pdm09-like virus
- A/Texas/50/2012 (H3N2)-like virus
- B/Massachusetts/2/2012-like virus

2014-2015 Influenza quadrivalent vaccine
- Trivalent preparation + B/Brisbane/60/2008-like virus

Photo credit: Dan Higgins
Influenza treatment

Symptom Control

Antivirals
Influenza treatment

- Minor infections
- Symptom management until the infection is resolved:
  - Rest
  - Fluids
  - Fever reduction
Influenza treatment

- Minor infections
- Symptom management until the infection is resolved:
  - Rest
  - Fluids
  - Fever reduction

- Severe infections complicated by underlying medical conditions
- Antivirals:
  - Neuraminidase inhibitors
  - M2 inhibitors
ATCC influenza research materials

Strains and reagents

**Influenza A** – 50+ strains from human, equine, and swine sources, including A/PR/8/34 and subtypes H10N7, H11N6, H12N5, H13N6, H1N1, H2N2, H3N2, H4N6, H4N8, H5N2, H5N9, H6N2, H6N3, H7N3, H7N7, and H8N4

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

**Genomic RNA** – 6 preparations of genomic RNA from Influenza A virus subtypes H1N1 and H3N2

**Antisera and monoclonal antibodies** – 4 preparations of antisera against Influenza A strains, and 18 preparations of monoclonal antibodies against various subtypes including H1N1, H7N7, H9N2, and H5N1

**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)
## ATCC influenza research materials

### New products

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Virus Type</th>
<th>Strain</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2008 and 2009 Southern Hemisphere influenza seasons</td>
</tr>
<tr>
<td>VR-1813™</td>
<td>Influenza B virus</td>
<td>B/Massachusetts/2/2012</td>
<td>2014-2015 Northern Hemisphere influenza season</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2014 Southern Hemisphere influenza season</td>
</tr>
</tbody>
</table>
Strain authentication and viability testing

Sequence

Viability

Sterility

Titer
Strain authentication and viability testing

- Generation and analysis of a 500-1200 bp amplicon
- Sequence comparison to NCBI
Strain authentication and viability testing

- Generation and analysis of a 500-1200 bp amplicon
- Sequence comparison to NCBI

- Mycoplasma detection
- BacT/ALERT 3D System

Sterility
Strain authentication and viability testing

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- Cultures grown in embryonated chicken eggs - Hemagglutination

- Cultures grown in tissue culture – IFA assisted plaque assay
Strain authentication and viability testing

Influenza B virus, B/Massachusetts/2/2012 (ATCC® VR-1813™)

<table>
<thead>
<tr>
<th>Test / Method</th>
<th>Specification</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viability (infectivity)*</td>
<td>PFU, IFU, TCID$<em>{50}$ or CEID$</em>{50}$ $\geq 5 \times 10^3$ per mL</td>
<td>Pass</td>
</tr>
<tr>
<td>Authentication**</td>
<td>Virus identity verified by Immunofluorescence, ELISA, and/or Sequencing</td>
<td>Pass</td>
</tr>
<tr>
<td>Test for Mycoplasma Contamination</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>Broth and agar culture (direct method)</td>
<td>None detected</td>
<td>None detected</td>
</tr>
<tr>
<td>DNA detection by PCR of test article nucleic acid</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>Sterility test (Bact/ALERT 3D)</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>iAST bottle (aerobic) at 32°C, 14-day incubation</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>INST bottle (anaerobic) at 32°C, 14-day incubation</td>
<td>No growth</td>
<td>No growth</td>
</tr>
</tbody>
</table>

*Titer notes: 2.3 x 10^9 CEID$_{50}$/mL in 2 days in 10 day old Specific Pathogen Free Chicken Eggs (intra-allantoic inoculation) at 33°C with humidity, as determined by Hemagglutinin Inhibition Assay using 0.5% Chicken Red Blood Cells in DPBS at room temperature for 20 minutes.

**Authentication notes: Molecular authentication was performed by RT-PCR. An amplicon of approximately 1000 bp was generated. A portion of the amplicon was sequenced and shown to have 99% homology to NCBI # KC892118.1 [Influenza B virus (B/Massachusetts/02/2012) segment 4 hemagglutinin (HA) gene, complete cds].
ATCC® Virology Guide

- Viral propagation in chicken eggs and tissue culture
- Growth media for tissue culture-adapted viruses
- Preservation via cryopreservation or lyophilization
- Titering by plaque assay, TCID$_{50}$, and CEID$_{50}$
- Viral authentication and viability testing

www.atcc.org/guides
Conclusion

• Influenza viruses are highly contagious airborne pathogens that undergo frequent adaption through antigenic drift and shift, resulting in yearly seasonal outbreaks and occasional pandemics.

• Influenza infection can be prevented through the administration of vaccines that represent strains that are in circulation.

• ATCC offers a number of influenza research materials to help support influenza research; the development, verification, and evaluation of novel influenza detection methods; and the analysis of therapeutic efficacy.

• ATCC influenza viral strains are analyzed for authenticity, infectivity, and viability through culturing, sterility testing, titering, hemagglutination assay, IFA-assisted plaque assay, and sequencing.
Thank you!

Register for more webinars in the ATCC “Excellence in Research” webinar series at www.atcc.org/webinars.

September 18, 2014
10:00 AM, 3:00 PM EST
Dr. Fang Tian, Dr. David H. Randle
ATCC® Genetic Alteration Cell Panels: Effective tools for high throughput screening using Corning® Epic® Technology

October 16, 2014
10:00 AM, 3:00 PM EST
Dr. Tigwa H. Davis
Using LUHMES cells as a model system to study dopaminergic neuron cell biology

Thank you for joining today!
Please send additional questions to tech@atcc.org