
Biosafety and Influenza Research

*Laboratory Biosecurity and Biosafety
for BSL3 Laboratories
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www.biosecurity.sandia.gov

Influenza

BLACK NOVEMBER

The 1918 influenza pandemic
in New Zealand



Geoffrey W. Rice

- 1918 pandemic (1 year)
 - >650,000 deaths in U.S.
 - 50-100 M deaths worldwide
- 1957 pandemic
- 1968 pandemic
- Seasonal influenza
 - >30,000 deaths/yr in U.S.
 - >100,000 hospitalizations/yr in U.S.
 - ~1M deaths globally/yr
- H5N1 ?
 - First reported in 1997
 - 29 May 2006; WHO
 - 224 confirmed cases
 - 127 deaths

Influenza Viruses

- Contemporary influenza viruses
- Low pathogenic avian influenza viruses (LPAI)
- Non-contemporary strains (WT H2N2)
- Reconstituted 1918 influenza virus
- Recombinant influenza viruses
- Highly pathogenic avian influenza viruses (HPAI)

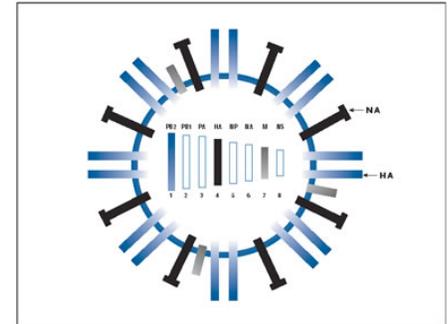
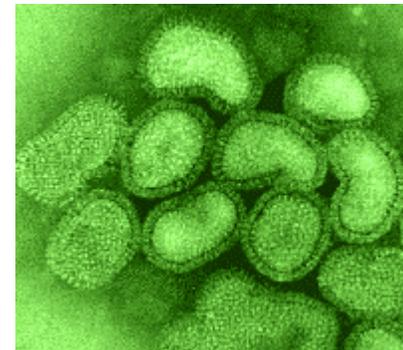
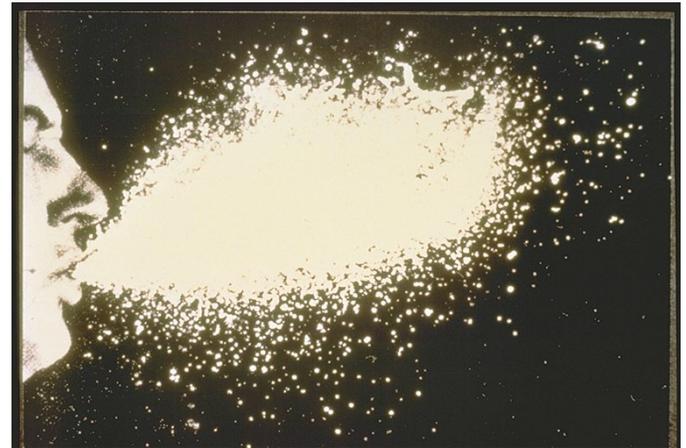


Figure 3. Model of the influenza virus showing the segmented nature of the viral genome and the two major surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA).



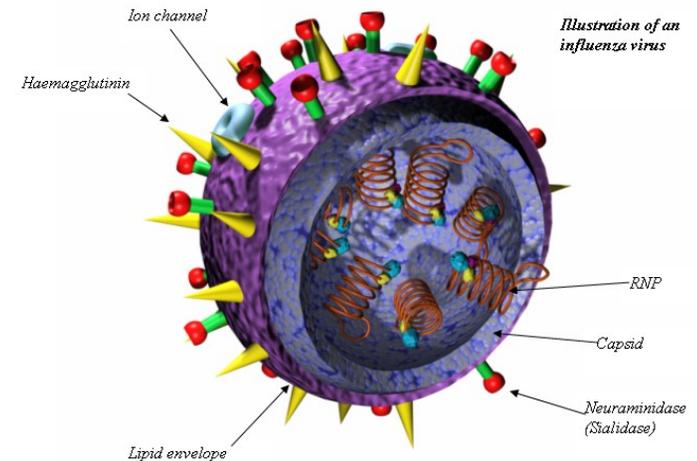
Natural Modes of Infection

- **Primarily airborne**
 - Crowded areas
 - Enclosed spaces
- **Direct contact**
 - **Virus persistence**
 - Hours on surfaces
 - Cold
 - Relatively low humidity
- **Human-human transmission**
- **Transmission from infected animals**
- **Incubation period: 1-3 days**
- **Treatment**
 - Vaccine
 - Tamiflu (oseltamivir)
 - Amantadine
 - Rimantadine
 - Zanamivir



Occupational Infection

- LAIs not routinely documented in literature but are known to have occurred
- Occupationally-acquired nosocomial infections are documented
- Lab animal-associated infections not reported though transmission between ferrets and humans is possible



Laboratory Hazards

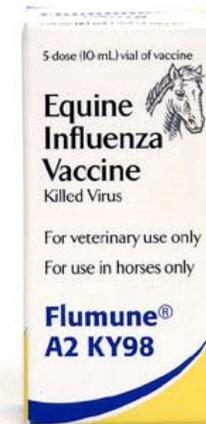
- **Primary hazards**
 - Aerosol from infected animals
 - Respiratory tissues and secretions
 - Aerosols from handling specimens
- **Other hazards**
 - Cloaca and intestines of infected avian species
 - Multiple organs in infected species
- **Procedural hazards**
 - Direct mucous membrane inoculation from contaminated gloves
 - Manipulation of infected samples
 - Strain identification
 - PCR
 - Virus isolation
 - Genetic manipulation can alter host range



University of Connecticut Pathobiology Lab

Contemporary Strains

- **3 Serotypes**
 - **A (2 subtypes)**
 - Hemagglutinin (H)
 - Neuraminidase (N)
 - **B**
 - **C**
- **Circulating WT human strains**
 - **H1/H3/B**
- **LPAI strains**
 - **H1-4, H6, H8-16**
- **Equine strains**
- **Swine strains**



Non-contemporary Strains

- **WT circulating H2N2 virus**
- **Handle with increased caution**
- **Risk assessment considerations**
 - **Number of years since antigenically related virus last circulated**
 - **Potential for susceptible population**
 - **Infectivity**
 - **Virulence**
 - **Transmissibility**
 - **Potential for lab acquired infection**

1918 Spanish Influenza Strain

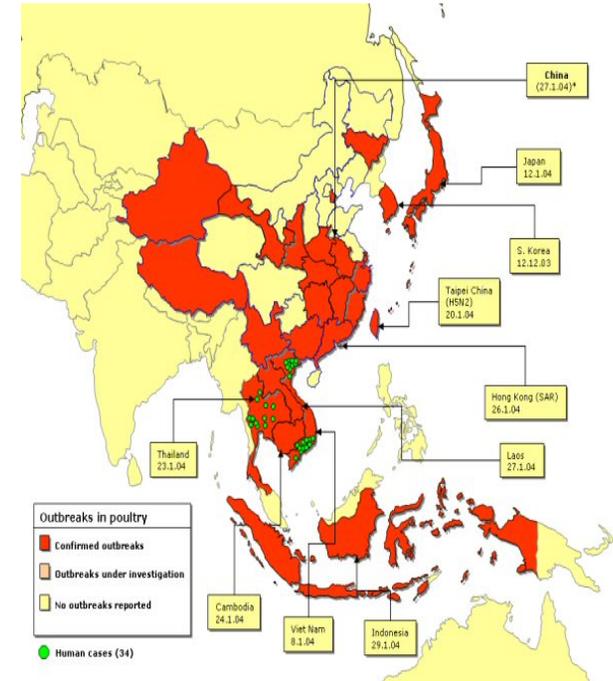
- **Genome sequence determined**
 - Reconstructed 1918 influenza virus
 - Infected lab animals
- **Risk to laboratory staff presently unknown**
- **Extreme caution for any research activities**
 - Manipulation of reconstructed virus
 - Handling infected laboratory animals or their tissues



Highly Pathogenic Avian Influenza (HPAI)

- Zoonotic
- Distribution
 - Spreading rapidly in environment
- Relatively unstable
 - But stable in water
- Highly infectious in poultry; apparently limited infectivity currently in humans
- Vaccine for animals available; human vaccine under development
- Treatment for humans:
 - Oseltamivir
 - Zanamavir

Outbreaks of H5 Avian Influenza in Asia
In the period January-March 2004
(with first dates of animal outbreaks reporting)



CDC-NIH Interim BSL Recommendations

- **Contemporary circulating and LPAI strains**
 - **BSL-2 and ABSL-2**
 - **Facilities, Procedures, Practices**
 - Diagnostics
 - Research
 - Production
- **Non-contemporary**
 - **BSL-3 and ABSL-3**
 - **Procedures, Facilities, Practices**
 - Respiratory protective equipment
 - Clothing change protocols
 - **Cold-adapted live attenuated H2N2 vaccine strains require BSL-2**



CDC-NIH Interim BSL Recommendations (cont.)

- **Reconstituted 1918 influenza**
 - **BSL-3 and ABSL-3 facilities, practices and procedures**
 - **Respiratory protection (negative pressure masks, PAPR)**
 - **HEPA treated exhaust air**
 - **Personnel clothing change and showers prior to exiting the lab**
- **H5N1**
 - **BSL-3 and ABSL-3**
 - **Respiratory protection (negative pressure masks, PAPR)**
 - **HEPA treated exhaust air**
 - **Personnel clothing change and showers prior to exiting the lab**
- **Recombinant or re-assorted viruses**
 - **IBC performs protocol driven risk assessment**
 - **Last circulating antigenically similar virus**
 - **Phenotypic stability and clonal purity**
 - **In absence of data, increase biocontainment level**



U.S. Guidance

	Select Agent	BSL	Risk Guidance
Contemporary (seasonal) strains, LPAI	No	BSL-2	BMBL 4 th ed.
Non-contemporary strains	No	BSL-3	CDC Interim Guidelines
H5N1 (HPAI)	USDA	BSL-3 BSL-3 Ag	BMBL 4 th ed.
Reconstituted 1918 virus	CDC	BSL-3 BSL-4	CDC 42 CFR 73
Recombinant viruses	No	BSL-2 BSL-3	NIH rDNA Guidelines

WHO Biosafety Guidelines for Suspected Avian Influenza A Infected Specimens (January 2005)

- Labs must meet basic BSL-2 standards and use **BSL-3 practices**
 - Specimen manipulation
 - Diagnostics
 - Nucleic acid extraction; slide preparation
- **BSL-3 Practices**
 - BSC required for all aerosol generating procedures
 - Personal protective equipment
 - Centrifugation with sealed sample cups; open rotors in BSC
 - Decontaminate work surfaces after work completion
 - Appropriate disposal of all biological waste
 - Appropriate decontamination practices
- Facilities not meeting basic BSL-2 conditions should send specimens to suitable reference labs for primary diagnostic testing

See WHO Laboratory Biosafety Manual for more information



WHO Guidelines for Production of Pandemic Human Influenza Vaccines (October 2005)

- **These guidelines:**
 - **Outline in detail international biosafety expectations for pilot and large-scale pandemic influenza vaccine production**
 - **Recommend and outline facility risk assessment**
 - Risk assessment considerations
 - Hazard identification
 - Assignment of containment level based on assessment
 - **Outline BSL-2 enhanced requirements**
 - **Outline BSL-3 enhanced requirements**

- **Provide a table of proposed containment requirements for influenza viruses**



BSL-2 Enhanced (BSL-2 en) Specifications

- **Organisms**
 - Low-pathogenic WT H5 or H7 viruses
 - Reassortants from non H5 or H7 viruses
- **Used in addition to WHO LBM BSL-2 requirements**
- **BSL-2 en specifications**
 - BSCs when possible
 - HEPA filtered exhaust
 - Positive-pressure with negative-pressure sinks prior to exhaust
 - Decontamination of all waste
 - Validated decontamination monitoring during and at end of production
 - Gaseous fumigation
 - PPE
 - Respiratory protection (N95 or FFP3 APRs)
 - Full-body lab clothing
 - Facility should provide a document recommending no contact with avian or porcine species for 14 days post departure from facility
 - Should be signed by employee
 - Prophylactic influenza vaccination



BSL-3 Enhanced (BSL-3 en) Specifications

- **Organisms**
 - Cell culture vaccine production
 - Highly pathogenic WT H5 or H7 viruses
- **Used in addition to WHO LBM BSL-3 requirements**
- **Recommendations include**
 - Negative pressure containment areas
 - HEPA filtered supply and exhaust air
 - On-site liquid effluent decontamination
 - Floor dams around bioreactors, large-scale equipment
 - PPE
 - Full-body lab clothing
 - Respiratory protection (N95 or FFP3 APRs)
 - Shower out (must be used for vaccine virus exposure)
 - No contact with avian or porcine species for 14 days post departure from facility
 - Prophylactic influenza vaccination



WHO Guidelines for Production of Pandemic Human Influenza Vaccines

Additional Recommendations

- **Require implementation of a comprehensive biosafety manual**
- **Recommends Biosafety Committee and Biosafety Officer**
- **Recommends a medical surveillance program**
- **Recommends development and implementation of guidelines for all emergencies**
- **Recommend that BSL implementation be verified through independent assessment**
- **Egg-based vaccine production from highly pathogenic H5 or H7 viruses is not recommended due to open nature of large scale production**

- **Facilities should meet OIE requirements for containment**



International Transfer and Laboratory Containment of Animal Pathogens (OIE)

- **Provides guidance on lab containment of animal pathogens**
- **Based on risk assessment**
- **Categorizes animal pathogens into risk groups**
- **Describes containment levels**
 - **Lab requirements**
 - **Entry and exit requirements**
 - **Specimen manipulation procedures**
- **Provides guidance on the laboratory requirements for the different containment groups**
- **Provides guidance for possession and handling**
- **Provides guidance for importation**

Conclusions

- **Discussed various types of influenza viruses**
- **Discussed recommendations for research and diagnostics**
 - **CDC-NIH interim guidelines**
 - **WHO**
- **Discussed recommendations for vaccine production practices**
 - **BSL-2 enhanced recommendations**
 - **BSL-3 enhanced recommendations**
 - **OIE recommendations for containment**