



**Recombinant Work with Human H2N2,
1918 H1N1 and Highly Pathogenic Avian
H5N1 Influenza Viruses**

NIH Guidelines: Influenza Viruses

- **Currently, all influenza viruses are classified as Risk Group (RG) 2 agents**
 - **No distinction between pandemic strains such as 1918 H1N1, H2N2, or potentially pandemic strains, such as highly pathogenic avian influenza (HPAI) H5N1 and potentially less dangerous influenza viruses**



Risk Groups Under the NIH Guidelines

■ Risk Groups

RG1

Agents are not associated with disease in healthy adult humans.

RG2

Agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available.

RG3

Agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available (high individual risk and **low** community risk).

RG4

Agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions *are not usually* available (high individual risk and **high** community risk).

■ Containment levels (BL, BSL)

- Containment level may be raised or lowered depending on a comprehensive risk assessment.



Public Health Impact of Influenza



- **Yearly epidemics**
 - ~36,000 deaths/yr in U.S.
- **Pandemics in the last century**
 - **1918 H1N1**
 - ~ 675,000 deaths in U.S
 - 20 - 40 million world wide
 - **1957 H2N2**
 - ~ 66,000 excess deaths in US
 - **1968 H3N2**
 - ~ 34,000 excess deaths in US



NIH Guidelines: Influenza Viruses

- While RG often correlates with the level of containment (e.g. a RG 2 agent will often be worked on at BL-2) it does not determine containment level and practices
- The RG is the starting point for the risk assessment which may result in a higher level of containment
- In 2007 the BMBL recommended Biosafety Level 3 with certain enhanced practices for work with 1918 H1N1, H2N2 and HPAI H5N1.
- Because under the current *NIH Guidelines* these agents are RG 2 an IBC could recommend work at BL2 or BL3 or BL3 enhanced for recombinant work, depending upon their risk assessment



Questions for the RAC

- **Risk Group Designations:**
 - Fully reconstructed 1918 H1N1?
 - Human H2N2 (1957-1968) ?
 - Highly pathogenic avian influenza H5N1?

- **What additional biosafety guidance should be provided regarding recombinant influenza viruses containing genes from these influenza strains?**
 - Consider current guidance provided by the CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) and USDA Animal and Health Inspection Service (APHIS)
 - Review additional scientific data



RAC Biosafety Working Group

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RAC Biosafety Working Group Meetings and Consultations

- **June 24, 2008 Safety Symposium: Recombinant DNA Research with Non-contemporary Influenza Viruses and Highly Pathogenic Avian Influenza Viruses**
- **Continued consultation with outside experts**
- **Proposed Changes Presented at September 2008 RAC meeting**
 - **Questions raised about recommendation of pre-exposure prophylaxis for 1918 H1N1 research**
- **December 2, 2008 joint RAC/Intragovernmental Select Agent and Toxin Technical Advisory Committee Safety Symposium: Public Health and Biosafety for Research with 1918 H1N1 Influenza Virus**



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(BMBL 5th edition)



- **Human H2N2 (1958-1967)**
- **Due to pandemic potential “increased caution”**
 - **Biosafety Level 3 plus the following enhancements:**
 - **Rigorous adherence to additional respiratory protections and clothing changes**
 - **Use of negative pressure HEPA- filtered respirators or PAPRs**
 - **Cold-adapted, live attenuated H2N2 vaccine strains may continue to be worked with at BSL-2**

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(BMBL 5th edition)



- **Fully Reconstructed 1918 H1N1**
- **Due to pandemic potential, “*extreme caution*”**
 - **Biosafety Level 3 plus the following enhancements:**
 - Rigorous adherence to additional respiratory protections and clothing changes
 - Use of negative pressure HEPA- filtered respirators or PAPRs
 - HEPA filtration for treatment of exhaust air
 - Personal showers prior to exiting the laboratory
 - Large laboratory animals such as NHP should be housed in primary barrier systems in ABSL-3 facilities.
- **In addition, fully reconstructed 1918 H1N1 is a Select Agent and registration with the CDC Select Agent Program is required**

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- **Highly Pathogenic Avian Influenza (HPAI) with the potential to infect humans**
 - **Biosafety Level 3 or ABSL 3 facilities, practices and procedures plus the following enhancements:**
 - **Rigorous adherence to additional respiratory protections and clothing changes**
 - **Use of negative pressure HEPA- filtered respirators or PAPRs**
 - **Personal showering protocols**
 - **Loose housed animals must be in BL3-Ag facilities**
 - **HPAI are regulated by the USDA's Animal and Plant Health Inspection Service (APHIS) and additional measures may be required**



HPAI H5N1-RG Determination

- **Under NIH Guidelines, a RG 3 agent is one that causes *serious or lethal disease in humans***
 - **HPAI H5N1 is primarily an avian influenza, but**
 - 400+ human cases
 - Mortality rate in humans is over 50%
- **To date very limited evidence for human to human transmission**
 - **Small, familial clusters of cases**



HPAI H5N1-RG Determination

Questions for the RAC: 1) How to handle those viruses under the *NIH Guidelines* that have the potential to cause human disease without restricting other research with HPAI H5N1 that are primarily avian viruses and 2) how does one handle those viruses isolated from human cases relative to the same virus isolated from the animal host?



CDC Recommendation to the BSWG

Highly pathogenic avian influenza H5N1 strains within the Goose/Guogdong/96-like H5 lineage

- It is important that this entire lineage be considered for RG-3 status.
 - Because multiple genetically distinct clades of the currently circulating Goose/Guogdong/96-like lineage of highly pathogenic H5N1 virus strains have caused documented human disease (including clades 0, 1, 2 and its subclades, and 7),
- Therefore a definition that encompasses all virus strains within this lineage and of either human or animal origin is desirable.
 - currently 10 clades of H5N1 virus strains recognized to date: clades 0-9



Proposed Recommendations: Risk Group Classifications

Risk Group 3

Associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available (high individual risk and **low** community risk).

- 1918 H1N1
- Human H2N2 (1957-1968)
- HPAI H5N1 strains within the Goose/Guogdong/96-like H5 lineage (HPAI H5N1)



Additional Considerations

HPAI H5N1

- Influenza viruses that contain the HA gene from a HPAI avian influenza (or characteristic of an HA gene) are Select Agents and are regulated by USDA
 - OBA will defer to USDA-APHIS on Biosafety Containment and Practices for Select Agent work involving HPAI H5N1



Additional Considerations HPAI H5N1

- The *NIH Guidelines* will address the minimum containment for recombinant influenza viruses containing segments or genes from HPAI H5N1 other than the HA gene; in *addition*
- Such work will also require a permit from USDA-APHIS specifying containment and may have additional practices beyond those required in the *NIH Guidelines*



Additional Considerations

1918 H1N1

- Fully reconstructed 1918 H1N1 is a recombinant molecule and is subject to *NIH Guidelines*
- Select Agent: Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (70 FR 61048)
- *NIH Guidelines* will set a minimum level of containment that is consistent with the BMBL



Proposed Containment: 1918 H1N1, H2N2* and HPAI H5N1

BL3 or BLN-3 Facilities, Practices and Procedures (Appendix G of *NIH Guidelines* and CDC/NIH BMBL),

- **PLUS** Enhancements outlined below (BL3+)

- **Facility: BL-3 enhanced facility includes:**

- HEPA filtration of exhaust air
- All work with virus conducted in class II biosafety cabinet (standard BL3)
- Shower facility ideally available especially for work with the full virus

* Research with the HA gene from H2N2 that circulated from 1957- 1968



Proposed Containment: 1918 H1N1, H2N2 HA* and HPAI H5N1

- **Personal Protective Equipment Enhancements**
 - Powered Air-purifying Respirator (PAPR)
 - Protective suit
 - Wrap-back disposable gown
 - Double gloving
 - Double shoe coverings
- **Practices and Procedures Enhancements**
 - Complete clothing change protocols with training to demonstrate proper procedure
 - Consider showers prior to exiting laboratory depending upon risk assessment of research activities

*Research with the HA gene from H2N2 that circulated from 1957- 1968



Proposed Containment: Animal Research

- **BL-3 or BL3-N (Appendix Q *NIH Guidelines*)**
 - Work with small animals (mice, ferrets, guinea pigs)
 - Animals housed in negative pressure bioisolators
 - All manipulations conducted in class II biosafety cabinet
 - Large laboratory animals such as NHP should be housed in primary barrier systems in BLN-3 facilities.
 - Specialized training program and proven competency in all assigned practices and procedures for laboratory staff, including staff involved in animal care



Proposed Containment: Animal Research

Additional Recommendations for H5N1:

- Showers should be considered for research involving animal studies in which animal to animal or animal to human transmission may occur.
- Defer to USDA-APHIS recommendations from on biocontainment practices for loose-housed animals



Proposed Recommendations: Training of Laboratory Workers

- Proper training of laboratory workers is the key to biosafety containment for all research
- Retraining and periodic reassessments (at least annually) in BSL3 enhanced techniques, especially in the proper use of respiratory equipment, such as PAPRs, shall be part of standard laboratory practices
- Reporting of all incidents to the PI and Biosafety Officer, even if relatively minor is required
 - *NIH Guidelines Appendix G-II-C-2-q*: “Spills and accidents which result in overt or potential exposures to [RG3] organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, and NIH/OBA...Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.”



Avoiding Inadvertent Cross Contamination of 1918 H1N1, HPAI H5N1 or H2N2 HA (1957-1968)

- **Between experiments adherence to:**
 - **Good biosafety practices, e.g., surface and biosafety cabinet surface decontamination according to standard BL3 enhanced procedures**
 - **Separate reagents to minimize risk of cross-contamination**
 - **30 minute wait period after decontamination before equipment can be used with other influenza A viruses**
 - **Containment facilities and practices appropriate for highest RG virus shall be used at all times even with lower RG viruses, when studied in the same lab**
 - **Tissue cultures with these viruses shall be conducted at separate times (temporal spacing)**



Avoiding Inadvertent Cross Contamination of 1918 H1N1, HPAI H5N1 or H2N2 HA (1957- 1968)

- A Laboratory worker shall not perform concurrent influenza experiments that carry the risk of unintended recombination among HPAI H5N1 viruses, 1918 H1N1, Human H2N2 HA (1957-1968) and other human influenza viruses
- Between experiments, in addition to decontamination of work area, clothing changes and PAPR disinfection shall be performed prior to handling a different virus in the same work area
 - Showers may be required by USDA-APHIS under permitting process for certain experiments with HPAI H5N1



Avoiding Inadvertent Cross Contamination of 1918 H1N1, HPAI H5N1 or H2N2 Influenza

- **Laboratory workers shall not perform within the same work area simultaneous influenza virus experiments that carry the risk of unintended segment reassortment among HPAI H5N1 viruses, 1918 H1N1, human H2N2 HA (1957-1968) and other human influenza viruses**



Maintenance of Antiviral Susceptibility

- **The availability of antiviral drugs as preventive and therapeutic measures is an important safeguard for research with HPAI H5N1, 1918 H1N1 and Human H2N2 and the basis for their classification as RG 3 agents rather than RG 4**
- **An influenza virus containing genes from these viruses that is resistant to current antivirals may require higher containment**



Maintenance of Antiviral Susceptibility

- **Susceptibility of the recombinant influenza viruses containing 1918 H1N1, HPAI H5N1, or H2N2 HA genes to antivirals shall be established:**
 - By sequence analysis to confirm susceptibility, or
 - By suitable biological assays
- **After genetic manipulations of genes that influence sensitivity to antiviral agents, susceptibility to these agents shall be reconfirmed**



High Risk Research

- Experiments with HPAI H5N1, Human H2N2 HA or 1918 H1N1 that are designed to create resistance to neuraminidase inhibitors or other effective antivirals (including investigational antivirals being developed for influenza) would be subject to Section III-A-1 (Major Actions) of the *NIH Guidelines* and require RAC review and NIH Director approval
- If regulated as Select Agents, the NIH defers to the regulatory body (CDC or USDA Select Agent Divisions).



Proposed Recommendations: Occupational Health

Key Elements

- **A detailed occupational health plan shall be developed in advance and be practiced**
 - **It shall include experts with appropriate clinical expertise**
- **The local health department or appropriate public health entity shall be consulted in this planning**
- **An incident reporting system shall be in place and laboratory workers shall report all incidents**



Proposed Recommendations: Occupational Health

- **Medical cards for laboratory workers;** information to include
 - **Strain characterization of influenza to which potentially exposed**
 - **24 hour contact numbers for principal investigator and institution's occupational health care provider(s)**

- **A detailed occupational health plan shall include:**
 - **Annual seasonal influenza vaccine as prerequisite for research**
 - Reduces the risk of influenza illness and need to isolate and rule out infection with one of these viruses
 - Reduces risk of possible co-infection with these viruses and circulating influenza strains
 - **If available, virus specific vaccine should be offered**



Proposed Recommendations: Occupational Health

- ❑ **Mandatory reporting** of respiratory symptoms and/or fever
- ❑ A **detailed occupational health plan** that provides 24 hour access to medical facility that is prepared to implement appropriate respiratory isolation to prevent transmission and able to provide appropriate antiviral agents
 - real time RT-PCR procedures should be used to discriminate these viruses from currently circulating human influenza viruses
 - For 1918 H1N1 and H2N2, specimens shall be sent to the CDC for testing (real time RT-PCR and confirmatory sequencing)



Proposed Recommendations: Occupational Health for 1918 H1N1 and Human H2N2

- Community risk from a laboratory release of H2N2 HA or 1918 H1N1 is expected to be higher than for HPAI H5N1
 - Both viruses caused pandemics with serious and lethal disease that demonstrated efficient human-to-human transmission
 - Vaccine not available and while antivirals exist, distribution may be limited in event of pandemic
 - Considerable portion of the population with no immunity to H2
 - There may be limited immunity in the human population to 1918 due to continued circulation of H1N1
 - Additional research into the degree of 1918 H1N1 immunity resulting from current H1N1 viruses/vaccines is needed



Proposed Recommendations: Occupational Health for 1918 H1N1 and Human H2N2

- In the case of a **known laboratory exposure**
 - The laboratory worker shall be informed in advance that as part of their duties they will be asked to isolate themselves in a designated facility until infection can be ruled out by adequate and appropriately timed specimens
 - e.g., negative RT-PCR for 1918 H1N1 or H2N2
 - Treatment with appropriate antiviral agents shall be initiated
 - The local public health department shall be notified



Proposed Recommendations: Occupational Health for **HPAI H5N1**

- Community risk is expected to be lower because virus is not efficiently transmitted human-to-human
- In the case of a **known laboratory exposure**
 - The laboratory worker shall be informed to **self isolate** until infection with HPAI H5N1 can be ruled out
 - RT-PCR testing with adequate and appropriately timed specimens
 - Option for isolation in a designated facility shall be offered
 - Treatment with appropriate antiviral agents shall be initiated
 - The local public health department shall be notified



Proposed Recommendations: Occupational Health

- **Development of influenza-like illness** in any person handling recombinant influenza viruses containing the H2N2 HA, or any gene from the 1918 virus or HPAI H5N1, or animals exposed to these viruses
 - If
 - Recent exposure (within 10 days)
 - Symptoms/signs of influenza infection (e.g. fever/chills, cough, myalgias, headache)
 - Then
 - Report via the phone to the supervisor/PI and the other individuals identified in the occupational health plan.
 - Transport laboratory worker to a healthcare facility that can provide adequate respiratory isolation, appropriate medical therapy and testing to determine whether infection due to recombinant influenza virus

- The local public health department should be informed whenever a suspected case is isolated



Use of Oseltamivir for Pre-Exposure Prophylaxis for Research with 1918 H1N1

- **Antiviral agents are generally safe for 6 weeks and some data indicate that oseltamivir may be safe for up to 12 continuous weeks**
- **The most common side effects of oseltamivir are mild and include nausea, vomiting and headache**
- **Reports of neuropsychiatric side effects largely from Japan and limited to younger individuals but have been seen up to age 21**
 - **Not definitively linked to the antiviral agents but FDA warning on oseltamivir and zanamivir**
- **Pregnancy Class C but very limited human data in post-marketing**
- **No data on safety of repeated courses of antiviral agents**
- **Emerging resistance to oseltamivir in community strains of H1N1**



Efficacy of Neuraminidase Inhibitors Oseltamivir and Zanamivir



- Pre-exposure prophylaxis can reduce symptomatic laboratory confirmed infection during community outbreak (efficacy 70-84%)
- Ability of post-exposure prophylaxis to reduce symptomatic laboratory confirmed infections in household contacts exposed to a laboratory confirmed influenza case ranges from 60-89%
- Ability of prophylaxis to prevent symptomatic and asymptomatic infection is considerably less approximately 30 - 60%

Considerations and Unresolved Questions



- **While generally safe, use of antiviral agents may lead to nausea, vomiting and headache which could adversely affect laboratory workers**
 - Possibility of more severe allergic and other reactions
- **Pre-exposure prophylaxis could potentially convert symptomatic cases to asymptomatic**
 - Loss of ability to identify sentinel case
 - Unknown to what degree asymptomatic cases of influenza are infectious
- **Unknown whether the potential reduction in viral shedding from prophylaxis leads to significant reduction in ability to transmit virus, even in asymptomatic cases**

Considerations and Unresolved Questions

While ongoing prophylaxis with antiviral agents can protect against complications and symptomatic infections in lab workers:

- ❑ they can not eliminate risk of transmission to the community;
- ❑ relative reduction in risk is not yet defined given the possibility of converting potentially symptomatic cases to asymptomatic ones.



Proposed Recommendation Regarding Antiviral Prophylaxis

- Pre-exposure prophylaxis with antiviral agents should not be mandated for work with 1918 H1N1
- Antiviral agents (e.g., oseltamivir) for pre-exposure prophylaxis shall be discussed with laboratory workers including a discussion of the data on the safety of 6-12 week exposure to these agents and their ability to reduce the risk of clinical disease and the limited data regarding protection of close contacts and the community
- Antiviral agents for *post-exposure* prophylaxis shall be provided only after medical evaluation
 - Home supplies shall not be provided in advance



Lowering of Containment for Influenza Virus Recombinants Under *NIH Guidelines*: **H2N2**

- Work with recombinant influenza viruses containing the human H2N2 specific HA (1957-1968) shall be conducted at BL3 enhanced containment (outlined previously)
- Work with the H2 HA gene in a cold-adapted, live attenuated vaccine strains (e.g., A/Ann Arbor/6/60 H2N2) may be conducted at **BL2** containment provided that:
 - Segments with mutations conferring cold-adapted, live attenuation must be retained in the recombinant virus
 - Maintenance of cold-adapted, live attenuation must be confirmed when recombinant virus generated



Lowering of Containment for Influenza Virus Recombinants Under *NIH Guidelines*: HPAI H5N1

- For non-select agent research, if an influenza recombinant virus contains a majority of gene segments from a Risk Group 3 influenza virus, this recombinant must be worked with at BL-3 enhanced unless in consultation with OBA a decision has been made to lower containment (see *NIH Guidelines* Section III-D).
- If an influenza recombinant virus contains a majority of genes segments from a Risk Group 2 influenza virus, the *NIH Guidelines* permit this research to be conducted under BL-2; however, the IBC may raise containment based on their risk assessment



Lowering of Containment for Influenza Virus Recombinants Under *NIH Guidelines*: HPAI H5N1

- OBA is available to IBCs to provide consultation with the RAC and Influenza experts when risk assessments are being made as to whether recombinant influenza viruses containing some gene segments (but less than a majority) from HPAI H5N1 should be done at BL3 enhanced containment or lowered to BL2, the level of containment for most work with other influenza viruses
 - As USDA-APHIS regulations will also apply, any decision on lowering of containment from BL3 to BL2 will also involve USDA APHIS



Lowering of Containment for Influenza Virus Recombinants Under *NIH Guidelines*: HPAI H5N1

- In deciding to lower containment, the IBC should consider whether, in two animal models (e.g., ferret, mouse, syrian golden hamster, cotton rat, nonhuman primate), there is evidence that the resulting influenza virus shows reduced replication and virulence at relevant doses compared to the risk group 3 parent virus.
 - as determined by reduced dependent measures appropriate for specific animal model
 - e.g. severe weight loss, elevated temperature, mortality or neurological symptoms



Containment Considerations for Recombinants containing Genes from 1918 H1N1

- 1918 H1N1 showed efficient human to human transmission when circulating
- The 1918 H1N1 pandemic caused significant morbidity and mortality
- Due to multigenic effects one can not predict virulence of recombinant influenza viruses containing genes from 1918 H1N1 in advance
- Identification of genes as virulence determinants is still ongoing
- Questions remain with respect to the animal model[s] that best predict virulence in humans



Containment Considerations for Recombinants containing Genes from 1918 H1N1

- Given the potential public health implications of inadvertent release of a recombinant influenza virus with enhanced virulence due to genes from 1918 H1N1, developing a framework for consistent containment decisions is a public health priority



Containment for Recombinants

1918 H1N1

- Recombinant influenza viruses containing any genes or segments from 1918 H1N1
 - Must be worked with at BL-3 enhanced until there is agreement upon a set of experiments that can be used to demonstrate, prior to lowering containment, that such influenza viruses are safe to work on at BL-2 (i.e. are not likely to cause serious disease in humans or have pandemic potential)
 - The identification of this set of experiments will be developed with input from scientific community through Federal Register comment



Next Steps

- **Immediate implementation under the *NIH Guidelines***
- **Publication in the Federal Register to obtain additional expert consultation and comment**
- **OBA will revisit these recommendations as new data emerge**

