Report of the IBC Subcommittee for Review of Potential Dual Use Research of Concern

A subcommittee of the University of Wisconsin-Madison Institutional Biosafety Committee (IBC) was formed and charged with evaluating the dual use issues associated with the research of Dr. Yoshiro Kawaoka, Professor, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison. Each member of the subcommittee was provided with the materials listed below. While this grant involves research using the reconstructed 1918 influenza virus, which is in itself a select agent and thus a bioterrorism risk, each member of the subcommittee independently determined that the research did not meet the criteria for Potential Dual Use Research of Concern (DURC) using the guidelines established by National Security Agency Advisory Board (NSAAB). Additionally, each member of the subcommittee independently determined that the described research provided considerable benefit to society. A report of the subcommittee's findings was presented at the July 11, 2012 meeting of the IBC. The IBC voted unanimously to accept the report. The specific aims of the proposed research, background information, and the response to each of the 7 criteria for DURC as outlined in "Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information" published by NSAAB is given below along with a risk benefit analysis of the proposed research.

The following individuals served as members of the subcommittee:

Susan E H. West, MS, PhD, is an Associate Professor of Microbiology, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, and has been a member of the University of Wisconsin-Madison Institutional Biosafety Committee since 2002. Dr. West has served as Chair of the Institutional Biosafety Committee since 2009. Her research interests are the regulation of virulence factor production by animal and human pathogens, molecular epidemiology of bacterial pathogens, and early detection of *Pseudomonas aeruginosa* lung infections in children with cystic fibrosis.

Matt Reynolds, PhD., is an Associate Scientist in the Department of Pathology and Laboratory Medicine at the University of Wisconsin-Madison and has been a member of the University of Wisconsin-Madison Institutional Biosafety Committee since 2010. His interests include investigating the cellular immune responses to Simian and Human Immunodeficiency Virus (SIV and HIV), pathogenesis of SIV/HIV infections, and SIV/HIV vaccine design. He has over 14 years experience working in BSL-3 facilities.

Kristen Bernard, DVM, PhD, is an Associate Professor of Virology, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, and has been a member of the University of Wisconsin-Madison Institutional Biosafety Committee since January 2010. Her research interests include viral pathogenesis of mosquito-borne, emerging and zoonotic viruses. Dr. Bernard has over 20 years experience working in BSL3 and ABSL3 facilities, including nine years with select agents. Her current research requires the use of BSL3 and ABSL3 facilities, and she has FBI clearance to work with select agents.

The subcommittee was provided the following materials:

1. A copy of NIH Grant **RO1AI080598,** "Molecular Mechanisms for the High Pathogenicity of 1918" awarded to Dr. Yoshiro Kawaoka, DVM, PhD
2. A copy of Dr. Kawaoka's most recent Biosafety Protocol, which was approved unanimously by the IBC.

3. A copy of "Responsible Communication of Life Sciences Research with Dual Use Potential: *A Set of Communication Tools Excerpted from the NSABB's* Proposed Framework for the Oversight of Dual Use Life Sciences Research" published by NSAAB.
4. A copy of "Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information" published by NSAAB.

Potential DURC Concerns: NIAID indicated that the entire grant should be evaluated.

Specific Aims of NIH Grant RO1AI080598:

The long-term goal of Dr. Kawaoka's research is to understand the emergence of pandemic influenza viruses such as the 1918 influenza virus. The 1918 influenza virus caused the most devastating outbreak of an infectious disease on record.

Aim 1. Assess the [redacted]
Aim 2. Assess the molecular features of HA that support the high virulence of the 1918 virus.
Aim 3. Assess the role of the 1918 virus replication complex in severe viral infection.

The basic approach taken in **Aim 1** will be to [redacted] **Aims 2 and 3** will study the role of the HA protein (**Aim 2**) and the viral replication complex (**Aim 3**) in 1918 virulence and pathogenicity. Studies in **Aims 1-3** will also include an assessment of host cell immune responses that are known to be atypical in 1918 virus-infected animals (19, 25). Collectively, the studies proposed in this application are designed to address two critical questions in influenza virus research – ***why was the 1918 virus highly pathogenic and what is the risk for emergence of another 1918-like virus?***

Background:

Humans can be infected by influenza A, B and C viruses. So far all major outbreaks have been caused by influenza A viruses. Influenza A viruses are further divided into subtypes based on the antigenicity of their hemagglutinin (HA) and neuraminidase (NA) glycoproteins. To date, 16 HA subtypes (H1-H16) and 9 NA subtypes (N1-N9) have been identified. While viruses of all subtypes are maintained in avian populations, only those of the H1-H3 subtypes have circulated widely in humans. Influenza A viruses possess eight negative-sense RNAs encoding 10-11 proteins. The virus particles are enclosed by a lipid envelope, derived from the plasma membrane of the host cell. Three viral proteins, the surface glycoproteins hemagglutinin (HA), and neuraminidase (NA), and the M2 ion channel, are embedded in the lipid membrane. Underneath the lipid bilayer lies the matrix protein (M1), a major structural protein. Within the virus shell are eight viral ribonucleoprotein (vRNP) complexes, each composed of viral RNA (vRNA) encapsidated by the nucleoprotein NP and the three components of the viral RNA polymerase complex (PB2, PB1, and PA). The NS1 protein, which counteracts the cellular interferon response, is synthesized from an unspliced mRNA, while a spliced mRNA gives rise to the NS2 (=NEP) protein, which mediates nuclear export of vRNP complexes. In addition, a protein (PB1-F2; encoded by the PB1 segment) has recently been identified that may play a role in viral pathogenicity. (4, 6, 32, 57).

In the last century, the world has experienced three influenza pandemics. Two of these pandemics were caused by genetic reassortment, which occurs when one cell is infected with different viruses, resulting in progeny viruses with various combinations of gene segments derived from the parental viruses. The pandemic of 1957 was caused when avian HA, NA, and PB1 genes were introduced into the genetic background of a human influenza virus. The pandemic of 1968 was characterized by the introduction of avian virus HA and PB1 genes into the human population (2a, 23, 27, 43). In addition, direct avian-to-human transmissions of highly pathogenic avian H5N1 viruses since 1997 demonstrate the potential of avian influenza viruses to directly infect humans and cause fatal disease (5, 46, ; reviewed in Ref. 55). For example, the available data suggest that the pandemic in 1918/1919 was caused by an avian influenza virus that became adapted to humans.

The influenza virus pandemic in 1918/1919 (as also called the '1918 pandemic' or 'Spanish influenza') killed an estimated 40-50 million people worldwide, making it the most devastating infectious disease ever recorded. This particular virus is unique in that it killed healthy individuals in the prime of their life instead of the majority of the victims being young or elderly as with other pandemics. Recently, the sequences of all eight RNA segments of the 1918 pandemic virus were determined from preserved lung tissue of three of its victims (2,36-39, 48, 49). Sequence analyses identified the pandemic strain as an H1N1 influenza A virus of avian origin (36, 39, 40, 47, 49). Interestingly, the 1918 virus sequences do not contain motifs associated with high virulence, such as multiple basic amino acid residues at the HA cleavage site (36). Reverse genetics has allowed Dr. Kawaoka and others (25, 52); to recreate the 1918 pandemic virus and to study the properties associated with its extraordinary virulence. These studies identified the HA and polymerase genes as important determinants of 1918 virus pathogenicity (26, 35, 52, 53).

Currently, it is not known which molecular features allow avian influenza viruses to replicate in and transmit among humans. HA executes two functions in the early stages of the viral life cycle; receptor binding and membrane fusion (reviewed in Ref. 56). The protein is synthesized as a single polypeptide precursor (HA0) that is posttranslationally cleaved into HA1 and HA2 subunits (56). Cleavage of HA into two subunits is required for infectivity (12, 45), because it generates the hydrophobic N-terminus of HA2 (also referred to as 'fusion peptide'), which mediates fusion between the viral envelope and the cellular membrane. The HAs of highly pathogenic avian influenza viruses play a critical role in virulence as they typically contain a multibasic sequence at the cleavage site, which is recognized by ubiquitous proteases and allows systemic infections (reviewed in Refs. 24, 45). The 1918 HA does not possess this feature (36). Rather, it possesses a single arginine at the cleavage site, which is typically recognized by proteases present in a limited number of organs (such as respiratory organs), restricting viral replication to those sites. Nonetheless, the 1918 HA gene itself confers high virulence when tested in the genetic background of conventional human viruses in a mouse model (26, 35, 53, 53).

The receptor-binding specificity of the HA protein is a major determinant in influenza viral host range (reviewed in Refs. 18, 33). In general, human influenza viruses preferentially bind to sialic acid-α2,6-galactose (SAα2,6Gal), the predominant sialyloligosaccharide species on epithelial cells in the upper respiratory tract of humans (44, 54). Dr. Kawaoaka demonstrated that avian viruses have higher affinity for sialic acid-α2,3-galactose (SAα2,3Gal), the major sialyloligosaccharide species in the avian intestinal tract (18a). It is generally assumed that efficient virus transmission among humans will require the ability to recognize SAα2,6Gal, i.e., human-type receptors.

In addition to the receptor-binding *specificity*, viral replication can also be affected by receptor-binding affinity (1). The three viral polymerase proteins (PB2, PB1, PA), in combination with NP, are responsible for the transcription and replication of the viral RNA genome. Recent findings suggest a role for the viral replication complex in influenza virus pathogenicity (8, 9, 13, 15, 16, 17, 30, 35, 41, 42, 52). For example, Lys at position 627 of the PB2 protein is a critical determinant of avian virus pathogenicity in mammalian species (15). Components of the 1918 virus replication complex allow the 1918 virus to grow more efficiently than a modern H1N1 virus in human respiratory epithelial cells and animals models (35, 52).

The innate immune response is the first line of defense against viral infections. Innate immune responses are triggered by the interaction of pathogens with 'pattern recognition receptors' (PRRs) that regulate the synthesis of interferons and pro-inflammatory cytokines. Currently, two major families of PRRs are recognized – Toll-like receptors (TLR) and RIG-I-like RNA helicases (RLH) (reviewed in Refs. 21, 22, 49). A variety of TLRs exist that are activated by different stimuli and signal via different signaling pathways (reviewed in Refs. 21, 21a, 22, 50). At least two – TLR3 and TLR7 – are activated upon influenza virus infection (7, 14, 28, 29, 31). TLR3 stimulation leads to the activation of NFκB and the expression of pro-inflammatory cytokines. TLR7 activation triggers the induction of the adaptor protein MyD88 (myeloid differentiation primary-response protein 88), and the subsequent stimulation of pro-inflammatory cytokines or type I IFN-stimulated gene products. RIG-I-like RNA helicases (RLH). RIG-I (retinoic acid-inducible protein I) activation results in the stimulation of IFN responses and cytokine/chemokine expression. The cellular interferon (IFN) responses are a critical defense mechanism against viral infections (reviewed in Refs. 10, 11, 20). IFN-α and IFN-β are induced upon viral infections, and stimulate the activation of many genes that encode cellular factors with antiviral activities, and/or immunostimulatory functions. Dr. Kawaoka's previous studies suggest that the 1918 virus induces fewer IFN-α genes than a contemporary human influenza virus in nonhuman primates (25).

Two classes of antivirals are effective against influenza: neuraminidase inhibitors oseltamivir and zanamivir) and amantadanes (amantadine and rimantadine). Neuraminidase inhibitors are effective against both influenza A and B strains; however, rare sporadic cases of resistance have been detected in recent years. Neuraminidase inhibitors competitively inhibit the action of the viral neuraminidase enzyme which cleaves sialic acid on the surface of normal host cells. Thus, these drugs prevent new viral particles from being released by infected cells. The amantadanes, which target the influenza M protein, are only effective against influenza A strains; they are not effective against influenza B strains. There is widespread resistance to the amantadanes and for this reason they are no longer recommended for clinical use.

The 1918 pandemic was unprecedented in its severity and its overall impact on life expectancy. In light of the finding that [redacted] it is essential to understand the features that define the pathogenicity of the 1918 virus. [redacted] With the ability of influenza viruses to mutate rapidly and current global travel habits it is essential to the worldwide economy and public health that the risk of emergence of a virus with properties similar to the 1918 influenza virus be determined.

Specifically, this research will establish [redacted] In addition, this research may [redacted] Such information is urgently needed to assess and monitor the pandemic potential of circulating avian influenza viruses. This information will also help regulatory agencies in their assessment of circulating and newly emerging strains and the need for emergency preparedness measures to reduce the consequences of a worldwide influenza pandemic caused by a 1918-like virus. For example, appropriate vaccines will need to be developed and distributed. This is a time consuming process that can take many months. Additionally, in addition to minimizing the spread within a localized community by closing schools and workplaces, extreme measures, such as prohibiting travel between countries, would need to be put in place to prevent the virus from rapidly spreading around the world. Such measures would have a significant impact on the world's economy. In summary, the likelihood of the emergence of a 1918-like virus is fairly high.

Criterion for Identifying Dual Use Research of Concern

Research that, based on current understanding, can be reasonably anticipated to provide knowledge, products, or technologies that could be directly misapplied by others to pose a threat to public health and safety, agriculture, plants, animals, the environment, or materiel. The NSABB categories are knowledge, products, or technologies that could enable any of the following:

1. **Enhance the harmful consequences of a biological agent or toxin.** The rationale for this category is that enhancing the pathogenic consequences of an agent or toxin could increase the likelihood of disease and compromise the ability to treat the disease(s) they cause if extant therapeutics are no longer effective.

In this proposal, reassortants, chimeras, and site-directed mutants will be constructed, using a 1918 influenza virus and [redacted]*. The parental and engineered influenza viruses will then be tested in all three aims of the proposal to identify genes, regions, and specific amino acids that are important in the virulence and transmission of the highly virulent 1918 influenza virus. The techniques for generating these reassortants, chimeras, and site-directed mutants have been in the public domain for many years.*

a. *AIM I: Dr. Kawaoka proposed to investigate* [redacted]

b. *AIM II: Dr. Kawaoka proposed to study the features of HA associated with the high virulence of 1918 influenza by investigating its glycosylation pattern and fusion kinetics. Part of their analysis will entail constructing HA hybrids by inserting successively larger fragments of HA from a closely related but low pathogenic swine influenza virus into the 1918 HA. The HA hybrids will be tested in both 1918 influenza and a contemporary H1N1 (K173) background. (Influenza strain A/Kawasaki/173/2001 (K173) is a human isolate that has low pathogenicity and does not kill mice.) It is anticipated that the 1918 chimeric viruses will be attenuated in comparison to the parental 1918 influenza strain. Conversely, it is anticipated that the K173 chimeric viruses will gain-virulence but not become highly pathogenic.*

c. *AIM III: Dr. Kawaoka proposed to investigate the role that the virus replication complex plays in the severe pathogenesis of 1918 influenza. They will construct chimeric viruses containing all combinations of the 1918 replication complex genes (PB2, PB1, PA, and/or NP) in the K173 background. They will further characterize the regions or specific amino acids in the 1918 replication complex responsible for increased replication. Once identified, they will construct chimeric 1918/K173 viruses with hybrid proteins in the replication complex. They will do this in both the 1918 and K173 backgrounds. It is anticipated that the chimeric K173 viruses will display varying degrees of increased replicative capacity in comparison to the parental K173 strain but will not become highly pathogenic. Conversely, it is anticipated that the chimeric 1918 viruses containing segments of the K173 replication complex will be attenuated in comparison to the parental chimeric 1918 strain.*

The engineered viruses are expected to be attenuated or of equal virulence compared to the parental 1918 influenza virus. Thus, the subcommittee believes that this research will not enhance the harmful consequences of the parental 1918 virus. The subcommittee does acknowledge that it is extremely doubtful that a more pathogenic strain would be constructed. It should be noted that

all of these experiments are conducted at BSL3AG which mitigates the risk associated with such an unlikely circumstance. Should there be an increase in virulence, UW-Madison would reconsider for potential DURC.

2. **Disrupt immunity or the effectiveness of an immunization without clinical and/or agricultural justification.** The rationale for this category is that immunity is a key component in a host's defense against pathogens and toxins, thus rendering an immunization ineffective or disrupting immunity could have harmful consequences for public health, agricultural crops and other plants, and animals.

The proposed research will examine the immune response to the parental 1918 and engineered influenza viruses, but will not disrupt the host immune response in any way. Preliminary results suggest an over reactive immune response contributes to the severity of 1918 pathogenesis. It is anticipated that the chimeric viruses constructed in this study will decrease the severity of disease and increase the ability of the host immune system to deal with the virus in comparison to the parental 1918 influenza strain. Specifically, the HA protein, one of two major antigenic determinants in vaccines, will be mutated in the proposed research. However, the current seasonal vaccine does not include the 1918 or avian influenza viruses; therefore, changes in the HA protein of these viruses will not affect the current annual seasonal influenza vaccines. In the case of an accidental or intended release of the engineered influenza viruses, the mutant HA could be used to generate new vaccines as is done for the production of the annual seasonal influenza vaccines. For the above reasons, the subcommittee believes that this research will not disrupt immunity or the effectiveness of an immunization strategy.

3. **Confer to a biological agent or toxin, resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitate their ability to evade detection methodologies.** The main concept is that anything that might compromise the ability to detect, treat, or prevent disease or illness (human or agricultural) caused by biological agents or toxins could result in a significant public health and/or economic burden.

There are two approved prophylactic/therapeutic classes of antivirals against influenza virus. One class (amantadanes, e.g. amantadine) targets the matrix 2 protein, and the other class (neuraminidase inhibitors, e.g. oseltamivir) targets the neuraminidase protein. Neither protein target will be mutated in the proposed research. Furthermore, the rapid diagnostic tests for influenza are designed to detect all influenza viruses (influenza A or influenza A and B viruses); therefore, these assays would be expected to identify the engineered influenza viruses in this proposal. Cell culture and broadly reactive RT-PCR assays would also be expected to identify all influenza viruses. For the above reasons, the subcommittee believes that this research is unlikely to confer influenza virus with resistance to prophylactic/therapeutic interventions or to facilitate the ability of influenza virus to evade detection.

4. **Increase the stability, transmissibility, or the ability to disseminate a biological agent or toxin.** The rationale for this category is that increasing an agent's stability, transmissibility, or ability to disseminate could facilitate the purposeful malevolent use of a biological agent or toxin and increase the rate or ease by which an agent could spread, impeding attempts to contain disease outbreak.

Transmission will be examined for the 1918 and engineered influenza viruses in order to identify important genetic determinants. In Aims I and II of the study Dr. Kawaoka will [redacted] *In Aim III of the study Dr. Kawaoka will construct chimeric viruses containing all combinations of the 1918 replication complex genes (PB2, PB1, PA, and/or NP) in the K173 background. Additionally, they will construct chimeric viruses with hybrid 1918/K173 replication complex proteins in both the 1918 and K173 backgrounds. Replacing all or parts of 1918 influenza replication complex with the comparable K173 fragments is anticipated to decrease the transmissibility of the chimeric viruses in comparison to the parental 1918 influenza. There are no proposed studies to select for greater transmissibility, stability, or dissemination properties of these viruses, and it is very unlikely that the engineered influenza viruses will have enhanced properties over the parental 1918 virus. For the above reasons, the subcommittee believes that this research is unlikely to result in an engineered influenza virus with greater stability, transmissibility or ability to disseminate.*

5. **Alter the host range or tropism of a biological agent or toxin.** The rationale for this category is that altering the host range or tropism of a pathogenic agent or toxin could endanger a host population that normally would not be susceptible.

A unique feature of 1918 influenza is its relatively broad host and tissue distribution in comparison to other influenza isolates. *Influenza viruses naturally infect humans, pigs, and birds.* [redacted] *In this proposal, studies will examine viral determinants of binding, fusion, and replication in host cells. These functions contribute to host range/tropism; however, it is very unlikely that the engineered influenza viruses will have enhanced host range/tropism compared to the parental 1918 virus.*

In Aim II of the study Dr. Kawaoka will construct 1918 influenza HA hybrids with contemporary low pathogenic HA fragments. In Aim III of the study Dr. Kawaoka will chimeric viruses containing all combinations of the 1918 replication complex genes (PB2, PB1, PA, and/or NP) in the K173 background. Additionally, they will construct chimeric viruses with hybrid 1918/K173 replication complex proteins in both the 1918 and K173 backgrounds. Replacing all or parts of 1918 influenza replication complex with the comparable K173 fragments is anticipated to have no impact on host range or cell tropism since these gene segments are not associated with cell attachment.

6. **Enhance the susceptibility of a host population.** Information about rendering host populations more susceptible to the pathogenic consequences of an agent or toxin could be used to compromise immune responses and enable the acquisition and spread of disease on an epidemic scale.

The proposed research will not involve enhancing the susceptibility of the host population. In Aim I of the study Dr. Kawaoka will [redacted]

[redacted] *is anticipated to decrease the susceptibility of humans to infection or severe disease in comparison to the parental 1918 influenza. In Aim II of the study Dr. Kawaoka will construct 1918 influenza HA hybrids with contemporary low pathogenic HA fragments. Replacing all or parts of the 1918 influenza HA with the comparable low pathogenic HA fragments are anticipated to decrease the susceptibility of humans to infection or severe disease in comparison to the parental 1918 influenza. In Aim III of the study Dr. Kawaoka will construct chimeric viruses containing all combinations of the 1918 replication complex genes (PB2, PB1, PA, and/or NP) in the K173 background. Additionally, they will construct chimeric viruses with hybrid 1918/K173 replication complex proteins in both the 1918 and K173 backgrounds. Replacing all or parts of 1918 influenza replication complex with the comparable K173 fragments is anticipated to decrease the susceptibility of humans to infection or severe disease in comparison to the parental 1918 influenza. For the reasons listed above we do not believe that the proposal will enhance the susceptibility of host populations to 1918 influenza.*

7. **Generate a novel pathogenic agent or toxin or reconstitute an eradicated or extinct biological agent.** The rationale for this category is that host populations may not be immune to novel agents and reconstituted eradicated agents and there may not be existing diagnostics or known or widely available prophylaxes or therapeutics for such agents.

The proposed research uses the 1918 influenza virus, which was previously constructed from an "extinct" virus. The studies to construct this virus were already published by the investigator and others. The 1918 influenza virus itself poses a pandemic threat if it was accidentally or intentionally released; however, as discussed above, the current diagnostics and therapeutics would be effective. In this proposal, [redacted] *however, it is very unlikely that any of the engineered influenza viruses will be more virulent than the parental 1918 influenza virus, which currently exists in secure laboratory facilities. Furthermore, the reconstruction of the 1918 influenza virus was already published. New publications from the proposed research may include the identification of virulence determinants for the 1918 influenza virus. The question then is whether this information could be used to render another influenza virus more virulent. Most likely the virulence determinants would have to be in the context of the closely related low pathogenic avian influenza virus. This is a similar situation to the recent controversial transmission study.*

In Aim I of the study Dr. Kawaoka will [redacted] *The constructed virus is also likely to remain sensitive to antivirals used to treat or prevent influenza infection. A key part of this portion of the study is to compare the pathogenicity of the 1918 influenza virus to, expectedly, lower pathogenic chimeric viruses* [redacted] *Dr. Kawaoka and colleagues will be using reconstituted 1918 pandemic influenza in this study for comparison to their constructed viruses. This group has the facilities (BSL3AG) and experience to work safely with this virus. The chimeric viruses with the 1918 influenza background are expected to be less pathogenic than the parental strain. In Aim II of the study Dr. Kawaoka will construct 1918 influenza HA hybrids with contemporary low pathogenic HA fragments. Both 1918 influenza and K173 are susceptible to neuraminidase inhibitors and to amantadanes. Replacing all or parts of 1918 influenza HA with the comparable low pathogenic HA fragments will not affect the susceptibility of 1918 influenza to*

either neuraminidase inhibitors or amantadanes as these antivirals target either the neuraminidase and M protein, respectively. Conversely, inserting all or portions of 1918 influenza HA into K173 will not affect its susceptibility to either neuraminidase inhibitors or amantadanes. (Please see the Background Section for additional information on influenza anti-virals and the response to Question 3 above). In Aim III of the study Dr. Kawaoka will construct chimeric viruses containing all combinations of the 1918 replication complex genes (PB2, PB1, PA, and/or NP) in the lowly pathogenic K173 background. Additionally, they will construct chimeric viruses with hybrid 1918/K173 replication complex proteins in both the 1918 and K173 backgrounds. Both 1918 and K173 are susceptible to both neuraminidase inhibitors and amantadanes. Thus, replacing all or parts of the 1918 influenza replication complex with the comparable K173 fragments will not affect the susceptibility of the 1918 influenza virus to either neuraminidase inhibitors or amantadanes as these drugs target neuraminidase and M protein, respectively. Conversely, insertion of 1918 influenza replication complex genes into K173 will not affect its susceptibility to neuraminidase inhibitors or amantadanes. . Dr. Kawaoka and colleagues will be using reconstituted 1918 pandemic influenza in this portion of the study for comparison to their constructed viruses. This group has the facilities and experience to work safely with this virus. The chimeric viruses with the 1918 influenza background are expected to be less pathogenic than the parental strain.

For the above reasons, the subcommittee believes that this research will not generate a novel more pathogenic strain of influenza virus that has become resistant to current anti-viral therapy. Rather, the described research will provide essential information for monitoring naturally evolving variants of influenza virus for the potential development of a naturally occurring highly virulent pandemic strain of influenza.

Risk versus Benefit Assessment

1. **Could this research yield information that could be intentionally misused to threaten public health and safety or other aspects of national security?**

   The proposed research is unlikely to provide any additional information that could be intentionally misused to threaten public health and safety. The 1918 pandemic strain has been recreated and sequenced.

   *The proposed research will provide information that will identify the features that define the pathogenicity of the 1918 virus and will help determine the risk of emergence of a virus with properties similar to the 1918 influenza virus. This information is essential to developing strategies to minimize the public health and economic risks associated with an outbreak caused by an influenza virus similar to the 1918 virus. The measures that would need to be developed are identification of viruses to include in a protective vaccine and production and stockpiling of ADEQUATE quantities of the vaccine. Additionally, adequate stockpiles of effective antivirals would be required as well as the implementation of particularly stringent public health measures. These are SIGNIFICANT undertakings. Either failure to react in a preventive manner or, likewise, an overreaction to a minimal threat could have a significant impact on the world economy and public health. Thus, **the information that will be obtained from Dr. Kawaoka's research is essential to making an informed risk assessment as to the re-emergence of a 1918 like influenza virus.***

2. **What is the nature of that information?**

   The proposed research will provide information that will identify the features that define the pathogenicity of the 1918 virus and will help determine the risk of emergence of a virus with properties similar to the 1918 influenza virus.

3. **Is the information novel?**

   The research does describe the construction of chimeric strains of influenza viruses. Construction of chimeric vaccines by reverse genetics is a well-established technique used by the influenza community. Additionally, reassortant influenza viruses occur naturally and are responsible for the continuing evolution of influenza viruses. Thus, the methods used are not novel. The nucleotide sequence of the pandemic 1918 virus is known.

4. **Is the information applicable to other, perhaps common, organisms, biologics, etc.?**

   No, the research is specific to influenza viruses.

5. **Could the information be directly misused to pose a threat? For example, even if the information would need to be combined with other information/technologies in order to pose a threat, is that other information/technology currently available?**

   Information that already is in the public domain is sufficient to pose a threat. The proposed research will not add new information that is likely to increase that threat.

6. **Does the information need to be combined with other information to pose a threat?**

   To pose a threat this information would need to be combined with a highly sophisticated knowledge of molecular biology techniques, protein chemistry, and influenza pathogenesis. Additionally, highly sophisticated laboratory facilities would be required.

7. **If so, is that other information already available?**

   Information that already is in the public domain is sufficient to pose a threat. The proposed research will not add new information that is likely to increase that threat.

8. **What is the nature of the threat that could be posed from intentional misapplication of the information, and what are the potential consequences?**

   Information that already is in the public domain is sufficient to pose a threat. The proposed research will not add new information that is likely to increase that threat.

9. **What is the potential nature (e.g., economic, agricultural, public health, and/or public terror), and what is the potential impact of the threat?**

   The University of Wisconsin-Madison is not in the position to determine the nature and impact of a potential threat.

10. **What is the scope of the potential threat (i.e., how many/which people, plants, animals might be adversely affected)?**

    The University of Wisconsin-Madison is not in the position to determine the scope of a potential threat.

11. **Are there currently countermeasures for this threat?**

    The following countermeasures to a threat from influenza are prior vaccination with an efficacious vaccine and/or the administration of appropriate antivirals.

12. **What type of technical expertise and/or physical resources would be needed to apply the information for malevolent purposes?**

    Information that already is in the public domain is sufficient to pose a threat. To pose a threat the new information that will be obtained from this proposal would need to be combined with a highly sophisticated knowledge of molecular biology techniques, protein chemistry, and influenza pathogenesis. Additionally, highly sophisticated laboratory facilities would be required.

13. **In what timeframe might the information be misused? Is there concern about immediate or near-future potential use, or is the concern about misuse in the distant future?**

The University of Wisconsin-Madison is not in the position to determine when this information could be used for a potential threat.

14. **Would it require a low or high degree of technical skill and sophistication to use the dual use information for harmful purposes?**

The proposed new information to be generated in the grant proposal would require a high degree of technical skill and sophistication, as well as special facilities including appropriate biological containment, if it is to be adapted for a harmful purpose

15. **Could this research yield information that could potentially benefit the life sciences and/or public health and safety and other aspects of national security?**

This research will yield information that could potentially benefit worldwide public health and safety. The research was undertaken to determine the risk of emergence of a virus with properties similar to the 1918 influenza virus.

A. **If so, what is the nature of that information?**

The proposed research will provide information that will identify the features that define the pathogenicity of the 1918 virus and will help determine the risk of emergence of a virus with properties similar to the 1918 influenza virus.

B. **What is the nature of the potential benefit?** *The proposed research will determine the likelihood of an influenza virus similar to the 1918 pandemic strain of emerging naturally. This research may also provide information about the specific characteristics of the 1918 virus that are associated with its high level of pathogenicity.*

C. **How much of a benefit might there be?** *This information is essential to developing strategies to minimize the public health and economic risks associated with an outbreak caused by an influenza virus similar to the 1918 virus. The measures that would need to be developed are identification of viruses to include in a protective vaccine and production and stockpiling of ADEQUATE quantities of the vaccine. Additionally, adequate stockpiles of effective antivirals would be required as well as the implementation of particularly stringent public health measures. These are SIGNIFICANT undertakings. Either failure to react in a preventive manner or, likewise, an overreaction to a minimal threat could have a significant impact on the world economy and public health. Thus, **the information that will be obtained from Dr. Kawaoka's research is essential to making an informed risk assessment as to the re-emergence of a 1918 like influenza virus to ensure that adequate supplies of vaccines and anti-virals are available as well as adequate planning for massive shutdowns of schools, basic services, travel, and businesses.** It is difficult to comprehend the negative effects of a world-wide pandemic caused by a naturally evolved influenza strain similar in pathogenicity to the 1918 pandemic strain.*

16. **Do the potential risks outweigh the potential benefits?**

It is the belief of the IBC subcommittee that the benefits to human health that will be obtained from this research will far outweigh any potential harmful benefits.

A. **If not, determine applicable risk management strategies.**

No new risk management strategies are needed. The Kawaoka laboratory is part of the University of Wisconsin-Madison's Select Agent program which is a registered entity with the CDC and APHIS.. The laboratory is regularly inspected by the University of Wisconsin Select Agent Program, CDC and APHIS. . The Kawaoka laboratory is housed in a dedicated stand-alone facility with multiple containment suites that were designed specifically for work with influenza viruses. This facility was most recently inspected and approved by the CDC and APHIS in February 2012. As a rule the University does not discuss biosecurity measures used in its select agent facilities, but we can assure the facility is monitored extensively. The work described in this grant is performed under BSL-3Ag conditions. Personnel working in Dr. Kawaoka's laboratory have undergone the Select Agent FBI background checks and are trained extensively before working in the containment facilities. In addition each researcher has undergone training for the NIH Guidelines for Recombinant DNA Guidelines. Additionally, the Kawaoka Laboratory biosafety protocol is reviewed at a minimum of every three years and more frequently if there are either changes to the research protocol and/or administrative changes. In the last three years alone, Dr. Kawaoka's influenza biosafety protocol has been revised and reviewed by the IBC eighteen times.

B. **If so, consider whether the research should be modified or discontinued.**

Not applicable. The research should not be modified or discontinued.

Relevant Literature Citations

1. Asaoka, N. Y., Tanaka, T. Sakai, Y. Fujii, R. Ohuchi, and M. Ohuchi. 2006. Low growth ability of recent influenza clinical isolates in MDCK cells is due to their low receptor binding affinities. Microbes. Infect. 8:511-519.
2. Basler, C. F., A> H. Reid, J. K. Dybing, T. A. Janczewski, T. G. Fanning, H. Zheng, M. Salvatore, M. L. Perdue, D. E. Swayne, A. Garcia-Sastre, and J. K. Taubenberger. 2001. Sequence of the 1918 jpandemic influenza virus nonstructural gene (NS) segment and characterization of recombinant viruses bearing the 1918 NS genes. Proc. Natl. Acad. Sci. U.S.A. 98:2746-2751.

2a. Beare, A. S. and R. G. Webster. 1991. Replication of avian influenza viruses in humans. Arch. Virol. 119:37-42.

3. Blumenthal , R. A. Ball-Puri, A. Walter, D. Covell, and O. Eldelman. 1987. pH-dependent fusion of vesicular stomatis virus with Vero cells. Measurement by dequenching of octadecyl rhodamine fluorescence. J. Biol. Chem. 262:13614-13619.
4. Chen, W., P. A. Clavo, D. Malide, J. Gibbs, U. Schubert, I. Bacik, S. Basta, R. O'Neill, J. Schickli, P. Palese, P. Henklein, J. R. Bennink, and J. W. Yewdell. 2001. A novel influenza A virus mitochondrial protein that induces cell death. Nat. Med. 7:1306-1312.
5. Claas, E. C., A. D. Osterhaus, B. R. van, J. C. de Jong, G. F. Rimmelzwaan, D. A. Senne, S. Krauss, K. F> Shortridge, and R. G. Webster. 1998. Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. Lancet 352:472-477.
6. Concnello, G. M.., D. Zamarin, L. A. Perrone, T. Tumpey, and P. Palese. 2007. A single mutation in the PB1-F2 of H5N1 (HK/97) and 1918 influenza A viruses contributes to increased virulence. PLoS. Pathog. 3:1414-1421.
7. Diebold, S. S., T. Kalsho, H. Hemmi, S. Akira, and Reis e Sousa. 2005. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science. 303:1529-1531.
8. Gabriel, G., M. Abram, B. Keiner, R. Wagner, H. D. Klenk, and J. Stech. 2005. The viral polymerase mediates adaptation of an avian influenza virus to a mammalian host. Proc. Natl. Acad. Sci. U.S.A. 102:18590-18595.
9. Gabriel, B. Dauber, T. Wolff, O. Planz, H. D. Klenk, and J. Stech. 2005. The viral polymerase mediates adaptation of an avian influenza virus to a mammalian host. Proc. Natl. Acad. Sci. U.S.A. 102:18590-18595.
10. Garcia-Sastre, A. 2001. Inhibition of interferon-mediated antiviral responses by influenza A viruses and other negative-stranded RNA viruses. Virology 279:375-384.
11. Garcia-Sastre. A. 2006. Antiviral response in pandemic influenza virus pathogenecity. Trends Microbiol. 7:99-100.
12. Garten, W. and H. D. Klenk. 1999. Understanding influenza virus pathogenicity. Trends Microbiol. 7:99-100.
13. Grimm, D., P. Staehell, M. Hufbauer, I. Koemer, L. Martinez-Sobrio, A. Solorzano, A. Garcia-Sastre, O. Halier, and G. Kochs. 2007. Replication fitness determines high virulence of influenza A virus in mice carrying functional Mx1 resistance gene. Proc. Natl. Acad. Sci. U. S. A. 104:6806-6811.
14. Guilloit, L., G. R. Le, S. Bloch, N. Escriou, S. Akira, M. Chignard, and M. Si-Tahar. 2005. Involvement of toll-like receptor 3 in the immune response of lung epithelial cells to double-sranded RNA and influenza A virus. J. Biol. Chem. 280:5571-5580.
15. Hatta, M., P. Gao, P. Halfman, and Y. Kawaoka. 2001. Molelcular basis for high virulence of Hong Kong H5N1 influenza A viruses. Science 293:1840-1842.

16. Hatta, M. Y. Hatta, J. H. Kim, S. Watanabe, K. Shinya, T. Nguyen, P. S> Lien, Q. M. Le, and Y. Kawaoka. 2007. Growth of H5N1 influenza viruses in the upper respiratory tracts of mice. PLoS Pathog. 3:1374-1379.
17. Hulse-Post, D. J., J. Franks, K. Boyd, R. Salomon, E. Hoffman, H. L. Yen, R. J. Webby, D. Walker, T. D. Nguyen, and R. G. Webster. 2007. Molecular changes in the polymerase genes (PA and PB1) associated with high pathogenecity of H5N1 influenza virus in mallard ducks. J. Virol. 81: 8515-8524.
18. Ito, T. and Y. Kawaoka. 2000. Host-range barrier of influenza A viruses. Vet. Microbiol. 74:71-75.
18a, Itoh, Y., Ozake, H. Tsuchiya, K. Okamoto, R. Toril, Y. Sakods, Y. Kawaoka, K. Ogassawara, and H. Kida. 2008. A vaccine prepared from a non-pathogenic H5N1 avian influenza virus strain confers protective immunity against highly pathogenic avian influenza virus infection in cynomolgus macaques. Vaccine 26:562-572.
19. Kash, J. C., T. M. Tumpey, S. C. Proll, V. Carter, O. Perwitassari, M.J. Thomas, C. F. Basler, P. Palese, J. K. Taubenberger, A. Garcia-Sastre, D. E. Swayne, and M. G. Katze. 2006. Genomic analysis of increased host immune and cell death responses induced by 1918 influenza A virus. Nature 443:578-581.
20. Katze, M. G., Y. He, and M. Gale, Jr. 2002. Viruses and interferon: a fight for supremacy. Nat. Rev. Immunol. 2:675-687.
21. Kawai, T. and S. Akira. 2006. Innate immune recognition of viral infection. Nat. Immunol. 7:131-137.
21a. Kawai, T. and S. Akira. 2007. TLR signaling. Semin. Immunol. 19:24-32.
22. Kawai, T. and S. Akira. 2007. Antiviral signaling through pattern recognition receptors. J. Biochem. 141:137-145.
23. Kawaoka, Y. S. Krauss, and R. G. Webster. 1989. Avian-to-human transmission of the PB1 gene of influenza A viruses in the 1957 and 1968. Pandemics. J. Virol. 63:4603-4608.
24. Klenk, H.D. and W. Garten. 1994. Host cell proteases controlling virus pathogenicity. Trends Microbiol. 2:39-43.
25. Kobassa, D., S. M. Jones, K. Shinya, J. C. Keith, J. Copps, H. Ebihara, Y. Hatta, J. H. Kim, P. Halfman, M. Hatta, F. Feldmann, J. B. Alimonti, L. Fernando, Y. Li, M. G. Katze, H. Feldman, and Y. Kawaoka. 2007. Aberrant innate immune response in lethal infection of macaques with the 1918 influenza virus. Nature 445:319-323.
26. Kobasa, D., A. Takada, K. Shinya, M. Hatta, P. Halfman, S. Theriault, H. Suzuki, NH. Nishimura, K. Mitamura, N. Sugaya, T. Usui, Y. Maeda, S. Watanabe, M. Suresh, T. Suzuki, Y. Suzuki, H. Feldmann, and Y. Kawaoka. 2004. Enhanced virulence of influenza A viruses with the haemagglutinin of the 1918 pandemic virus. Nature 431:703-707.
27. Laver, W. G. and R. G. Webster. 1973. Studies on the origin of pandemic influenza. 3. Evidence implicating duck and equine influenza viruses as possible progenitors of the Hong Kong strain of human influenza. Virology 51:383-391.
28. Le, G.R., V. Balloy, M. Lagranderie, L. Alexopoulou, N. Escriou, R. Flavell, M. Chignard, and M. Si-Tahar. 2006. Detrimental contribution of the Toll-like receptor (TLR) 3 to influenza A virus-induced acute pneumonia. PLoS Pathog. 2:e53.
29. Le G. R., J. Pothichet, D. Vilour, T. Fujita, E. Meurs, M. Chignard, and M. Si-Tahar. 2007. Cutting Edge: Influenza A virus activates TLR3-dependent inflammatory and RIG-2-dependent antiviral responses in human lung epithelial cells. J. Immunol. 178:3368-3372.
30. Londt, B.Z., J. Banks, R. Gardner, W. J. Cox, and I. H. Brown. 2007. Induced increase in virulence of low pathogenic avian influenza by serial intracerebral passage in chickens. Avian Dis. 51:396-400.
31. Lund, J. M., L. Alexopoulo, A. Sato, M. Karow, N. C. Adams, N. W. Gale, A. Iwasaki, and R. A. Flavell. 2004. Recognition of single-stranded RNA viruses by Toll-like receptor 7. Proc. Natl. Acad. Sci. U.S.A. 101:5598-5603.

32. McAuley, J. L., F. Hornung, K. L. Boyd, A. M. Smith, R. McKeon, J. Bennink, J. W. Yewdell, and J. A. McCullers. 2007. Expression of the 1918 influenza A virus PB1-F2 enhances the pathogenesis of viral and secondary bacterial pneumonia. Cell Host. Microbe 2:240-249.
33. Neumann, G. and Y. Kawaoka. 2006. Host range restriction and pathogenicity in the context of influenza pandemic. Emerg. Infect. Dis. 12:881-886.
34. Neumann, G., T. Watanabe, H. Ito, S. Watanabe, H. Goto, P. Gao, M. Hughes, C. R. Perez, R. Donis, E. Hoffman, G. Hobom, and Y. Kawaoka. 1999. Generation of influenza A viruses entirely from cloned cDNAs. Proc. Natl. Acad. Sci. U.S.A. 96:9345-9350.
35. Pappas, C., P. V. Aguilar, C. F. Basler, A. Solorzano, H. Zeng, L. A. Perrone, P. Palese, A. Garcia-Sastre, J. M. Katz, and T. M. Tumpey. 2008. Single gene reassortants identify a critical role for PB1, HA, and NA in the high virulence of the 1918 pandemic influenza virus. Proc. Natl. Acad. Sci. U.S.A. 105:3064-3069.
36. Reid, A. H., T. G. Fanning, J. V. Hultin, and J. K. Taubenberger. 1999. Origin and evolution of the 1918 "Spanish" influenza virus hemagglutinin gene. Proc. Natl. Acad. Sci. U.S.A. 96:1651-1656.
37. Reid, A. H., T. G. Fanning, T. A. Janczewski, R. M. Lourens, and J. K. Taubenberger. 2004. Novel origin of the 1918 pandemic influenza virus nucleoprotein gene. J. Virol. 78:12462-12470.
38. Reid, A. H., T. G. Fanning, T. A. Janczewski, S. McCall, and J. K. Taubenberger. 2002. Characterization of the 1918 "Spanish" influenza virus matrix gene segment. J. Virol. 76:10717-10723.
39. Reid, A. H., T. G. Fanning, T. A. Janczewski, and J. K. Taubenberger. 2000. Characterization of the 1918 "Spanish" influenza neuraminidase gene. Proc. Natl. Acad. Sci. U. S. A. 97:6785-6790.
40. Reid, A. H. and J. K. Taubenberger. 2003. The origin of the 1918 pandemic influenza virus: a continuing enigma. J. Gen. Virol. 84:2285—2291.
41. Salomon, R., J. Franks, E. A. Govorkova, N. A. Ilyushina, H. L. Yen, S. J. Hulse-Post, J. Humbred, M. Trichet, J. E. Rehg, R. J. Webby, R. G. Webster, and E. Hoffman. 2006. The polymerase complex genes contribute to the high virulence of the human H5N1 influenza virus isolate A/Vietnam/1203/04. J. Exp. Med. 203:689-697.
42. Salomen, R., P. Staehell, G. Kocha, H. L. Yen, J. Franks, J. E. Rehg, R. G. Webster, and E. Hoffman. 2007. Mx1 gene protects mice against the highly lethal human H5N1 influenza virus. Cell Cycle 6:2417-2421.
43. Scholtissek, C., W. Rhode, H. von, V. and R. Rott. 1978. On the origin of the human influenza virus subtypes H2N2 and H3N2. Virology 87:13-20.
44. Shinya, K., M. Ebina, S. Yamada, M. Ono, N. Kasai, and Y. Kawaoka. 2006. Avian flu: influenza virus receptors in the human airway. Nature 440:435-436.
45. Steinhauer. D. A. 1999. Role of hemagglutinin cleavage for the pathogenicity of influenza virus. Virology 258:1-20.
46. Subbarao, K., A. Klimov, J. Katz, H. Regnery, W. Lim, H. Hall, M. Perdue, D. Swayne, C. Bender, J. Huang, M. Hemphill, T. Rowe, M. Shaw, X. Xu, K. Fukuda, and N. Cox. 1998. Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. Science 279:393-396.
47. Taubenberger, J. K. 2006. The origin and virulence of the 1918 "Spanish" influenza virus. Proc. Am. Philos. Soc. 150:86-112.
48. Taubenberger, J. K., A. H. Reid, A. E. Krafft, K. E. Bijwaard, and T. G. Fanning. 1997. Initial genetic characterization of the 1918 "Spanish" influenza virus. Science 275:1793-1796.
49. Taubenberger, J. K., A. H. Reid, R. M. Lourens, R. Wang, G. Jin, and T. G. Fanning. 2005. Characterization of the 1918 influenza virus polymerase genes. Nature 437:889-893.
50. Thompson, A. J. and S. A. Locarnini. 2007. Toll-like receptors, RIG-I-like RNA helicases and the antiviral innate immune response. Immunol. Cell Biol. 85:435-445.

51. Tsuchlya, E., K. Sugawara, S. Hongo, Y. Matsuzaki, Y. Muraki, Z. N. Li, and K. Nakamura. 2002. Effect of addition of new oligosaccharide chains to the globular lead of influenza A/H2N3 virus haemagglutinin on the intracellular transport and biological activities of the molecule. J. Gen. Virol. 83:1137-1146.
52. Tumpey, T. M., C. G. Basler, P. V. Aguilar, H. Zeng, A. Solorzano, D. E. Swayne, N. J. Cox, J. M. Katz, J. K. Taubenberger, P. Palese, and A. Garcia-Sastre. 2005. Characterization of the reconstructed 1918 Spanish influenza pandemic virus. Science 310:77-80.
53. Tumpey, T. M. A. Garcia-Sastre, J. K. Taubenberger, P. Palese, D. E. Swayne, snf C. F> Basler. 2004. Pathogenicity and immunogenicity of influenza viruses with genes from the 1918 pandemic virus. Proc. Natl. Acad. Sci. U. S. A. 101:3166-3171.
54. van, R.D., V. J. Munster, W. E. de, G. F. Rimmelzwaan, R. A. Fouchier, A. D. Osterhaus, and T. Kuiken. 2007. Human and avian influenza viruses target different cells in the lower respiratory tract of humans and other mammals. Am. J. Pathol. 171:1215-1223.
55. Webster, R. G., and E. A. Govorkova. 2006. H5N1 influenza-continuing evolution and spread. N. Engl. J. Med. 355:2174-2177.
56. Wiley, D. C., and J. J. Skehel. 1987. The structure and function of the haemagglutinin membrane glycoprotein of influenza virus. Annu. Rev. Biochem. 56:365-394.
57. Zamarin, D., M. B. Ortigoza, and P. Palese. 2006. Influenza A virus PB1-F2 protein contributes to viral pathogenesis in mice. J. Virol. 80:7976-7983.