ATCC INFLUENZA RESEARCH MATERIALS

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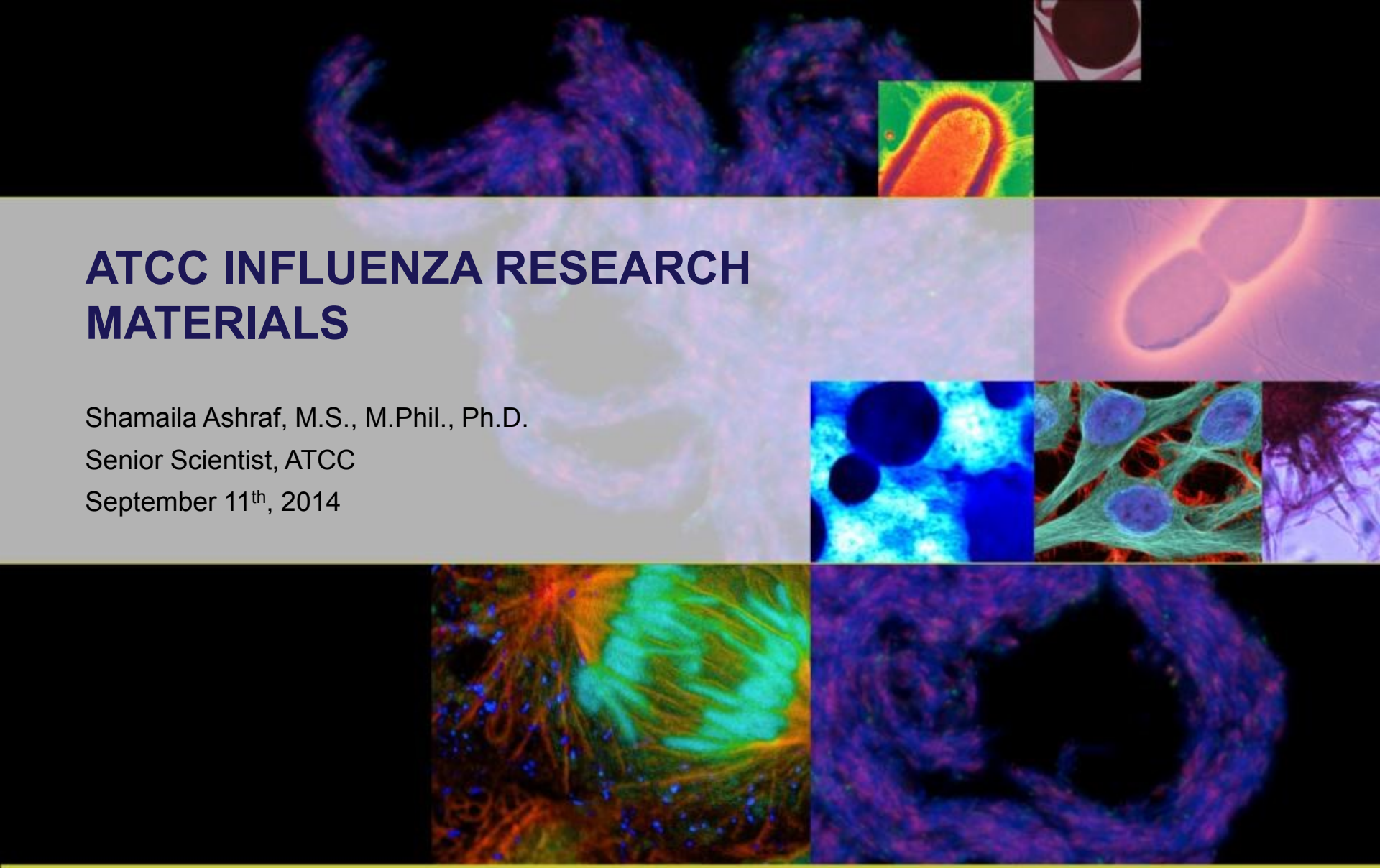
September 11th, 2014



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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

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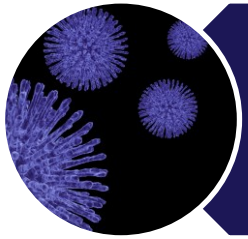
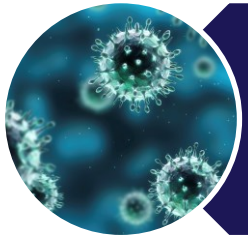
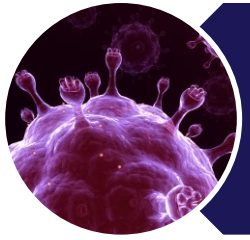
Outline



Introduction and clinical significance



Prevention and treatment



ATCC influenza research materials

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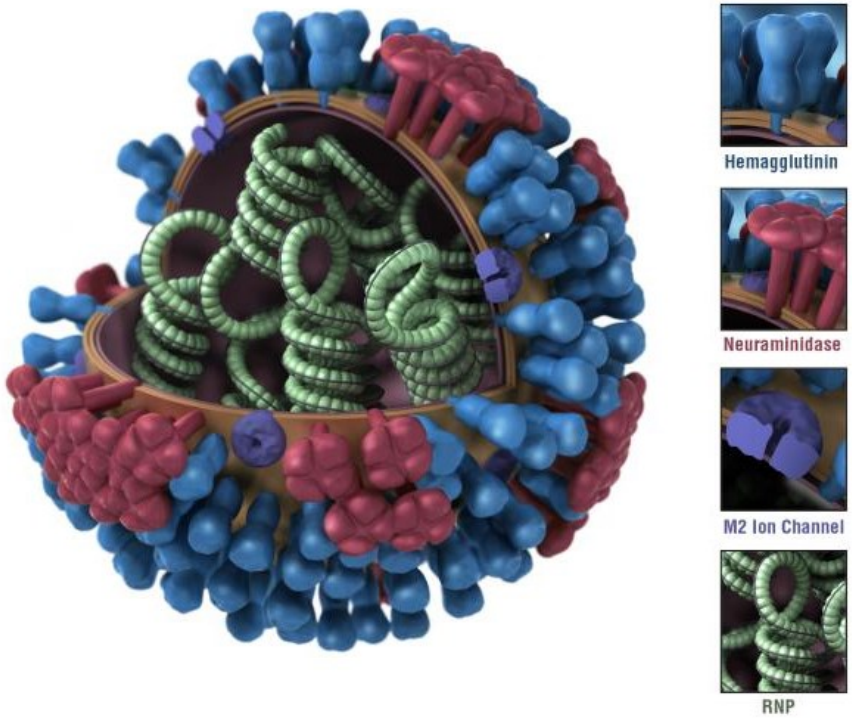
Influenza virus

- 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

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Photo credit: Dan Higgins.

Life cycle

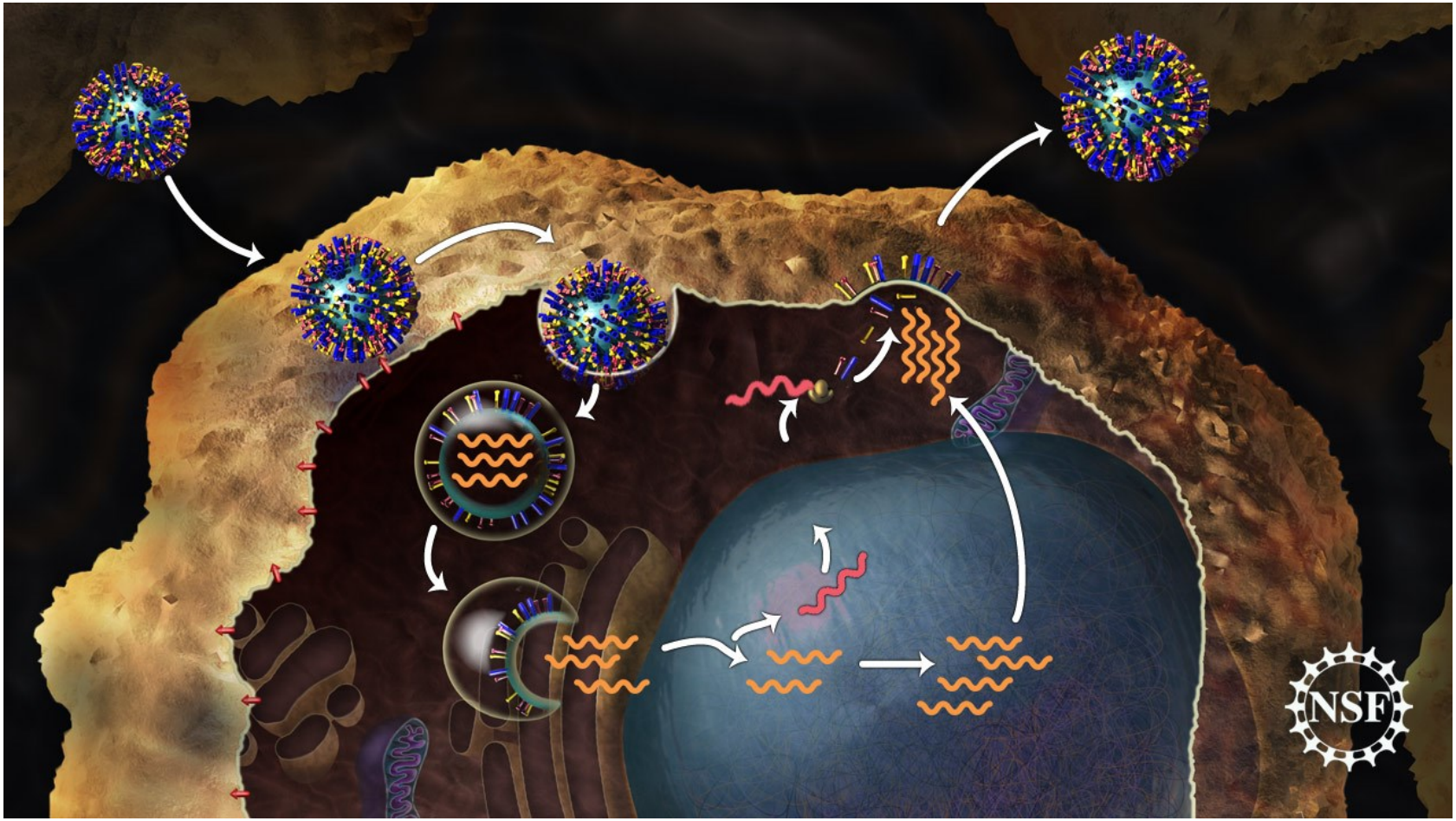
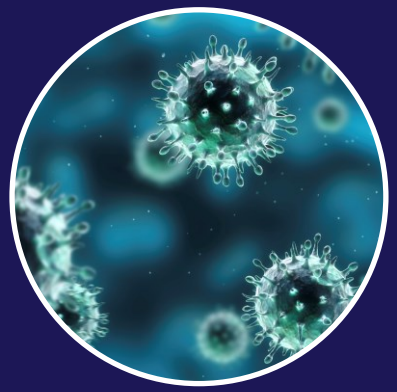


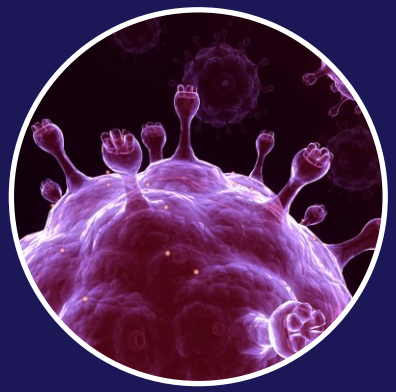
Photo credit: Nicolle Rager Fuller, National Science Foundation

Subtypes and strains



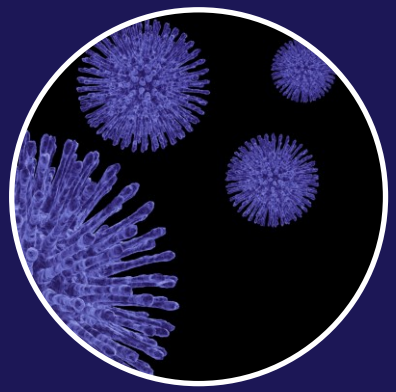
Influenza A

- 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

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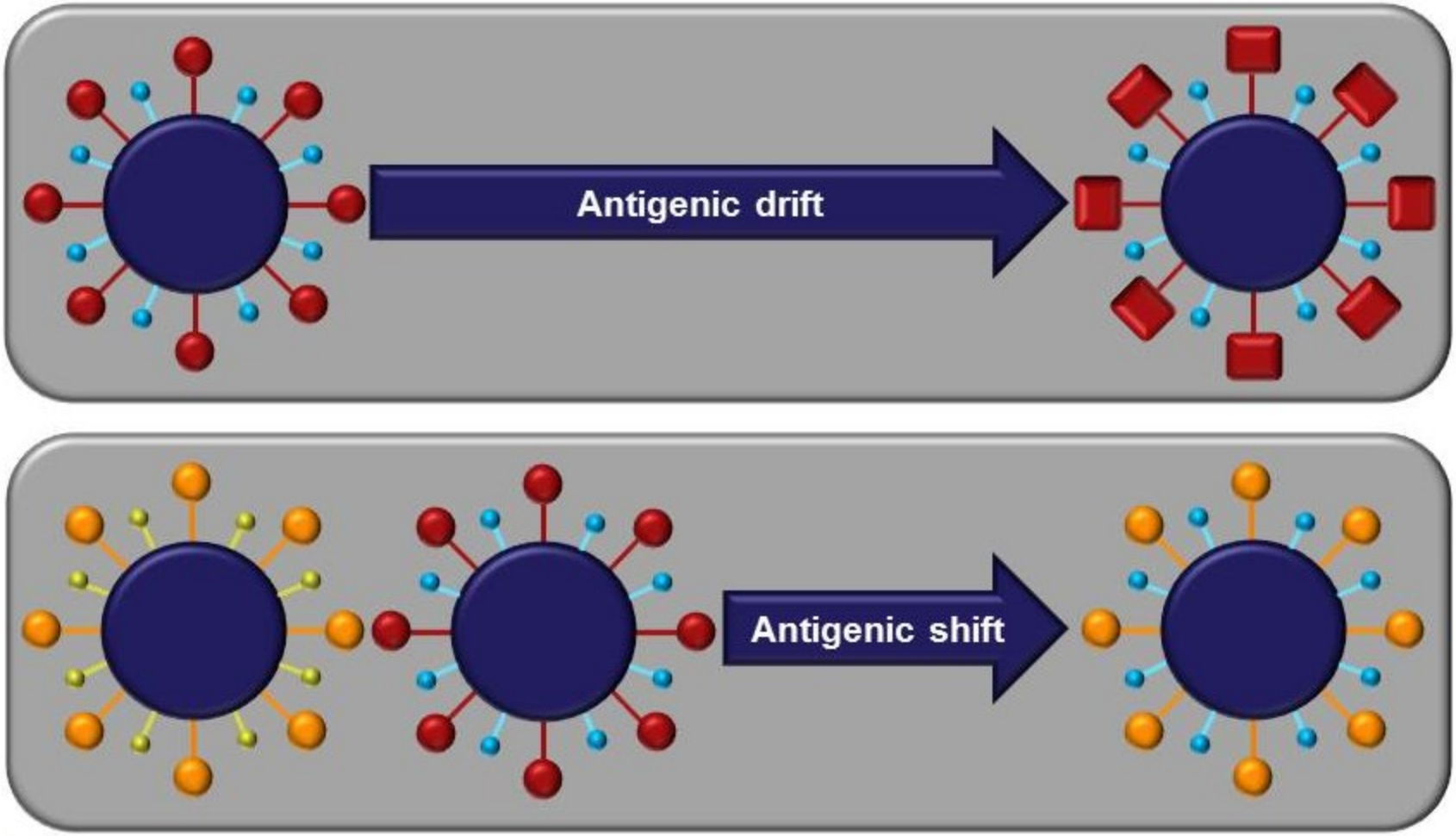
Antigenic drift and shift



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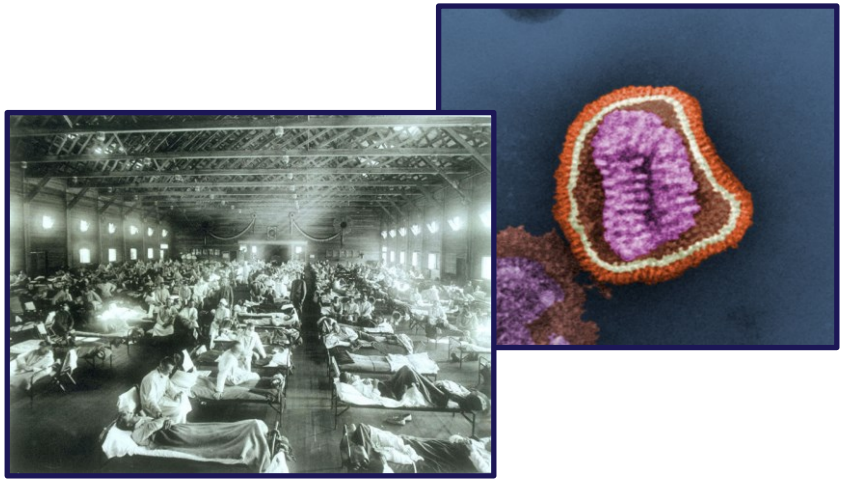
Influenza pandemics

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.



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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

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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.

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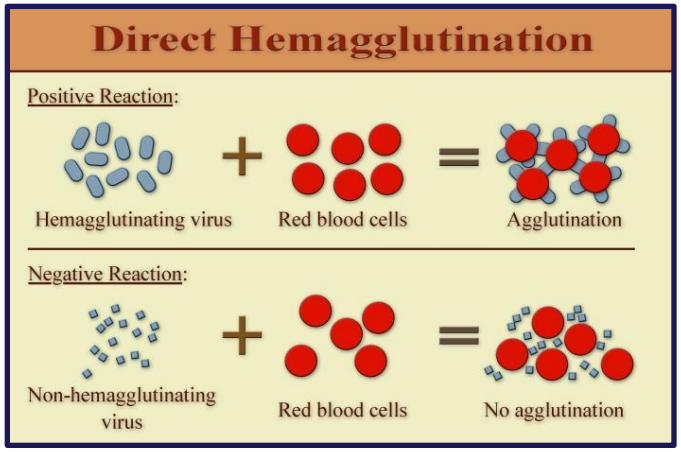
Diagnosis

- 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



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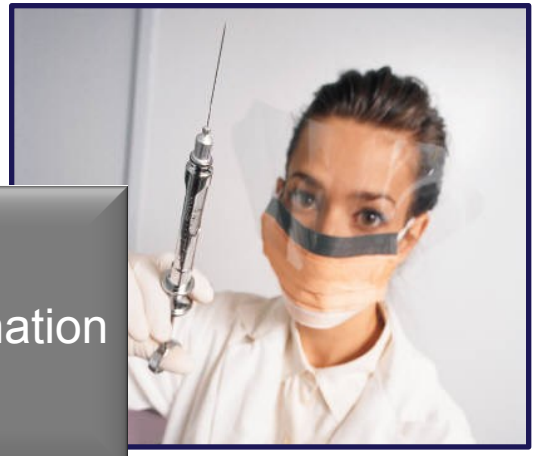
Prevention

Social Distancing

Personal Hygiene

Vaccination



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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

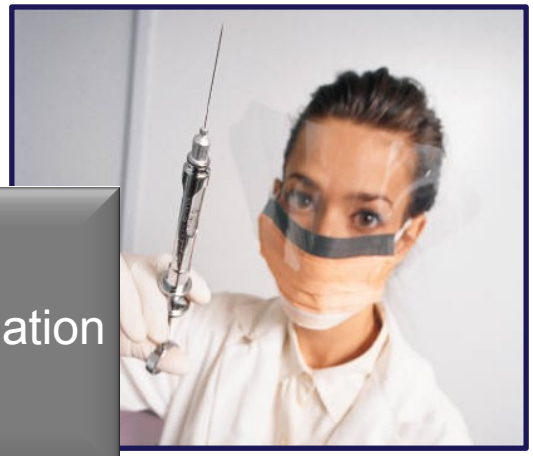
ATCC®

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



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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

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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

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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

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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

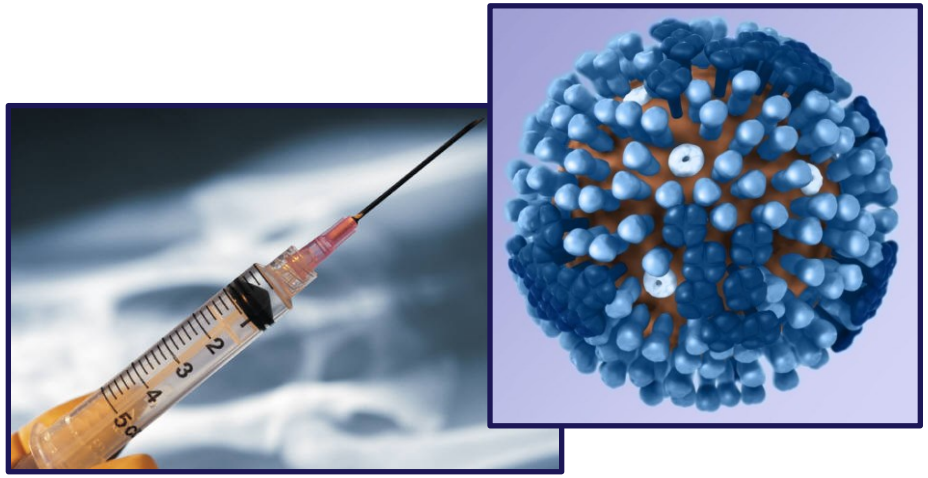

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Photo credit: Dan Higgins

Influenza treatment

Symptom Control

Antivirals

Influenza treatment

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

Symptom Control

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

Antivirals

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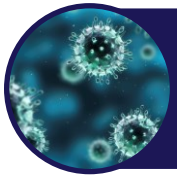
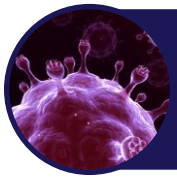
ATCC influenza research materials

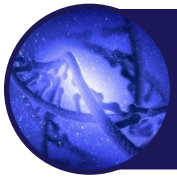
Strains and reagents



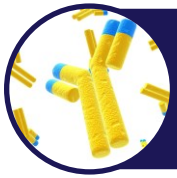
Influenza A – 50+ strains from human, equine, and swine sources, including A/PR/8/34 and subtypes H10N7, H11N6, H12N5, H13N6, H1N1, H2N2, H3N2, H4N6, H4N8, H5N2, H5N9, H6N2, H6N3, H7N3, H7N7, and H8N4



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

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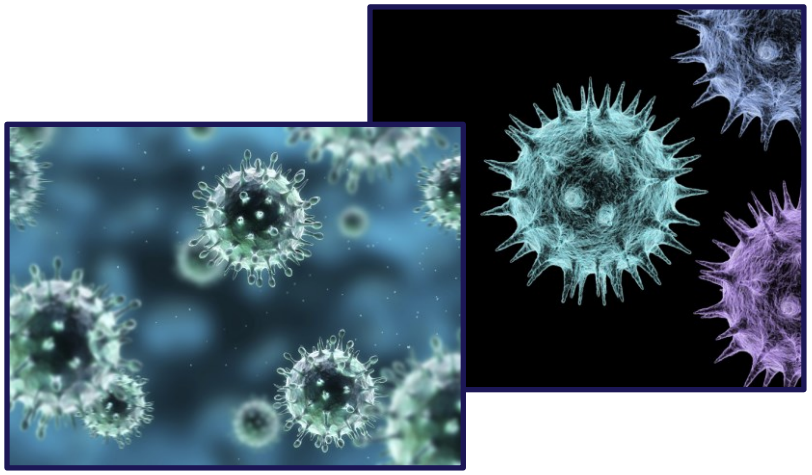
ATCC influenza research materials

New products

ATCC® No.	Virus Type	Strain	Significance
VR-1804™	Influenza B virus	B/Florida/4/2006	2008-2009 Northern Hemisphere influenza season 2008 and 2009 Southern Hemisphere influenza seasons
VR-1813™	Influenza B virus	B/Massachusetts/2/2012	2014-2015 Northern Hemisphere influenza season 2014 Southern Hemisphere influenza season



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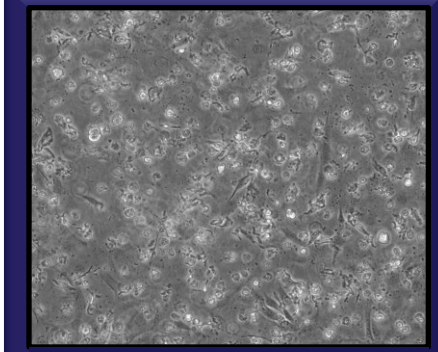
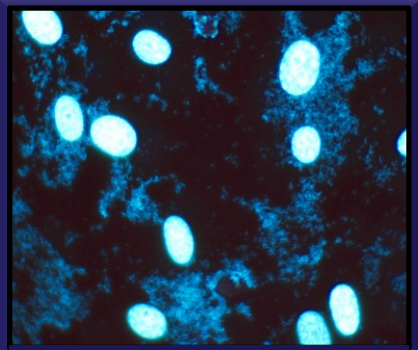
Strain authentication and viability testing

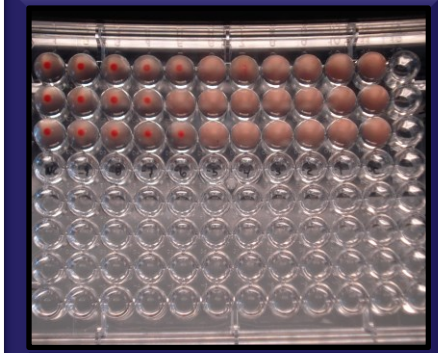
Sequence



Viability



Sterility



Titer

ATCC®

Strain authentication and viability testing

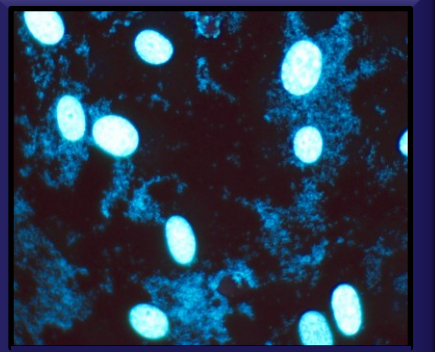
- Generation and analysis of a 500-1200 bp amplicon
- Sequence comparison to NCBI

ATCC®

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

ATCC®

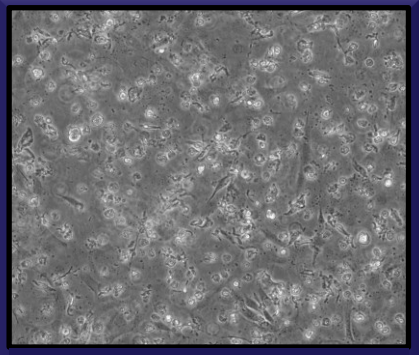
Strain authentication and viability testing

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

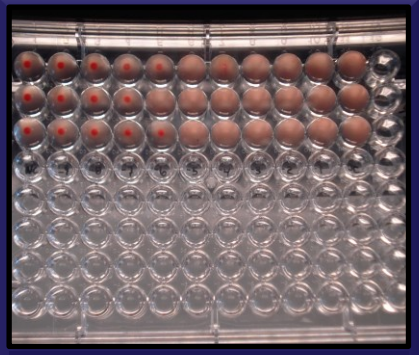
- Mycoplasma detection
- BacT/ALERT 3D System

- Cultures grown in embryonated chicken eggs - Hemagglutination

- Cultures grown in tissue culture – IFA assisted plaque assay



Viability



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Strain authentication and viability testing

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

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

*__Titer notes:__ 2.3×10^9 $CEID_{50}$/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].

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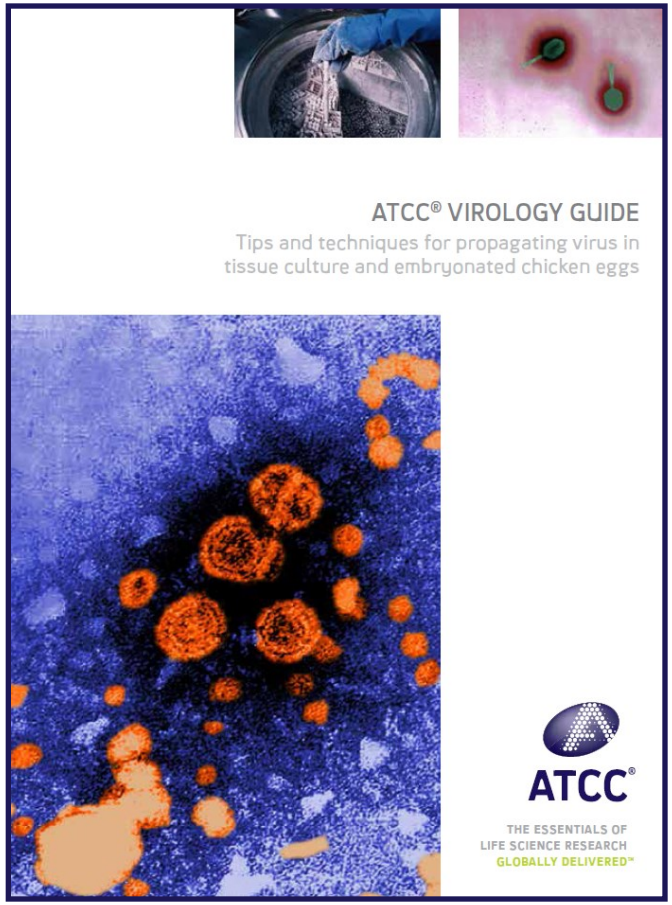
ATCC® Virology Guide

- 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



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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!

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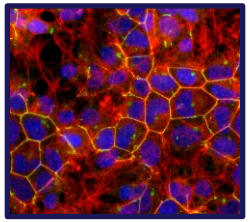
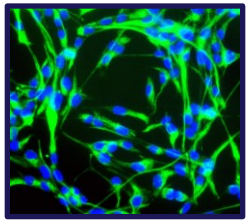


September 18, 2014
10:00 AM, 3:00 PM EST
Dr. Fang Tian, Dr. David H. Randle
ATCC® Genetic Alteration Cell Panels: Effective tools for high throughput screening using Corning® Epic® Technology



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

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Photo credit: Yirui Sun