MEETING SUMMARY OF THE WORKSHOP

"APPROACHES TO BETTER UNDERSTAND HUMAN INFLUENZA TRANSMISSION"

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CENTERS FOR DISEASE CONTROL AND PREVENTION

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Background for the Meeting

The impact of influenza epidemics and occasional sporadic pandemics has been clinically and historically recognized for centuries, although influenza viruses were identified first in 1933. During annual influenza epidemics in the United States, approximately 3,000 – 49,000 influenza-related deaths have occurred from 1976-2007 and the death toll from pandemics has ranged from estimates of approximately 12,000 in the 2009 H1N1 pandemic to 500,000 during the 1918 pandemic (Kasowski et al, 2011; Thompson et al, 2009). In addition, an estimated 24.7 million influenza cases, 31.4 million outpatient visits, and 220,000 to 334,000 hospitalizations attributable to influenza may occur in the United States each year (Molinari et al, 2007). The global burden of influenza, of course, is much higher. In view of influenza's importance, it is surprising that, despite numerous studies dating back to 1918, the predominant mode or modes of transmission of influenza between humans remain to be clearly documented. This fact has been summarized by a number of authors (Aiello et al, Am J Infect Control 2010; Bell et al, 2006; Tellier et al, 2009; Brankston et al, 2007; Bridges et al, 2003; Influenza team, European Centre for Disease Prevention and Control, 2007). Understanding the relative contributions of different modes of influenza transmission would provide an important conceptual basis for designing optimal prevention strategies. These strategies

could differ significantly depending on the relative importance of direct and indirect contact transmission (occurring when a person with influenza-contaminated hands touches the mucous membranes of the face), droplet spray transmission (occurring when large infected droplets are expelled by an infected person, travel a short distance through the air and impact on the mucous membranes of another person, but are too large to remain suspended in the air), and aerosol transmission [occurring when droplets or droplet nuclei suspended in air are small enough (< about 100μm aerodynamic equivalent diameter) to be inhaled into the oronasopharynx or lung]. Lacking this conceptual basis, prevention recommendations have been based on uncertain assumptions about influenza transmission. This uncertainty has contributed to a lack of consensus about best approaches to prevention and to intense controversies about implementation of some interventions, a notable example being the use of respiratory protection by healthcare workers (Institute of Medicine, 2009).

Many major health organizations, such as the World Health Organization, the US Institute of Medicine (IOM) and the European Centers for Disease Control have concluded that more information on influenza transmission is critical to pandemic planning. In a 2007 report on preventing pandemic influenza in healthcare settings, the IOM stated that: "… efforts to appropriately protect healthcare workers and their families are greatly hindered by the paucity of data on the transmission of influenza (Institute of Medicine, 2007). Two years later, after the emergence and spread of the 2009 pandemic influenza A (H1N1) virus, another IOM panel came to similar conclusions, stating that "The need for research in a number of areas was striking". Due to the lack of a strong and conclusive evidence base, the committee concluded that determination of the relative contribution of each route of influenza transmission is essential for long-term preparedness planning…" (Institute of Medicine, 2009).

The lack of definitive evidence on the primary mode or modes of transmission greatly hampers the development of evidence-based seasonal and pandemic influenza prevention recommendations for reducing transmission of influenza viruses. Clearly, the availability of better information would guide the development of future guidance and recommendations. Also, improved understanding of transmission would inform the development of more robust infectious disease models of influenza that could help in improving estimates of the impact of various interventions aimed at reducing influenza transmission. Despite the major gaps in information on the relative contribution of different modes of influenza

transmission and the role of environmental contamination, national, state and local planning and guidance for pandemic and seasonal influenza outbreaks has usually assumed that most transmission during the symptomatic phase occurs mainly, but not exclusively, via large droplet spread. (CDC healthcare influenza infection control guidance can be found at: http://www.cdc.gov/flu/professionals/infectioncontrol/healthcaresettings.htm, the 2005 National Strategic Plan for influenza pandemic preparedness can be found at: http://www.flu.gov/professional/federal/pandemic-influenza.pdf, and the 2007 Community Strategy for Pandemic Influenza Mitigation in the United States can be found at: http://www.pandemicflu.gov/professional/community/community_mitigation.pdf). Thus, CDC and other public health entities have proposed the use of school closures, limiting the use of public transportation, and avoiding large public gatherings to reduce person to person contact and as measures to mitigate the spread of influenza during a pandemic. The use of hand washing and cough etiquette are promoted each influenza season as well as during the recent 2009 H1N1 pandemic as ways to reduce transmission of influenza. Routine cleaning of commonly touched surfaces has also been advocated. In healthcare settings, CDC has recommended a variety of administrative, environmental, and personal protection measures to prevent transmission of large droplets containing viable influenza virus. However, CDC guidance has largely side-stepped issues of the potential benefit of mask use in the community setting, and the role, if any, of interventions to reduce aerosol exposures such as increasing ventilation in preventing the spread of influenza in home or community settings.

Clarification of the contribution of different modes of transmission of influenza would provide an evidence base for guidance on the most effective ways to reduce transmission. Evidence-based guidance is needed, not only for healthcare settings, but for households, schools, and other settings as well.

Decisions about what preventive interventions to use and when to use them can have important social and economic costs. For example, measures such as social distancing (e.g., school closures, workplace administrative controls and cancellation of large scale gatherings) while intended to reduce transmission and mitigate the impact of the disease, have substantial secondary impacts such as educational interruption, child care-related workplace absenteeism, social disruption, and negative economic consequences. Understanding the relative contribution of different modes of transmission to

disease spread may enable modifications of social distancing measures that reduce these costs, e.g., recommendations for spacing of desks and other interventions in a school environment versus school closure.

To more clearly identify modes of influenza transmission during symptomatic infection periods, it is critical that additional studies be undertaken. However, the optimal study designs to elucidate the relative contributions of various potential modes of influenza transmission in humans are not obvious. Previous studies of influenza transmission have encountered a number of challenges and limitations. In view of these issues, CDC held a meeting on November 4-5, 2010, of international experts from a wide variety of disciplines to share their individual expertise and opinions about what future study designs should be considered to better understand influenza transmission. Our conviction was that this issue would best be addressed by engaging the experience and wisdom of a group of persons who have a wide range of perspectives in a discussion forum. The presentations and discussions from the workshop are summarized in this document, allowing anyone with an interest in this important area to benefit from a summary of the workshop.

Opening of the workshop

Welcoming comments to the group were made by Dr. Stephen Redd, Chief, Influenza Coordination Unit, CDC and Dr. Nancy Cox, Chief, Influenza Division, National Center for Immunization and Respiratory Diseases (NCIRD). Both Drs. Redd and Cox remarked on the need for better information on influenza transmission and how this lack of information complicated the development of influenza prevention guidelines during the pandemic and they thanked participants for their attendance.

Dr. Dixie Snider, former Chief Science Officer at CDC and current Senior Advisor to CDC Director Dr. Thomas Frieden, provided opening comments and background information for the meeting. He reviewed the chronology of events during the 2009 H1N1 pandemic and highlighted areas where the limited information on the relative contribution of different modes of influenza transmission led to difficulties in the development of evidence-based guidance for infection control in a variety of settings. However, the controversy was likely greatest for the health care setting in regards to the optimal use of personal protective equipment to be recommended. He also reported the deliberations of the Institute of Medicine,

as is also noted above. Dr. Snider noted the potential benefits of greater information on influenza transmission would be to improve the evidence base for guidance on infection control in many settings, including health care, schools, workplaces and public transportation; to better information purchases for the Strategic National Stockpile; to better serve the needs of other agencies and professional organizations and other stakeholders for decision making about interventions during influenza outbreaks, and to meet the expectations of other parts of government which have indicated that resolving issues about influenza transmission are a priority activity.

Dr. David Weissman, Director, Division of Respiratory Disease Studies, National Institute for Occupational Health and Safety (NIOSH), CDC provided additional background about the importance of better understanding influenza transmission from the perspective of the healthcare sector which includes approximately 18.38 million persons in the United States and for which safety at work is a key issue. He cited results from a September 2001 American Nurses Association NursingWorld.org Health & Safety Survey which reported that only 19.5% of nurses felt very safe at work. In addition, 88% of nurses reported that health and safety concerns influenced their decision to remain in nursing and the kind of work they chose to perform. Dr. Weissman also noted the 2007 and 2009 IOM reports cited above, both of which indicated a great need for influenza transmission research to understand the relative contributions of different modes of transmission. This information is needed to form the conceptual basis for the development of interventions to prevent transmission. Other research that would need to follow this fundamental research includes randomized controlled trials of preventive interventions to understand the efficacy of various interventions and then effectiveness research, or "real-world" trials, of preventive interventions to better understand how such interventions perform in the workplace.

Dr. Carolyn Bridges, Associate Director for Science, Influenza Division, NCIRD, CDC presented the charge to workshop participants. The stated purposes of the workshop were:

1) To review state of the art science in influenza transmission
2) To identify gaps in understanding influenza transmission in humans
3) To identify the best study approaches to resolve continuing questions about the relative contributions of contact, droplet, and airborne transmission of influenza in humans.

The stated goals of the workshop were to:

1) To identify scientific gaps in understanding of influenza transmission from multiple perspectives
    - Influenza virology and epidemiology
    - Infection control
    - Health and safety
    - Aerobiology
    - Industrial Hygiene /Engineering
2) To gain input from individuals regarding best scientific approaches and study designs to determine relative contributions of different modes of transmission among humans, considering multiple perspectives
3) To provide information from this workshop to other government agencies, investigators and potential funders
    - Summary and slides to be posted on CDC website, including results of breakout sessions

Topics that were explicitly mentioned by Dr. Bridges as not being a focus of this workshop were issues of transmission of animal-adapted viruses in humans, any policy or policy implications that may arise with emphases on different modes of transmission, discussions of funding opportunities for work on transmission, large-scale, population-based social distancing measures, such as school closures, or current infection control guidance or policy. Participants were also reminded that this was a workshop designed to elicit individual input from workshop participants, but that no consensus on any issue was being sought.

Dr. Bridges asked workshop participants to keep in mind the interaction of influenza virus pathogen-specific characteristics, host characteristics and behaviors and environmental factors that may result in influenza infection. Pathogen factors include Infectious dose, receptor binding, antigenicity, pathogenesis, strain/subtype characteristics, and aerobiology. Environmental factors include humidity, temperature, air exchange rates, physical barriers and other means that may reduce amount of virus, including UV lights and HEPA filtration of air. Host factors include the degree of viral shedding, immunity from prior influenza virus exposures and vaccination, illness severity, symptoms and symptom severity such as degree of cough, behaviors, e.g. hand washing, and the distance between people and the amount of time spent in close proximity.

Scientific Presentations on Thursday, November 4, 2010

Molecular determinants and strain variability in influenza transmission/virus pathogen factors that may influence transmission – Dr. Terrence Tumpey (CDC)

In Dr. Tumpey's opinion, the ferret is a better model for human influenza transmission and pathogenesis than the mouse or the guinea pig. Ferret's are susceptible to all human influenza viruses (developing both immunological and clinical responses), the clinical course of infection is similar to that in humans, and the distribution of sialic acid receptors for influenza viruses in ferrets is similar to their distribution in humans. Both humans and ferrets have a predominance of alpha 2,6 sialic acid receptors in the upper airway and both alpha 2,6 and alpha 2,3 sialic acid receptors in the lower airway. Dr. Tumpey cited studies from the literature which showed that the infectious dose of influenza virus from nasal instillation was 127-320 TCID50 whereas the infectious dose from aerosol inhalation was much lower (3 TCID50) (Couch et al, 1971; Couch et al, 1974; Alford et al, 1961).

He has done respiratory droplet transmission studies in ferrets to examine differences between influenza strains and to explore pathogen genetic characteristics associated with efficient transmission. It was acknowledged that, because his studies are done among ferrets in cages in close proximity to one another, it is not possible to distinguish between large droplet transmission and droplet nuclei (airborne) transmission.

His studies have shown that, as in humans, avian influenza strains are not transmissible from ferret to ferret. However, two 1918 virus genes, HA and PB2, can confer transmissibility when inserted into avian strains. There are ten amino acid differences between the PB2 of the avian Dk/NY//96 virus and the 1918 virus and the sites of these changes are believed to participate in a variety of important functions. Of particular interest is PB2 627K which has been suggested to allow for more efficient growth at the lower temperatures found in the upper airway of mammals (Hatta et al, 2007). A single amino acid change in PB2 at 627, i.e. substituting a glutamic acid for a lysine residue (PB2-K627E), can abolish transmissibility.

Dr. Tumpey has also studied the transmissibility of various 2009 H1N1 influenza viruses. He found that, in general, 2009 strains were less transmissible in the ferret model than a variety of seasonal influenza

strains. Interestingly, he found molecular changes at the PB2 637 site which could explain this. When influenza virus CA/04/2009 had a lysine residue substituted at the 637 position for the existing glutamic acid residue (CA/04/2009 PB2 627K), the virus became much more transmissible to contact ferrets. Furthermore, a naturally occurring E627K strain, i.e., a strain isolated from a patient that had the lysine substitution, was shown to be as transmissible as seasonal strains. Thus, the PB2 gene appears to be an important determinant of the transmissibility of an influenza strain. He cited a number of epidemiologic articles that found a relatively low secondary attack rate among people which may indicate that the 2009 H1N1 virus was suboptimal in terms of transmission efficiency (Cauchemez et al, NEJM 2009; Doyle and Hopkins, 2011; Lessler and Reich, 2009; Leung et al, 2010).

The HA gene also appears to play a role in transmissibility and in pathogenesis. Amino acid position 222 resides in the receptor binding site of the HA protein and may influence binding specificity and cellular tropism of the virus. An HA mutation in which aspartic acid was substituted for glycine (HA D222G) was found with increased frequency in fatal and severe cases in Norway (Kilander et al, 2010) and, in another report, D222G mutants were detected more frequently in viruses isolated from patients with fatal outcomes and in lung samples (Glinsky 2010). In Dr. Tumpey's studies, the HA (D222G) mutation enhanced transmission of the CA/04/2009 strain to contact ferrets. Another HA mutation, at position 219, improves the human-receptor binding affinity of CA/04 HA. This mutation, the substitution of an isoleucine for a lysine, has also been shown by Dr. Tumpey and colleagues to enhance "droplet transmission" in the ferret model. Thus, both the HA and the PB2 genes appear to play important roles in the transmissibility of influenza virus strains.

In discussions regarding this presentation, participants noted that the terminology being used by presenters (and authors) from various disciplines was inconsistent and not standardized and contributed to difficulties in discussing transmission across disciplines. This issue is addressed later in this document.

H1N1: Surface survival and hand hygiene. Drs. Judith Noble-Wang, Angela Coulliette, Kate Ellingson (CDC)

This group is currently reviewing the literature on, and in the process of conducting studies aimed at, understanding the transmission and survival of influenza virus on surfaces and hands. Important questions include: How long will the virus remain infectious on surfaces? Under what environmental conditions and substrate conditions will the virus survive and remain infectious? What is the survival and infectivity of the virus on personal protective equipment (e.g., N95 respirators, gowns)? What is the importance of hand hygiene in preventing the transfer of the virus to receptor sites, e.g. from contaminated N95 respirators to the hands and mouth or nose?

Factors that affect surface survival include the size of particles in which influenza viruses are found, sample matrix, influenza envelope composition, porosity of the surface on which influenza viruses are deposited, temperature, and absolute humidity. With regard to studies of surface survival and infectivity of 2009 H1N1 influenza virus, there have been three published studies of survival on stainless steel at various temperatures and humidities. One of these studies (Bean et al,1982) reported longer survival (over 48 but less than 72 hours) on plastic and stainless steel than on pajamas, a handkerchief, a magazine, and a tissue suggesting that porosity is an important determinant of the potential infectivity of a surface over time. Another study (McDevitt et al, 2010) showed that viral viability on stainless steel decreased with increasing temperature, increasing relative and absolute humidity, and increasing exposure time. A third study showed that the virus is relatively quickly inactivated on a copper surface (Noyce, et al, 2007). CDC investigators are currently examining the effects of temperature, humidity, and deposition in matrices containing fetal bovine serum or mucin on the viability of the 2009 H1N1 virus on stainless steel.

Dr. Ellingson reviewed the logic for hand hygiene in infection control and some of the literature which supports that intervention for the prevention of transmission of respiratory viruses, including influenza. For example, it was shown that influenza A virus remained stable on the hands of vaccinated healthcare workers for one hour (Grayson et al, 2009). In another study, influenza viruses were shown to survive up to three days on banknotes and up to 17 days in the presence of mucus (Thomas et al, 2008). Other circumstantial evidence noted was that people frequently touch target mucous membranes in the course of their work (Nicas et al, 2008). Randomized controlled trials of hand hygiene (with or without other interventions) have yielded mixed results with some showing a benefit in preventing respiratory infections (e.g. White et al, 2005; Luby et al, 2005) but more showing little or no benefit (e.g. Larson, 2006; Sandora et

al, 2005; Cowling et al, 2009) in contrast to GI infections, where hand hygiene generally shows a benefit. However, because of the proven general benefits of hand hygiene in reducing transmission of multiple pathogens, hand hygiene will continue to be a recommended intervention for infection control intervention, particularly in the healthcare setting for all healthcare personnel. .

In summary, the contribution of contact transmission to influenza transmission is unknown. Previous studies of interventions to prevent contact transmission, e.g., hand hygiene, have yielded contradictory results and have been plagued with numerous difficulties, including limitations in compliance with hand hygiene and insufficient differences in behavior between intervention and control groups in terms of hand hygiene adherence (Aiello et al, Am J Infect Control 2010). As elaborated upon below, there is a need for better study designs to address the role of contact transmission and interventions for preventing contact transmission, if it plays a significant role.

Influenza Aerobiology: Characterization of human source aerosols. Dr. Donald K. Milton, University of Maryland

Dr. Milton covered the following topics: 1) general considerations regarding the aerobiology of communicable respiratory infections, 2) experimental influenza aerosol stability, 3) experimental human infection with aerosols, 4) human source influenza aerosols, and 5) transmission at a distance and levels of evidence. He showed a figure published in 2004 (Roy and Milton, 2004), which illustrated the various sources and sizes of infectious particles, factors influencing transport and dispersion of these particles, and the various sites of deposition of various particle sizes in the human respiratory tract. In 2004, Dr. Milton proposed that agents' abilities to transmit infection via the airborne route could be characterized as obligate (airborne transmission required, example *M. tuberculosis*), preferential (airborne transmission a preferred route, but other routes also possible), or opportunistic (airborne transmission only when unusually conducive circumstances exist). He noted that it was probably more useful to think of respiratory pathogens' abilities to transmit via the airborne route along a continuum from anisotropic (airborne route preferred for transmitting respiratory infection) to isotropic (multiple potential routes other than airborne for transmitting respiratory infection). He showed a graph characterizing microorganisms according to

(aerosol/contact) transmission occurrence ratio and as isotropic or anisotropic based on sensitivity to route of entry. For example, *B. anthracis* and *M. tuberculosis* were anisotropic, Variola major and influenza A were intermediate, and respiratory syncytial virus and human rhinovirus were isotropic.

Dr. Milton also reviewed previous studies (Edward et al, 1943; Hemmes et al, 1960; Harper, 1961; Shaman and Kohn, 2009; Schaffer et al, 1976) which demonstrated that influenza A virus PR8 decay/death is greater at higher relative and absolute humidity, with absolute humidity being the stronger determinant. However, he cautioned that the effect of absolute humidity may vary by strain and the aerosolization medium.

He reviewed previously published human challenge studies, i.e. experimental infection of human research subjects with influenza virus (Alford et al, 1966; Couch et al, 1971; Couch et al, 1974; Douglas, 1975). Although these studies present ethical challenges and are logistically difficult and expensive, they have the potential to provide more useful and relevant information than other types of studies. Studies to date were done primarily to establish the human infectious dose. The data indicate that the TCID50 required to establish infection from intranasal instillation (large droplet) is in the range of 127-320 viral particles whereas the TCID50 for aerosol challenge into the lower respiratory tract is in the range of 0.6-3.0.

Moving on to human source influenza aerosols, Dr. Milton cited a study by Johnson et al (2009) which showed that placing either an N95 respirator or a surgical mask on the patient-source eliminated the capture of influenza virus from the source. He continued by summarizing experiments he had been involved with which used a piston spirometer device to collect aerosolized particles from patients with influenza who were asked to cough into the device. The purpose of these studies is to measure the number and size distribution of exhaled influenza viruses and to measure the effect of wearing a surgical mask on the release of virus aerosol by coughing patients. In one study, 32 of 38 patients (84%) of the cough aerosol samples contained influenza virus RNA (although the viability of these particles was not determined). In 2007-2008, an exhaled breath collection system was used for sampling patients in Hong Kong and at the University of Massachusetts Lowell. The conclusions from these studies were that: 1) influenza virus RNA was detectable in exhaled aerosols, 2) influenza virus RNA was more frequent with cough (0.1-419 RNA copies per cough) than with tidal breathing alone (<3.2-20 RNA copies/min), and that

influenza virus RNA was likely contained in fine exhaled breath particles during tidal breathing (Fabian et al, 2008).

More recent studies have been conducted using the Gesundheit Machine II. This machine was used in a study of persons who responded to an invitation to come to the Student Health Services or study research site during the 2009 influenza pandemic. Those with a positive rapid test for influenza or with a fever of >100 degrees F plus cough and presented within 3 days of onset were eligible to participate. Each one exhaled particles collected while wearing an ear-loop surgical mask and while not wearing a mask, with 10 forced coughs every 10 minutes for 30 minutes. Thirty-eight of 41 cases tested positive for influenza by RT-PCR from a nasal swab and exhaled breath was successfully collected from 37. Viral particle numbers varied among individuals by as much as a log for any given scenario, but fine particles, on average, contained more influenza virus RNA copy numbers by RT-PCR (about two logs) than coarse particles. However, virus was recovered through virus culture on MDCK cells from only the two subjects with the highest RNA copy numbers. Of note, in these two cases, samples with and without the mask were culture positive. Fractionation of the coarse particles was not feasible using the technology employed for these studies and a new design is under development to address this issue. In summary, masks were highly effective for coarse particles, overall there was a 5-fold reduction in virus aerosols, fine particle aerosols can contain infectious virus, but there is great individual variation in viral aerosol generation rates.

Dr. Milton ended his talk by reviewing two published studies that he interpreted as supporting a role for aerosol (droplet nuclei) transmission. One study by Hall et al. (2007) was interpreted by the authors as supporting large droplet transmission because the attack rate among susceptible children in the same neonatal ICU room as index cases was extremely high (OR=55.4) compared to children in other rooms. However, Dr. Milton noted that if one looks at all nosocomial cases among susceptible children with near or distant exposure to an index case, the attributable risk is 63%, suggesting that distal aerosol spread may not represent a high individual risk but might be a significant population risk. The other study he cited was a 1957-1958 Livermore VA study where a hospital building that had ultraviolet lights for reducing transmission of TB experienced a 90% reduction in influenza cases compared to the other hospital building. (McLean,1961). However, this study has multiple limitations and it is unfortunate that there has been no attempt to replicate it in over 50 years.

The guinea pig transmission model for influenza. Dr. Peter Palese (Mount Sinai School of Medicine)

Dr. Palese has long experience in working with the guinea pig model of infection. Guinea pigs are very susceptible to infection with human influenza viruses and help inform transmission questions, but they do not show symptoms or signs of disease and are not a good model for human pathogenesis. When challenged intranasally, these animals develop high titers of virus in the nose (~10^7 PFU/ml), but growth in the lungs was more moderate (~10^5 PFU/ml) and shorter-lived. While human influenza viruses will achieve high nasal titers when inoculated into the nostrils of guinea pigs, avian strains will not. The 2009 H1N1pandemic strain was also efficiently transmitted in guinea pigs as occurred in humans. Both contact (infected and susceptible animals in the same cage) and "aerosol" (animals in adjacent cages) transmission of human influenza viruses have been studied in these animals, although the same caveat is noted here as with Dr. Tumpey's studies in that placing animals in adjacent cages does not distinguish between large droplet and aerosol transmission.

Dr. Palese gave some examples of particular virus mutations which resulted in changes in transmission characteristics. He studied PB2 mutations in influenza strains and demonstrated their importance in increasing or decreasing the probability of transmission. He has also done studies of "aerosol" transmission at various temperatures and relative humidities and concludes from these studies that influenza viruses are more effectively transmitted at low temperatures and low relative humidity which may partially explain the seasonality of influenza in temperate climates.

Other experiments reviewed by Dr. Palese concerned the use of whole inactivated virus (WIV) to block transmission in the guinea pig model. He found that whole inactivated virus (WIV) vaccination 1) led to reduced titers of virus shed upon homologous challenge by the intranasal route in vaccinated animals and 2) reduced transmission from vaccinees to naïve cage-mates, however 3) vaccinated guinea pigs who were then inoculated were still able to transmit influenza to unvaccinated animals in the same cage (i.e. contact exposure). Dr. Palese also reviewed studies of oseltamivir-resistant viruses with different resistance-conferring mutations derived using reverse genetics. The different mutations resulted in different levels of resistance. A wild type resistant strain, A/Panama/2007/1999 (H3N2), was more efficiently

transmitted by aerosol than other strains with induced mutations which conferred greater drug resistance. However, unlike aerosol transmission, "resistance-inducing mutants" continued to be transmitted efficiently via the contact route of transmission. Dr. Palese believes that continued study of oseltamivir-resistant strains is important in part because H1N1 viruses have the capacity for acquiring oseltamivir resistance as occurred with the human seasonal influenza H1N1 strain in 2007-2009. Dr. Palese's lab conducted experiments using two seasonal H1N1 virus Isolates from 2008: one that was sensitive to oseltamivir (NY/1253/08) and one that was resistant to oseltamivir (NY/1326/08). Both grew equally well in MDCK cell culture. NY/1326/08 is about 1000-fold more oseltamivir resistant than NY/1253/08. In contrast to some of the other viruses where resistance resulted in reduced transmission, the resistant H1N1 virus used in these guinea pig transmission studies was more efficiently transmitted by both the contact and the "aerosol" route than the sensitive strain. This data points to the variability between specific viruses that may influence transmission by different routes in guinea pigs.

Meteorologic determinants of influenza survival, transmission, and seasonality. Dr. Jeffrey Shaman (Oregon State University)

Dr. Shaman is with the College of Oceanic and Atmospheric Sciences and therefore brought a unique perspective to the meeting. He began by reviewing a guinea pig experiment published by Lowen et al in 2007 which demonstrated that colder temperatures and lower relative humidity (RH) favored transmission and was also discussed by Dr. Palese. However, Dr. Shaman pointed out that RH is not a well-constrained variable; it varies as both a function of air water vapor content and temperature. Saturation vapor pressure, i.e. 100% RH, rises exponentially with increasing temperature. Absolute humidity (AH), i.e., the vapor pressure calculated from the temperature and humidity, was found to have a much more statistically significant association with influenza transmission (Lowen et al, 2008). This association appears to be non-linear. To explain the association of RH with influenza transmission, Lowen et al put forth two hypotheses: 1) virus-laden aerosols (droplet nuclei) are more efficiently produced at lower RH due to increased evaporation of expelled droplet particles, such that more virus-laden particles remain airborne longer, and/or 2) influenza virus survival (IVS) increases as RH decreases, such that the airborne

virus remains viable longer at lower RH. It is not known which of these hypotheses is true or if both (or neither) are true, but it does appear that AH is more important to measure than RH (Shaman et al, 2009).

Dr. Shaman then turned to the issue of the seasonality of influenza. He pointed out that, in the winter, RH is minimal indoors but maximal outdoors whereas AH is minimal, both indoors and outdoors. AH provides a single, coherent, more physically sound explanation for the observed variability in the yearly timing of influenza seasonality in temperate regions. He then posed three questions: Can we model the seasonal cycle of influenza incidence using observed AH conditions? Does AH variability explain the annual geographic spread of influenza? And, what will happen to influenza transmission in a warming world?

To begin to address these questions, Dr. Shaman first reviewed a SIRS (susceptible-infected-recovered-susceptible) model published by Dushoff et al in 2004. Dushoff et al showed that a small perturbation in the contact rate could produce a large seasonal cycle (dynamical resonance). This resonance occurs when the endogenous, or instrinsic, oscillation period is about one year and a one-year period forcing is applied in the model. By putting in various endogenous periods and running the model multiple times, they showed that runs when the endogenous period neared one year showed the largest seasonal peak-to-trough amplitude. Therefore, they concluded that a slight forcing could account for the pronounced seasonality of influenza in temperate regions. He then presented data which support the hypothesis that AH could be that seasonal forcing.

He also delved into the potential effect of AH on R_o which is the basic reproductive number or the average number of secondary infections a single infected person causes in a fully susceptible population. Dr. Shaman's group has revised the SIRS model used by Dushoff et al in several ways. Specifically, they have broadened the range for L, the duration of immunity, from 4-8 years to 2-10 years; they also used a range for D (the infectious period) (0.005-0.0192) based on reviews of several other studies; they have made the contact rate dependent upon the humidity; and they have reduced R_0 from 8-10 cases to 0.8-4 cases based on other published literature. The primary rationale for making R_0 a function of humidity was that, by surviving for a longer period once expelled into the environment, contact rates with influenza virus increase and thus R_0 increases. They have run 5000 1972-2002 simulations for five states (AZ, FL, IL, NY and WA). Each run used a different combination of the parameters for L, D, R_{0max} and R_{0min}. The best fit

simulations at each state were characterized by high R_{0max} (generally >2.8), high R_{0min} (>1), and low mean infectious period (2<D<4.2 days). The ratio of the average number of model infected persons to average observed excess pneumonia and influenza (P&I) mortality was the same at all five sites. (It was mentioned that, if this were done again, the number of influenza-like illnesses (ILI) at sentinel sites might be a better measure than P&I mortality.) When they applied the ten best parameter combinations to the remaining 43 contiguous states and D.C., 32 of 44 sites produced good fits. The 12 states that did not tended to have low population densities and/or low exchange of commerce with other states. His conclusion is that humidity forcing alone is able to produce a realistic climatology of influenza infection throughout the U.S. and largely explain influenza seasonality in the U.S.

Dr. Shaman also showed data which indicates that epidemic influenza onset is associated with changes in humidity. For all states except Hawaii and Alaska, and for Washington, D.C., the model identified the timing of epidemic influenza onset for each 1972-2002 winter season based on P&I mortality. .

Influenza outbreaks: What can they tell us about routes of transmission? Dr. Ben Killingley, University of Nottingham, UK

Dr. Killingley provided the context for the outbreak reports/investigations selected for this discussion, provided some examples, and addressed what, if anything, we can infer about routes of transmission from reports of outbreak investigations. He noted that outbreak investigations generate a wealth of information, that the workload of outbreak management and investigation is significant, and, most importantly, there are public health benefits such as interrupting transmission, improving knowledge, and providing lessons for the future. However, because the primary purpose and design of these investigations is not to study routes of transmission, can they tell us anything about routes of transmission?

There are many influenza outbreaks reported in the literature. Those that Dr, Killingley selected for this presentation met three criteria:

1) Influenza was laboratory confirmed (at least in some patients)
2) The outbreak occurred in a confined setting with minimal risk of confounding imported disease, and

3) Inferences about transmission routes can be made.

The first report examined an outbreak on a hospital ward in 1957, published by Blumenthal et al. in 1959. This was a prospective observational study in a ward with 29 patients and 33 healthcare personnel. Within seven days of illness in the index case, 14 patients and 14 healthcare workers had developed an influenza-like illness (ILI). Based on clinical illness and serological testing, the overall attack rate (AR) was 58%. Case patients were clustered, but in two different areas of the ward. The epidemic curve seemed to indicate a point source of infection mostly, but not exclusively, near the index case in the first 1-2 days after exposure, with possible secondary cases occurring 4-6 days after the index case became ill. Side rooms were not affected. These data suggest contact and/or droplet spread. The wide dissemination supports aerosol transmission, but the epidemic curve tends to negate that route. Healthcare personnel could have acted as primary or secondary vectors. The report does not address what infection control precautions were used and whether ill staff members were sent home. Dr. Killingley concluded that it is difficult to exclude any route of transmission from this report.

The next report Dr. Killingley examined is the Livermore VA hospital experience in 1957-1958 which was reported by McLean in 1961. There were two hospital wards – one 150 ward had UV lights for prevention of tuberculosis transmission and the other 250 bed ward was not irradiated. In the irradiated ward during a December and January period of influenza activity, there were no clinical cases of influenza based on respiratory symptoms and four patients with respiratory symptoms attributed to other causes. In contrast, in the non-irradiated ward, there were 39 cases of clinical influenza and 28 clinical cases of other respiratory diseases. Serologic data for the entire period of study (week of Dec. 15 through week of Jan. 26) revealed that, among patients, 4/209 (2%) in the irradiated ward and 75/396 (19%) in the non-irradiated ward had evidence of infection (4 fold rise in titer). Among personnel, who moved from ward to ward, the serologic attack rate was 92/511 (18%). This report is a compelling argument for the potential effectiveness of UV light. It also suggests that aerosol transmission was the predominant route of spread in this facility in 1957-1958. However, other modes of transmission cannot be completely excluded. There is no mention of staff movements in the facility and the length of stay of patients. Index cases and their location(s) were not described and other environmental controls, e.g., open windows, were not described. No other studies or

outbreak investigations have been published which assess the potential impact of UV radiation on the transmission of influenza.

The next study of interest is a report by CDC investigators of an outbreak of influenza on an aircraft grounded in Alaska for maintenance (Moser et al, 1979). Of the 54 persons on board, 53 were followed up. During the 4.5 hours the plane was grounded, the ventilation system was turned off. Passengers were allowed to disembark during this time - 24 did and 30 did not. The index case, a 21 year old female with fever and cough, remained on board - primarily reclining on seats near the lavatories. Thirty-eight of 53 passengers became ill (AR=72%); 8/31 tested were culture positive and 20/22 tested were seropositive. Passengers who remained on board for >3 hours had an AR of 86% whereas those who remained on board < 3 hours had an attack rate of 54%. The epidemic curve suggests a "point source", i.e., a single source, for all infections (other than the index case) since virtually all the infections were clinically manifest within 36-60 hours of exposure to the index case in this poorly-ventilated environment. There was no discussion of the temperature or humidity on the airplane while it was grounded. The strong evidence for a single source for the secondary infections, the high attack rates, and the exposure of passengers in a relatively small and poorly ventilated space support a role for aerosol spread in this circumstance. However, no route of infection can be completely ruled out because passengers were free to move about the cabin. Dr. Killingley raised the possibility that the index case may have been a super producer (of aerosols) or a super shedder (of virus). However, because a persistent cough is not a common manifestation of influenza, it may not require this radical an explanation. It may be, for example, that the subset of patients with influenza who produce aerosols (e.g., through coughing, sneezing, etc.) are the subset most likely to produce particles in the droplet nuclei range, usually in the range of < 10 μm.

The fourth report examined was published by Morens and Rash in 1995. An influenza outbreak occurred in a nursing home with 39 residents. Eleven of the 39 (28%) developed an ILI; six were laboratory confirmed as influenza. Infection control measures were instituted after > 2 weeks from symptoms in the first case. Observations of note included: 1) an AR of 34% among skilled care residents versus only 10% among intermediate care residents, 2) an AR of 45% among non-mobile residents compared to 21% among mobile residents, and 3) an AR of 38% among tube-fed or frequently suctioned patients compared to an AR of 13% among all others. Increased staff-patient contact was noted to be associated with clinical illness.

The authors stated that they suspected staff spread of the virus by hands or fomites. However, there was very little illness among the staff so they would have been asymptomatic vectors acting as sources of secondary infection. No serologies were done on staff, so it is possible that there were asymptomatic or mildly ill infected staff who shed virus. The fact that mobile residents were at lower risk of infection supports large droplet spread or even droplet nuclei spread depending upon where they spent their time. Furthermore, since the body of existing literature does not provide strong evidence of spread by hands or fomites as a major mode of transmission, the fact that non-mobile residents were at higher risk can be taken as evidence for aerosol and/or droplet transmission. However, the authors argue that the "spatial and temporal patterns of onset were not typical of airborne spread".

An outbreak of influenza in a neonatal unit, published by Cunney et al, 2000, was covered next. This outbreak occurred in Ontario during the 1998 epidemic. Of 54 neonates present in the unit over 18 days, 19 (35%) were laboratory-confirmed cases, although only six were symptomatic. Sixteen percent of the staff reported an ILI during the outbreak. Risk factors for infection were twin pregnancy (n=8, OR=7), mechanical ventilation (OR=6.2), and >100% bed occupancy. It was noted that ventilator tubing was being changed in a manner that produced aerosols. Staff influenza vaccination rates were low (15%) and only 4/28 took time off when they were ill. It is difficult to draw any conclusions about transmission from this report. Parents and healthcare personnel may have been sources of infection through either the contact or droplet route and there was some evidence that aerosol generation was taking place.

In 2001, Awofeso et al published a report of a prison inmate index case who had received a visitor who was without symptoms during the visit, but developed ILI symptoms the next day (i.e. was pre-symptomatic), although influenza was not confirmed in that visitor. There were 22 suspected secondary cases, nine of which were laboratory confirmed influenza. The ARs among inmates, healthcare personnel, and prison officers were 35%, 13%, and 0%, respectively. The authors speculated that the inmates were infected through close contact with each other. They stated that one healthcare worker handled soiled linen and one had face-to-face contact with an infected inmate that likely led to their illnesses. The guards/officers presumably did not get infected because they had little direct contact with the inmates. Given the limited data in this report and the difficulty of interpreting it, Dr. Killingley felt that conclusions about modes of transmission were speculative.

Han et al, 2009 recently published a report concerning a U.S. tour group who took a trip to China over a period of four days. There were 30 members of the group, which took four separate flights. The index case was a female tourist and there were 10 secondary cases, 9 among the tour members and one among other airplane passengers. Talking with the index case was associated with an increased risk of infection. The AR among those who talked for more than 2 minutes at less than 2 meters distance was 56% compared to 0% among those who did not talk with the index cases. Talking with the index case for > 10 minutes was associated with a five-fold higher risk than talking for 2-9 minutes. Dr. Killingley noted that this report definitely supports possible contact or large droplet transmission. Furthermore, it indicates that long distance (i.e. airborne or aerosol) transmission on well-ventilated airplanes is unlikely. The authors concluded that there was "no evidence of airborne transmission." However, there was no mention or discussion of short range aerosol transmission and talking does generate aerosols as well as large droplets.

In 2010, Baker et al. reported an outbreak involving a school group of 24 persons travelling via a "long haul" flight back home to New Zealand from Mexico (via Los Angeles). Persons considered "at risk", included 102 passengers in the rear section of the plan. Of these 102, 97 were contacted. A post-flight case was defined as an ILI within 3.2 days of arrival home; there were four such cases and 3/4 were considered probable or possible. During the flight, there were nine confirmed cases and three suspected cases. The risk of infection among passengers was calculated to be 1.9% and, if the susceptible individual sat within 2 rows of a symptomatic person during the flight, the risk was 3.5%. However, the seating plan seems to suggest that persons a slightly greater distance away may have been at risk as well. It is clear that long range transmission was not evident in this instance. However, all other modes of transmission, including short range aerosol transmission, were possible modes of transmission.

The final study presented was published by Wong et al in 2010, although the outbreak occurred in 2008. It happened on a 30 bed medical ward that had 59 patients and 29 healthcare personnel. The index case had chronic obstructive pulmonary disease and was treated with non-invasive ventilation (NIV) for 16 hours on the ward. Nine inpatients subsequently developed confirmed influenza and two healthcare workers were diagnosed by symptoms only. All secondary cases were treated with oseltamivir within 24 hours of symptom onset. The epidemiologic curve showed that the index case developed disease three days before the onset of disease in the second case. However, seven of the secondary cases occurred 2-4

days after the NIV treatment of the index case. Perhaps importantly, an air conditioning outlet was in the ceiling above the index patient's bed. The overall configuration of the HVAC system resulted in a net flow of air from Bay C, where the index patient was, to the corridor which led to Bay B. This was verified by tracer testing and modeling using computational fluid dynamics (CFD). The overall patient attack rate on the ward was 13.6%. However, the attack rate was 22.2% on Bay B, 20% on Bay C and was 0% in the side room and Bay A (which was at a similar distance as Bay B but did not receive airflow from Bay C). So, this particular outbreak was temporally related to a likely aerosol generating procedure (non-invasive positive pressure ventilation) performed on a patient with influenza and imbalanced airflow. Although contact and large droplet spread is possible, it cannot explain the epidemic curve and the spatial distributions of cases. Furthermore, the authors report that patient interaction was minimal and that there was little evidence for healthcare personnel as vectors. In these specific circumstances, aerosol transmission appears to have played a role.

In summary, outbreak investigations are useful but cannot be used to definitively identify the relative contributions of the various potential modes of transmission of influenza. Among the outbreaks there are differences in the viruses and, as shown earlier, these differences can affect transmissibility. As noted above, there are differences between hosts with regard to multiple factors such as symptoms and immunity. And there are differences in the environments of exposure, e.g., their size, temperature, humidity, ventilation, movements of persons, etc. Outbreaks are never controlled and are therefore subject to observation and recall bias, confounding, co-interventions, etc. Complete ascertainment of cases may not occur because of the difficulty in tracing individuals in a timely manner, overlooking asymptomatic cases, and incomplete testing. With regard to routes of transmission, however, it does seem reasonable to conclude that the combination of confined spaces, especially poorly ventilated spaces, and a large number of susceptible individuals can lead to high ARs. Close proximity to an index case, especially for more than a couple of minutes, appears to substantially increase the risk of infection. However, this does not prove that large droplet transmission is the primary mode because short range aerosol transmission is an equally plausible mode and can occur at the same time. It is possible that all routes play a role but the relative significance of each is unknown. It may be that the significance of each mode of transmission is dependent on the specific host-virus-environmental circumstances. Dr. Killingley noted that, for the future, an

emphasis on an interdisciplinary team to study the mode, or modes, of transmission offers the best promise for success.

Community Intervention studies – hand washing, face masks Dr. Benjamin J Cowling, University of Hong Kong

Dr. Cowling began by reviewing the various non-pharmaceutical interventions (NPI). The interventions have been applied 1) at the national and international level, 2) at the national/state/local level, and 3) at the household and/or individual level. Examples of 1) include travel restrictions and entry screening, of 2) include social distancing (such as school and workplace closures), liberal leave policies, isolation of cases, and quarantine of the exposed, and of 3) include hand hygiene and face masks. Many uncertainties remain about influenza virus shedding and infectiousness, and the effects of NPI. Examples of questions which remain unanswered include: 1. What is the infectious profile over time from infection to onset of clinical illness? (e.g., Is there pre-symptomatic infectiousness and are asymptomatic individuals infectious?) 2. Why do children seem to be more infectious than adults? And 3. How does influenza spread more commonly – contact, large droplet, or aerosol? Dr. Cowling reviewed the levels of evidence that must be obtained for a drug approval, such as a novel antiviral agent, and pointed out that there is no agreed-upon equivalent process for a NPI.

According to Ferguson et al, Nature 2006, most influenza transmission occurs in community settings, about 37% in schools, about 30% in households, and the rest in the general community. Schools appeared to be particularly important to transmission of 2009 pandemic H1N1 (Wu et al, 2010).

Most NPI intervention studies have been designed to compare an intervention and a control arm. They may be aimed at primary prevention of infection, secondary prevention among contacts, or both. Community studies of hand hygiene alone have been recently been reviewed by Aiello, et al 2008. These studies have given mixed results, with some studies showing prevention of infection but many not showing an effect.

Community studies of face masks, with or without hand hygiene, have also been done in households, schools, and university dormitories. Larson et al, 2010 studied 509 households randomized to

education, hand hygiene, and hand hygiene plus face mask. The households were followed for up to 19 months. For the combined outcomes of primary and secondary transmission, there was no statistically significant difference between the groups in terms of acute respiratory infections (ARI) or influenza-like illness (ILI). For the secondary prevention outcome alone, there were reduced ARI secondary attack rates in the hand hygiene plus face mask arm, but not the hand hygiene only arm. MacIntyre et al, 2009 studied 143 households, each with an index case aged 0-15 years of age with an ILI. Households were followed for seven days. Contacts were randomized to education, surgical mask, or N-95 respirator. In this study of secondary transmission, there was no difference between the arms in the intent-to-treat analysis. However, there was a statistically significant reduction in ILI (OR=0.26) in the per-protocol analysis when comparing the surgical mask and N-95 respirator groups combined to the education control. A recent study by Aiello et al in the Journal of Infectious Disease, 2010 involved 1430 university students in seven residence halls. These halls were randomized to control, face mask, and face mask plus hand hygiene. There was some evidence for a reduction in ILI late in the study in both the face mask and face mask plus hand hygiene arms.

Dr. Cowling then reviewed his group's recent randomized controlled trial of secondary prevention among household members of index cases presenting to outpatient clinics with ARI (Cowling et al, 2009). There were 306 households of index cases with laboratory-confirmed influenza. Of these, 47 households had to be excluded because there was a laboratory-confirmed co-index case at the time the intervention was to be applied. The three interventions were 1) health education, 2) health education plus hand hygiene (soap dispensers and alcohol hand rub), and 3) health education plus hand hygiene plus surgical mask. Outcome measures were laboratory-confirmed influenza, ARI and ILI. ARI was defined as at least two of the following symptoms – fever $>37.8^0$ C, cough, headache, sore throat, aches or pains in muscles or joints. ILI wasdefined as fever $>37.8^0$ C plus cough or sore throat. Among 259 analyzed households, the secondary attack ratios were not different for any of the three outcomes among the three arms. However, there were inevitable delays between the onset of disease in the index case and the application of the intervention. Among contacts in 154 households where the intervention was applied within 36 hours of symptom onset in the index case, there was a statistically significant reduction in laboratory-confirmed influenza in both the hand hygiene and hand hygiene plus surgical mask arms. The same was true for the

outcome of ARI but not for ILI. Adherence with hand hygiene in this study appeared to be good but adherence with mask use was much less than optimal. Further, differences in hand hygiene practice were not substantially different between control and intervention groups.

Dr. Cowling concluded by commenting that, in the Hong Kong study, hand hygiene and wearing surgical masks combined appeared to prevent secondary influenza transmission within households if implemented early. In other studies, there is some evidence to support hand hygiene and face mask effectiveness in primary prevention of ARI. However, many studies did not have laboratory outcomes and most studies had low power to detect all but large effect sizes. He then reviewed the advantages of conducting community-based studies and problems in conducting community-based studies. It is worth noting that Dr. Cowling stated that: "If we can identify which control measures are likely to work, large community-based studies are the best way to evaluate whether those interventions work in real-life settings." He asserted that one needs competing-risk style models for drawing accurate inferences about modes of transmission, e.g., one must separate the other modes of transmission so that contact is the only possible route of transmission. He noted that, in examining the question of whether face masks reduce transmission, one must consider many factors such as filtration efficacy, leakage, and the feasibility and safety of wearing masks for prolonged periods, among other factors. And efficacy in controlled settings may be quite different than effectiveness in real-world settings. These types of issues exist for other NPIs and not just masks. In summary, he concluded that community-based intervention studies, like outbreak investigations, cannot tell us the mode or modes of transmission of influenza. They can only tell us if an intervention reduces primary or secondary transmission (not the route) and we can only infer the mode or modes of transmission from those data.

Investigations of respiratory protection in health care settings. Dr. Mark Loeb, McMaster University, Canada

Dr. Loeb reviewed the possible modes of transmission of influenza that may be elucidated through human clinical trials of respiratory protection. He tried to include data on influenza A (2009) H1N1 whenever possible. He restricted his presentation to studies published in peer reviewed journals. He noted a recent

CDC report (CDC, MMWR 2010) of influenza vaccine coverage among healthcare personnel (HCP) August 2009-January 2010 which showed that only about a third had received both a seasonal influenza vaccination and a 2009 monovalent vaccination. In earlier years, seasonal vaccination for influenza in HCP was even lower (historical HCP vaccination coverage rates can be found at: http://www.cdc.gov/flu/professionals/pdf/NHIS89_08fluvaxtrendtab.pdf).

In support of what has already been said in the Background section, he reiterated the uncertainty around the use of N-95 respirators and surgical masks during the 2009 pandemic and the conflicting recommendations that were made. He then reviewed a "pyramid" of evidence that is used for decisions, ranging from in vitro research and animal research on the bottom to randomized controlled double-blind trials and systematic reviews and meta-analyses on the top.

Dr. Loeb then reviewed a randomized trial of surgical masks versus N95 respirators that he and his colleagues had done and recently published (Loeb et al, 2009). Study participants were nurses who were fit-tested and who worked full-time in the emergency department, medical units, and pediatric units in eight Ontario tertiary care hospitals. Nurses were randomized by an independent group, stratified by center, and investigators were blind to the randomization procedure and group assignment. Laboratory personnel were blinded to allocation. Nurses assigned to wear a surgical mask used a mask already in use at their hospital and were required to wear a mask when providing care to, or within one meter of, a patient with a febrile respiratory illness. For aerosol-generating procedures, nurses continued to use the device they were assigned to as long as TB was not suspected. Nurses assigned to wear an N95 respirator were fit-tested. Nurses wore gowns and gloves when entering the rooms of patients with febrile respiratory illnesses. All nurses were followed for signs and symptoms in themselves and asked if household members experienced an influenza-like illness. The primary outcome for this study was laboratory-confirmed influenza which was defined as either a) a positive RT-PCR test from a nasopharyngeal swab or flocked nasal specimen OR b) a four-fold or greater rise in serum antibodies to a circulating influenza strain using hemagglutination inhibition (HI) serologic assay. Secondary outcomes were a) the detection of other respiratory viruses by PCR, b) ILI, c) work-related absenteeism, and d) physician visits for respiratory illness.

The study was powered to have a 90% chance (two-sided type 1 error of 5%) of detecting an absolute risk reduction in the N95 respirator group of greater than -9%, the prespecified non-inferiority limit.

A 10% drop out rate was assumed. These parameters indicated that a total of 420 participants would be needed (210 in each group). A total of 478 nurses were assessed for eligibility and 32 were excluded, leaving 446 who were randomized. After losses to follow up, there were 212 nurses included in the analysis of the surgical mask arm and 210 nurses in N95 respirator arm of the study.

The results of this study are very interesting, intriguing, and perplexing. A comparison of laboratory-confirmed influenza between the surgical mask group and the N95 respirator group revealed that there was no statistically significant difference between the two groups. This was true regardless of which method of laboratory diagnosis was used. However, it is noteworthy that only 10 of the 98 laboratory-confirmed cases were diagnosed by RT-PCR. The remainder were diagnosed by serological testing. There was also no significant difference between the two groups with regard to the secondary objective of other respiratory viruses isolated; 9.4% of the surgical mask group and 10.5% of the N95 respirator group had other viruses isolated. A comparison between the two groups regarding clinical outcomes (physician visits for respiratory illness, ILI, and work-related absenteeism) revealed no statistically significant differences, but there was one finding of potential clinical significance. Nine (4.2%) of the nurses in the surgical mask group developed ILI compared to only two (1.0%) in the N-95 group. Dr. Loeb noted that the ILI findings were driven by a difference in fever incidence between the groups. Twelve of the nurses in the surgical mask group developed fever compared to two in the N95 group. The p value for this difference was 0.007. Audits and reports of household exposures revealed that participants were more likely to wear a surgical mask than an N95 respirator and that there were no statistically significant differences in the two groups with regard to exposure to a spouse, roommate, or child with ILI.

Dr. Loeb then showed results from the Cowling, et al 2010 systematic review of six studies from the literature on mask use for prevention of respiratory illness among healthcare personnel. One was an observational study, three were cross-sectional studies, and two were randomized trials, although the RCT by Jacobs et al,2009 involved only 32 participants and compared surgical masks with no masks. Only one of these studies, an observational study conducted in 1918, even suggested a protective effect of a mask – in that case a gauze mask and natural ventilation. Prior to the 2009 Loeb study, there were no comparisons of surgical masks vs. N95 respirators in healthcare settings. The remaining studies had many limitations and none showed a protective effect of surgical masks in the healthcare environment.

Subsequent to the Cowling et al systematic review, there was an observational study of HCP influenza cases at Tan Tock Seng Hospital, Singapore during the 2009 pandemic (Ang et al, 2010). HCP wore N95 respirators during the peak of local transmission and surgical masks as the pandemic receded. Anecdotally, cases in HCP rose while they wore N95 respirators and during a time inpatient cases decreased. Cases in HCP spiked again when HCP changed to surgical masks but then rapidly decreased as both inpatient and outpatient cases decreased. A recent study by Cheng et al, 2010 attempted to look at risk factors for infection during the 2009 pandemic, but there were too few infections among healthcare personnel to draw any conclusions.

Dr. Loeb also showed data from the paper by Murray et al, 2010 which demonstrated the high demand and use of facial protective equipment during the 2009 pandemic as compared to earlier periods. Peak use occurred during peak admissions for influenza and the peak period of HCP calling in sick. He also showed tables from the study by Baig et al,2010 showing the concerns HCP have about using N95 respirators and the characteristics they would desire in a new respirator. Finally, in contrast to influenza, Dr. Loeb showed the pooled estimates of case control studies on the use of masks and respirators for reducing the spread of SARS (Jefferson et al, 2009); wearing a mask was associated with a 68% reduction in risk and wearing a respirator was associated with a 91% reduction. So, it is quite clear that different respiratory viruses may require different interventions. However, none of these data can exclude any putative mode of transmission of influenza. Dr. Loeb concluded that existing evidence suggests that the surgical mask may offer similar protection to HCP against influenza as N95 respirators and that more randomized controlled trials are needed to address this issue.

Respiratory protection for influenza. Dr. Howard Cohen, Yale University

Dr. Cohen began by defining N95 respirators. NIOSH 42 CFR Part 84 defines N95 respirators. “N95” is a term often used to describe a specific type of N95 respirator, a filtering facepiece respirator with an N95 filter. This is the type of N95 respirator that is the focus of this discussion. It is designed to protect the entire respiratory tract. It requires a tight seal to the face to function properly. There are many different models on the market. In contrast, surgical or face masks are loose fitting devices worn over the nose and

mouth. Surgical masks are cleared by FDA for sale under their 510-K approval process. Masks were originally designed to protect patients and surgical areas from HCP. Both N95 respirators and surgical masks are personal protective devices in some settings, e.g., under OSHA's blood borne pathogen standard masks may provide protection to the upper respiratory tract.

There have been three published IOM studies of respirators and masks for protecting HCP from influenza and a fourth is in preparation. A 2006 IOM report dealt with the reusability of face masks during an influenza pandemic. The key findings were that 1) there is no simple way to decontaminate N95 respirators, 2) there is no simple way to eliminate fit-testing of N95 respirators, and 3) there is no documented method for decontaminating respirators. The key recommendations were to 1) avoid contaminating the surface of the respirator to extend its life, 2) conduct research on disinfection of respirators and masks, and 3) determine the routes of exposure and risks of transmission to HCP. A 2008 IOM report was entitled "Preparing for an Influenza Pandemic: PPE for Healthcare Workers. (PPE is personal protective equipment.) The key recommendations in that report were:

- Define evidence-performance requirements for PPE
- Conduct research on the design and engineering for the next generation of PPE
- Establish measures to assess and compare the effectiveness of PPE
- Strengthen pre- and Post-market testing and evaluation of PPE, and
- Expand research and resources for PPE research.

A 2009 letter report from an IOM panel requested by CDC and OSHA (Occupational Safety and Health Administration) had two basic recommendations: 1) Use fit-tested N95 respirators for the ongoing 2009 pandemic and 2) conduct research to determine the relative contribution of the mode or modes of transmission of influenza and to determine optimal personal respiratory protection. As was the case with previous IOM panels, this panel was struck with the paucity of high quality data on the mode or modes of influenza transmission.

Dr. Cohen said what we know is that N95 filters will stop 95% of the most penetrating particles (and often 97-99%) and that N95 filters will stop particles the size of virions. In contrast, masks have a wide range of filter efficiencies ranging from 0-90%. However, the protection afforded by a respirator is strongly influenced by its fit. At present, NIOSH certification does not include the quality of the fit. Thus, any

specific respirator model cannot fit all face sizes found in the workforce. A particular model may fit only 5% of the workforce or 50% of the workforce although some higher-priced models have facial seal enhancements that probably allow for fitting a higher proportion of workers. Purchasers generally cannot determine the quality of the fit of respirators before they buy them. We also know that current respirator fit test methods are cumbersome, complicated and may discourage some employers from choosing to purchase a respirator. Non-N95 respirator models that don't require fit-testing, e.g. powered air purifying respirators (PAPRs), are not designed for healthcare workers and present problems in terms of comfort, tolerability, and communication. Masks, which are somewhat more comfortable and tolerable and allow for better communication, do not protect the respiratory tract against aerosolized viruses both because of their poor seal against the face and unpredictable filtration efficacy.

What we don't know, Dr. Cohen said, is a lot. We do not know the modes of influenza transmission, including how the modes of transmission might differ depending upon the work activities. We do not know if masks and respirators might enhance protection from contact transmission. We do not know how masks and respirators might affect an infectious dose of virus, especially from aerosol transmission. We do not know if, and how well, masks provide protection from large droplet transmission. We do not know if eye protection is important for prevention of influenza transmission or may be more important during aerosol generating procedures such as during bronchoscopy, sputum induction, and elective intubation and extubation than at other times when near an influenza-infected patient. If eye protection is important, at least in some instances, can face shields provide an alternative? Would face shields in general provide an alternative and equivalent protection as respirators or masks? We do not know if the surface of respirators or masks is a source of transmission. We do not know if anti-viral coatings of either masks or respirators would provide useful protection. We do not know if effective decontamination methods can be developed. We do not know how long a mask or respirator can be safely used. We do not know if face masks (or respirators) provide protection to the patient when worn by workers. We do not know if face masks worn by patients provide protection to workers.

As his final topic, Dr. Cohen spoke about clinical studies of respirators and face masks. He noted that the data from the available published studies are not generally known by HCP who use PPE. He also noted that surveillance data on morbidity of influenza among HCP are not available. Limited data on

workers in other industries is available but has little relevance to HCP. He noted that surveillance data from any site will be in the context of whatever the "complete program" is at that site and, thus, may not be generalizable to other sites. He agreed with Dr. Loeb that clinical studies of respirators and masks cannot determine the transmission routes of influenza although such studies may be able to determine the relative effectiveness of one intervention, e.g. N95 respirators, versus another, e.g., face masks. He noted, however, that there are major challenges in doing these studies. PPE is likely to be worn only during a portion of the potential exposure period, e.g., community and household exposures will confound the studies. PPE compliance is inconsistent and has never been very high in studies to date. Also, since N95 respirators and masks come in a variety of models, the results may not be generalizable. Today, ethical considerations are likely to preclude any study that proposed a true control group, i.e., no PPE. Finally, Dr. Cohen noted that all studies to date have not been powered to detect important population differences in outcomes. This is unfortunate given the significant resources that are put into providing PPE. Despite the challenges, Dr. Cohen believes that more and better clinical studies should be done and that surveillance data in HCP should be collected.

He summarized by saying: 1) the transmission route(s) of influenza is the most important piece of information needed to determine PPE requirements, 2) clinical studies and surveillance data are needed to determine the risk of transmission to health care workers and patients and the effectiveness of PPE, 3) better respirators are needed that meet the needs of health care workers, and 4) simpler fit test methods are needed to encourage the use of respirators when they are required.

Considerations for evaluating the survival of airborne microbes and bioaerosol aging in the context of implementing engineering controls for buildings and workplaces. Dr. Mark Hernandez, University of Colorado at Boulder.

The stated goals of Dr. Hernandez's presentation were 1) to provide background and paradigm overview of inactivation or removal of airborne microbes, 2) to provide a contextual synopsis of selected bioaerosol research, and 3) to discuss emerging paradigms for full scale assessment, all in the context of bioaerosol characterization for wide area surveillance. He began by describing a paradigm being used for

assessing the performance of bioaerosol disinfection systems. In this paradigm, a bioaerosol chamber is used to receive an aerosol and allow it to equilibrate. The chamber can have various disinfection devices such as UV lights, precipitators, filters, and electric fields. It can also house animals to be exposed to a bioaerosol. The temperature and humidity in the chamber can be controlled. There is an aerosol generator device outside the chamber. Samples can be taken from the chamber at various times for analysis, e.g., microscopy, culture, PCR, etc. For example, the decay, or loss of viability of an aerosolized microbe at a given temperature and humidity can be studied in this system.

Dr. Hernandez pointed out that biopolymers take time to equilibrate with the water vapor in their surroundings and used the drying of human hair after a shower as a "teaching model" for this phenomenon. In studying issues such as what happens as bioaerosols age, he noted that physical equilibrium with chamber conditions was a critical parameter because it appears to affect survival of the microbe as judged by culturable recovery. He showed data from an article by Peccia et al, 2001 which investigated the kinetics of physical equilibrium. He described a water vapor sorption isotherm for three model organisms. (A water vapor sorption isotherm is the relationship between water content of a material and humidity at equilibrium.) In the examples shown, W, the mass of water divided by the total dry mass of cells, is plotted at various equilibrium humidities. Paccia et al. showed that water vapor sorption is physiology dependent and that different bacterial bioaerosols have different equilibrium kinetics with respect to environmental humidity and physiology. Air exchange rates would also have an effect on physical equilibrium.

Dr. Hernandez then summarized in one slide generalizations that can be concluded about the environmental behavior of airborne viruses from several studies:

1. Lipid-enveloped viruses are more stable in aerosols at low RH than at high RH
2. Viruses not enveloped by lipids are more stable at high RH than at low RH
3. Humidification in sampling devices increases the recovery of viruses that are not enveloped, and
4. When viable viruses can no longer be cultured after aerosol collection, some of their nucleic acid can be isolated and amplified but the results are highly dependent upon environmental conditions, and
5. At present, quantitation from these studies should be considered tentative.

Dr. Hernandez then went on to say that particle size distribution is another critical parameter. He raised the question if we really know where the virions are in indoor air. In searching for answers to the mode(s) of transmission and ways to mitigate them, he said one must be cautious. For example, UV irradiation of upper room air might be a way to mitigate influenza transmission and one might infer that aerosol transmission is an important mode of transmission if UV irradiation was shown to be effective. However, he showed data from Peccia et al. 2001 which demonstrates that the UV inactivation rate is highly sensitive to changes in RH so that RH needs to be taken into account in any study attempting to show an effect (or lack of effect) of UV irradiation on influenza transmission. The specific UV response for influenza viruses at various RHs is unknown.

Dr. Hernandez spoke about a case study conducted at the Mountain West Montessori School. The site was a combined grades 4,5 and,6 school with 18 students and 2 teachers. It had a dedicated HVAC system and nearly symmetric air supply and return airflow configuration with a 10% outside air “make up”. The site air flow was studied using CO_2 tracer for flood and decay tests of mixing of air. Regardless of the use or nonuse of 4 oscillating fans or the opening or closing of doors, the mean residence time of CO_2 was about 18 minutes or a little more than 3 air exchanges per hour. In the summer of 2007, they conducted a field test of enhanced filtration (using in-line electrically enhanced filtration) on indoor air quality (IAQ) parameters. These parameters included particulate matter, ozone, carbon monoxide, airborne bacteria, culturable microbes, phylogenetic patterns of bioaerosols, and potential pathogens (forensic DNA evidence). The field test showed that enhanced filtration effectively reduced all IAQ parameters. The parameters were reduced when the filtration unit was operational but rose when it was not. There was a significant drop in airborne (fine) particle number when the filtration unit was operational and a rise when it was off.

One reason for Dr. Hernandez’s presentation emphasizing engineering controls is because engineering controls, like the unit described above, are easier to implement in a workplace than PPE. However, there is the “sustainable engineering challenge” which means one must achieve high particle removal efficiency at low pressure loss within the heating, ventilation, and air conditioning (HVAC) system with high inactivation potential (*in-situ* kill rate). Electronically enhanced mechanical filtration appears to be

worth further investigation as a means of mitigating airborne microbes including viruses such as measles and influenza.

Another case study presented by Dr. Hernandez was bioaerosols in a confined hospital therapy pool area. The motivation for this study was clusters of mycobacterial lung disease in persons exposed to this pool. They focused their attention on bioaerosols since the hypothesis was that the organisms had been inspired into the lungs implicating fine particle aerosols. They placed aerosol samplers at the therapy pool and, using both membrane filter capture and liquid capture, looked for DNA to sequence. They then compared their isolated sequences to the National Science Foundation library of sequences. A report of this case study has been published (Agenent et al, 2005). There was a marked difference in the distribution of different genera of organisms identified in winter indoor air as compared to summer indoor air and both distributions were quite different from the distribution of genera in the pool water itself. Strikingly, in winter, 82% of organisms were classified as mycobacteria, the genus responsible for the lung infections. The installation of upper air UV lights was effective in reducing the number of culturable airborne bacteria (Kujundzic, et al 2005).

These study methods are not easily adaptable to viruses, however. For example, the capture and recovery of viruses is tenuous and dependent upon the type of sampler and environmental conditions, e.g., RH. Furthermore, extended collection times to increase recovery numbers can reduce collection efficiency. Although many highly infectious viruses appear to be very unstable in air, there is great variability and information about the stability of influenza viruses in air under various conditions of temperature, humidity, etc. will need to come from future studies.

Use of human challenge studies and human exposure studies to ascertain modes of influenza transmission and transmission of other respiratory viruses. Dr. Jonathan Van-Tam, University of Nottingham, UK

Dr. Van-Tam began by noting that the first successful human challenge infection with influenza virus occurred in 1936 (Smorodintseff et al, 1937). Seventy-two volunteers were challenged by inhalation of an atomized virus suspension (presumably “fine droplets”) of two H1N1 strains (Leningrad and WS) for 15-60 minutes. Volunteers had pre- and post-challenge antibody assays done and their secretions were also

used to challenge mice and therefore obtain virus recovery of the same strains. Notably, the severity of symptoms was related to pre-existing antibody titers and most viral shedding occurred within the first 48 hours after symptom onset. A review of more than 200 volunteer exposures to both influenza A (PR8, F-12, F-99) and influenza B (Lee) was reported by Henle et al in 1946. These studies were performed to evaluate the role of pre-existing immunity and early vaccines. The studies also showed that the route of inoculation influences the likelihood of fever. Fifty-eight of 65 (89%) volunteers infected by inhalation developed fever compared with 2/16 infected by nasal instillation of drops (undiluted allantoic fluid). Dr. Van-Tam stated that this suggests that exposure to particle sizes capable of deep lung deposition may be a more potent inducer of febrile illness. The typical inoculum concentration was 10^s to 10^7 mouse LD_{50}/ml. Ten boys were exposed to subjects inoculated with influenza B but there were no secondary illnesses. Another human challenge study was reported in 1966 by Alford et al. Twenty-three young male "volunteers" from correctional institutions were challenged with viral doses estimated to range from 126 to 5 $TCID_{50}$. The virus, which had been pre-passaged five times, was delivered as 1-3 μm, i.e., aerosolized, particles in 10 liters (10 breaths) of air. Seven of the 23 became infected. Asymptomatic infections occurred in two subjects. Clinical illness was more dependent on pre-exposure antibody titers than on viral dose (3 of 5 clinically ill persons received very low doses) which suggests that aerosol delivery of influenza virus can be very potent. The aerosolized infectious dose in seronegative subjects was estimated to be about 3 $TCID_{50}$. This estimate was later confirmed by Knight et al, 1979.

Little et al (1979) reported a comparison challenge of wild-type A/H3N2 strains, A/Port Chalmers/74 (21 subjects) and A/Victoria/75 (21 subjects), and experimental A/H3N2 strains, A/England/42/72 (8 subjects), A/Scotland/74 (8 subjects), and A/Victoria/75 (8 subjects). All subjects had no evidence of immunity (by HI titers) to the viruses they were challenged with. Infection was attempted via intranasal inoculation by drops at a dose of 10^4 $TCID_{50}$ and was confirmed by culture and/or serology in all cases. Of those infected by a wild type strain, 100% developed fever (>100^0 F) as compared to 67% of those infected with an experimental strain and 100% infected with a wild type strain developed cough versus 50% of those who were infected with an experimental strain ($p<0.001$). Those infected with wild type strains had evidence of lower airway disease (resistance and/or reactivity on pulmonary function tests) whereas those infected with experimental strains had either no or mild disturbances on pulmonary function testing.

Possible explanations offered for these differences were a) pre-passage of experimental virus in eggs or cell culture and b) the route of inoculation.

In 2008, Carrat et al published a review of virus shedding in experimentally-induced influenza infection. They reviewed 56 studies involving 1280 untreated participants, 1091 of whom were deemed to be susceptible. Their conclusions were that 1) viral shedding increases sharply from 12 hours post inoculation peaking on day 2, 2) symptom scores peaked on day 3, 3) the average duration of shedding was 4.8 days, 4) 67% developed clinical illness, 5) 40% develop fever (>100^0 F or 37.8^0 C), and 6) virus shedding correlated with symptoms with a lag period of 1 day.

A case report by Ison, et al 2005 has raised concerns about the potential risk of influenza complications after experimental influenza infections. In July 2000, one of 75 adult volunteers infected with influenza B/Yamagata/88 developed presumed myocarditis after experimental infection. The volunteer was reported to be "close to full recovery" at 5 months and "fully well" at 12 months and in retrospect was noted to have an abnormal ECG prior to study enrollment. A subsequent study of 30 young adults infected with wild type strains revealed that 53% had abnormal ECG findings on day 4 after infection; this had dropped to 23% by day 28. All were considered clinically insignificant findings. However, it does suggest that asymptomatic myocarditis may be a feature of influenza infection and, in Dr. Van-Tam's words, the "incident has affected the medico-legal/IRB landscape for challenge studies" and emphasized the importance of screening for pre-existing cardiac conditions prior to virus challenge.

In further discussing experimental influenza infections, Dr. Van-Tam noted that, in some respects, aerosol inoculation would be ideal because it produces a convincing symptomatic illness. Intranasal inoculation via drops produces a milder infection with a lower incidence of fever. The infectious dose for aerosol inoculation might well be about 3 $TCID_{50}$ whereas the infectious dose for nasal inoculation might well be 5-10 fold higher. Safety concerns induce wariness about future aerosol challenge studies whereas intranasal challenge appears relatively safe.

The UKCRC Influenza Transmission Research Strategy Development Group has conducted human challenge studies under controlled conditions. It is possible to achieve high attack rates in the index cases by following a number of procedures including pre-screening for immunity. Compliance with any interventions can be closely monitored and quantified. The source of transmission is known and it is

possible to obtain detailed data on transmission parameters and interventions. Environmental sampling can be added to obtain a more complete picture of the circumstances wherein transmission does or does not occur.

Although many challenge studies have been conducted, none have been conducted to evaluate transmission from one person to another. To determine if human challenge studies could be conducted to better understand influenza transmission, a "Proof of Concept Study", funded by the Department of Health in England, was recently done. There were 24 volunteers, 9 donors and 15 recipients, who were screened by HI for susceptibility to infection. Donors were experimentally infected, via intranasal droplets, with influenza A (H3N2) Wisconsin on day 0. Symptomatic donors and recipients were mixed on days 2 and 3 in 3 groups of seven. They were then monitored in quarantine for 11 days where safety assessments and laboratory tests were done to detect infection. Preliminary data from this proof of concept study finds that approximately 20% of recipients became infected after 2 days of interaction with donors. However, only 2 recipients developed symptomatic illness. All recovered without incidence. Three recipients and one donor were immune prior to the start of the experiment, which was determined retrospectively. From this pilot, Dr. Van Tam concluded that a number of alterations for future studies may be considered to optimize the study design, including use of additional pre-screening serologic tests to better ensure subjects are susceptible to the challenge strain, increasing the number of donors, and considering other environmental alterations, such as conducting the study in the colder months rather than during the summer when this study was done.

Influenza virus transmission: Lessons from antiviral studies. Dr. Frederick G. Hayden, University of Virginia, US, and Wellcome Trust, UK

Dr. Hayden began by citing information from the previously mentioned study by Henle et al. (1946) and again noted that inhalation of virus leads to a higher incidence of fever than intranasal instillation. In their paper, Henle et al speculated that "This difference between the results obtained by inhalation and intranasal instillation implies that the portal of entry for the virus of influenza may not be the nasal mucosa, but rather the lower regions of the respiratory tract." Dr. Hayden then showed several studies of the 50%

Human Infectious Dose of Influenza (HID_{50}) (Knight et al, 1965; Jao et al, 1965; Alford et al, 1966; Keitel et al, 1990; Hayden et al, 1996). The estimated HID_{50} (in $TCID_{50}$) via the aerosol challenge route from the study by Alford et al. was 0.6-3.0. Estimated HID_{50} via the intranasal challenge ranged from 40 - <80,000.

Dr. Hayden then turned to studies of antiviral agents against influenza virus infection. He showed one graph from a study he had authored (Hayden, Hall, and Douglas, 1980) which showed that aerosolized amantadine slightly reduced respiratory symptom scores in patients with naturally acquired influenza A infection. He then went on to discuss studies of interferon (IFN) in experimental and natural influenza infection. In a study by Merigan et al, published in 1973, intranasal IFN did not reduce infection or illness rates among volunteers challenged with influenza B/Hanover/1/70. However, Phillpots et al (1984) used IFN derived from a different source and a different challenge virus and they were able to demonstrate significantly decreased symptom scores on 3-5 days post-challenge with influenza A/Eng/40/83 (H3N2). A 1987 study by Treanor et al found that intranasal IFN reduced viral replication markers and there was a trend toward less illness 2-4 days post-challenge of volunteers with influenza A/California/78(H1N1). In a 1986 paper, Hayden et al found that once daily administration of intranasal IFN-alfa2b to household contacts reduced the total number of respiratory illnesses in these contacts and eliminated rhinovirus specific illness. Tannock et al (1988) found no reductions in upper respiratory tract infections, ILI or proven influenza during 4 weeks of intranasal treatment with recombinant IFN-α2a. Monto et al (1986) found recombinant IFN-α2b provided 76% efficacy against human rhinovirus infections but no protection against parainfluenza virus infections. A randomized controlled trial of 1449 military recruits was conducted in 2005 by Gao et al (2010). Recombinant IFN-2b or placebo nasal sprays were sprayed into the nostrils and throat twice daily for 5 days. Etiologic diagnoses were made by measuring IgM antibodies by ELISA on days 0 and 15. There were no reductions in respiratory symptoms with IFN and more epistaxis and dry throat in IFN recipients. However, short-term protection rates against influenza A, influenza B, and parainfluenza virus-3 were 76.4%, 76.2%, and 77.4% respectively. Dr. Hayden concluded this portion of his presentation by saying that 1) intranasal IFN is partially protective against experimental influenza at high doses, 2) intranasal IFN does not prevent natural influenza virus infection or illness in most studies, and 3) a possible protective effect of combined intranasal and pharyngeal dosing or topical application of IFN to the pharynx alone requires further study.

He then turned to studies of zanamivir. He began by showing data from one of his studies of human experimental influenza infection (Hayden et al, 1996). Volunteers were challenged with 10^5 $TCID_{50}$ of influenza A/Texas/91(H1N1). Intranasal zanamivir or placebo was administered intranasally 2-6 times a day as prophylaxis. Zanamivir was shown to reduce viral shedding from 73% to 3%, overall infection from 73% to 13% (82% efficacy), and upper respiratory illness from 61% to 26%. Calfee et al, 1999 subsequently showed that a single intranasal dose of zanamivir given 4 hours before challenge with influenza A/Texas/91 (H1N1) significantly reduced viral load and tended to reduce illness measures, e.g., fever and symptoms, and suggested that once daily dosing of zanamivir is protective against influenza virus challenge. Zanamivir's pharmacologic properties limit its utility, however. It does not penetrate cells, i.e., it has an extracellular or cell surface effect; it has low oral bioavailability; and it is rapidly eliminated in the urine (Cass et al, 1999). Therefore it is administered as a dry powder inhalation in humans. Zanamivir is detectable in nasal lavages after oral inhalation but levels are more than 50-fold lower than after nasal dosing (Calfee et al, 1999). A study by Kaiser et al. (CID, 2000), found that intranasal zanamivir is ineffective in preventing influenza illness or infection in household contacts, but inhaled zanamivir did appear to reduce the risk of infection, although the study had few influenza-positive patients. However, Monto et al (2002) found that if the index case tested positive for influenza, inhaled zanamivir did reduce symptomatic influenza in household contacts from 13% to 2% (efficacy = 80%). In another paper, Monto et al (1999) showed that seasonal prophylaxis with inhaled zanamivir at a dose of 10 mg once daily for 4 weeks, reduced laboratory confirmed clinical influenza by 67% and influenza infection with or without illness by 31%. Dr. Hayden's own study (1997) showed that inhaled, with or without intranasal, zanamivir reduced fever and symptoms when given early to patients with influenza. Unpublished data from that study was shown which indicated that inhaled plus intranasal zanamivir had a significant effect on reducing viral titers in nasal washes. A study by Kaiser et al (Arch Intern Med, 2000) showed that inhaled zanamivir alone did not reduce upper respiratory complications, such as sinusitis and otitis media, but intranasal plus inhaled zanamavir appeared to have an effect. A paper published by Halloran et al (2007) reviewed four studies of zanamivir and oseltamivir and their impact on prevention of secondary cases. Halloran et al reported zanamivir given to the index case and the contacts or given to contacts only, reduced the number of secondary illnesses. Oseltamivir, (an influenza antiviral medication administered with greater oral

availability and given as a capsule or oral solution, but in the same class of drugs as zanamivir) when given to index cases and their household contacts or given to contacts only reduced secondary illnesses. There was a high protection efficacy of post exposure prophylaxis in all four studies and for both antiviral drugs. However the estimated effect of index case treatment on reducing the infectiousness of the index case was only 19% for zanamivir and 80% for oseltamivir.

In summarizing his presentation, Dr. Hayden made the following points:

- Zanamivir is detectable in nasal washing after oral inhalation.
- Orally inhaled zanamivir treatment of natural influenza
  - does not consistently decrease nasal viral replication (Puhakka et al, 2003),
  - does not reduce upper respiratory tract complications in adults, and
  - does not importantly reduce the likelihood of secondary infections in household contacts.
- An effect of orally inhaled zanamivir on decreasing the risk of nasal acquisition of influenza remains possible.
- Intranasal IFN is partially protective against experimental influenza but does not prevent natural influenza virus infection or illness in most studies.
- Intranasal zanamivir is highly protective against experimental human influenza but not against natural influenza.
  - Antiviral prophylaxis of the nose alone does not prevent natural influenza.
- Orally inhaled zanamivir is highly protective against natural influenza illness.
  - The pharynx and/or tracheobronchial tree are key sites for virus acquisition and replication.

Influenza transmission: Insights from Modeling. Dr. Mark Nicas, University of California, Berkeley.

Dr. Nicas began by stating the following accepted facts:

1. Coughing and speaking release respiratory particles with diameters < 1µm to > 1000 µm.

2. The particles decrease in one second to one half their initial diameter due to H_2O evaporation
3. Respirable particles ("droplet nuclei") are < 10 µm
4. Inspirable particles (not respirable) are 10 µm to 100 µm
5. Respiratory particles, mostly those > 10 µm, settle onto room surfaces.
6. Secretions can be placed onto room surfaces
7. Viruses die off at different rates i) on hard surfaces, ii) on porous surfaces, iii) on the hands, and iv) in the air.
8. Fingers can touch room surfaces, and then the fingers can touch the eyes, nostrils and lips.
9. Facing (being in close contact with) a coughing or talking infector can permit large particles (> 100 µm) from the infector to strike the eyes, nostrils and lips (droplet spray).
10. Normal breathing anywhere in a room can lead to inhaling respirable particles < 10 µm.
11. Normal breathing while facing (in close contact with) a coughing/talking infector can lead to inhaling inspirable particles 10 µm to 100 µm.

Dr. Nicas stated that there are four possible infection pathways: 1) hand contact with the eyes, nostrils and lips, 2) droplet spray onto these same targets, 3) inhaling respirable (< 10 µm) particles, 4) inhaling inspirable (10 to 100 µm) particles. The relative importance of any pathway for influenza infection is not known, although some may feel certain it is known.

Dr. Nicas has developed a mathematical model to address the apportionment question, i.e., what proportion of infections can be attributed to each of the four routes. This room-scale engineering-type model has been published (Nica and Jones, 2009). The model considers the mechanics of exposure to virus in environmental media (in a simulated room) over a single 15-minute period while attending to an infector (an ill, infectious person). This model is similar to a model published by Atkinson and Wein (2008). One benefit of mathematical modeling is the systematic organization of diverse information. In addition, the numerical values of nearly all the inputs (what epidemiologists might term "risk factors") are fairly uncertain but sensitivity analysis can show which inputs have the greatest impact. These latter inputs (risk factors) deserve priority for funded research activities. First-order rates are assigned to the various transfer steps leading to (i) hand contact with the face and (ii) inhaling respirable particles containing virus. Exposure

episodes involving close-contact events, such as droplet spray and breathing inspirable particles, are modeled separately. The parameters in the model and the equations are described further in Dr. Nicas' paper. There are four infection risk estimates (R_1, R_2, R_3, and R_4), one for each of the four pathways described above. Nicas and Jones found that the most important factors for the magnitude and apportionment of risk were:

1) the virus concentration in the cough fluid,

2) α value (virus infectivity) for the pathways, and

3) the ε value, the fraction of virus deposited in the eyes, on lips, and on nostrils, that reach receptors capable of resulting in an infection.

Data from the 1960s suggest that α for the lower respiratory tract is 0.18 versus 0.000057 for the upper respiratory tract (Nicas, 2009; Alford, et al 1966). If ε is set at 1 and the preceding values for α are used in the model, then droplet spray plays a major role in transmission at virus concentrations of 10^4 – 10^6 per ml. However, if the value of ε=1 and the value of α is set at 0.18 for both the upper and lower respiratory tracts, then the hands play a major role in transmission. Risk apportionment is affected by the value of ε. For example, given an α=0.18 for the upper and lower respiratory tracts and a virus concentration of 10^6 per ml, the proportion of transmission attributable to the hands decreases from 93% at ε=1 to 79% at ε=0.1 while the proportion attributable to inspirable particles increases from 3.3% to 13%. If α is set at 0.000057 for the upper respiratory tract and 0.18 for the lower respiratory tract, at a concentration of virus of 10^6 and an ε=1, the hands contribute 31%, respirable particles 17%, and droplet spray 52%. Changing the value of ε to 0.1, the hands contribute 12%, respirable particles 62%, and droplet spray 25%. For ε=0.01, respirable virus contributes over 90% of the infection risk. In a new uncertainty analysis, uncertainty distributions were constructed for all of the input factors (Jones RM, 2010, In submission to Risk Analysis). For each factor, the risk outcomes were compared when the 10^{th} percentile versus the 90^{th} percentile value of the risk factor was used, with all of the other factors set equal to their median values. The factors with the greatest impact were:

1) virus concentration in cough fluid,

2) fluid volume per cough,

3) cough frequency,

4) the α value,

5) the ε value, and

6) the hand-to-face contact rate.

Another recent uncertainty analysis **(**Spicknall et al, 2010) used a model with interactions in a space represented by a lattice structure with multiple susceptible persons sharing the space with the infector. Uncertainty distributions were constructed for 19 parameters. Ten thousand unique parameter sets were created via Latin hypercube sampling. Pathway-specific R_0 values were obtained via 500 simulations for each parameter set. R_0 >1.7 was operationally defined as high transmission because it was estimated to be the same R_0 as in the 1918 pandemic. The ε value was constrained to the range 0.05 to 0.25. A little more than one-half of the parameter sets resulted in high transmission. Among these:

- 3,039 sets were due to hand contact only,
- 121 were due to respirable particles only,
- 66 were due to droplet spray only, and
- none were due to inspirable particles only.

There was overlap such that two or more exposure routes in a set each led to high transmission.
The factors with the greatest impact were:

- the density of susceptible persons in the space
- virus emission (shedding) rate
- the α value
- the hand-to-face contact rate.

In another study, Drs. Papinchak and Nicas (2010, manuscript in preparation) investigated the fraction of cough particle volume that deposits via droplet spray onto the eyes, nostrils and lips at distances of 1, 2, and 3 feet from the coughing subject. There was great variability among 20 subjects in the cough particle volume striking the face targets (most values were zero) and a small average decrease with increased distance.

Dr. Nicas concluded by suggesting research at three levels. Level 1 are those that he feels are relatively easy and doable now: 1) characterize the distribution of cough fluid volumes, 2) characterize the distribution of virus concentrations in cough and nasal fluids, and 3) characterize the fraction of virus

emitted in a face-to-face cough that deposits on the eyes, lips and nostrils. Level 2 is not so easy but doable now: 1) characterize the die-off rates of virus on different surfaces at different environmental conditions, 2) characterize the virus transfer efficiencies from the fingers to different surfaces and from these same surfaces to the fingers, and 3) characterize the virus numbers in different cough particle size fractions. Level 3 research is difficult: 1) characterize the α values (virus infectivity) via different exposure pathways and 2) characterize the ε values for virus deposited in the eyes, on the nostrils and on the lips.

Scientific Proceedings on Friday November 5, 2010

The day began with presentations of three recent studies which are relevant to the workshop topic. Two of the three were conducted in Thailand and were presented by Dr. Sonja Olsen. The third study was conducted in Egypt and was presented by Dr. Maha Talaat.

Findings from a randomized controlled trial of nonpharmaceutical interventions to reduce household influenza transmission: The Bangkok "HITS" study. Dr. Sonja Olsen, CDC, Thailand.

Dr. Olsen presented findings from a study among Thai households with an influenza-infected child where the households were randomized to either hand washing intervention, or hand washing plus masks, or smoking cessation education. The objectives of this study were 1) to provide data on the effectiveness of nonpharmaceutical interventions (NPI) in reducing household transmission of influenza, 2) to understand the incidence of asymptomatic secondary influenza infections, and 3) to describe the duration and intensity of viral shedding in index case children. This was a household randomized controlled trial in which households from metropolitan Bangkok, Thailand, were randomized in a ratio of 1:1:1 to 1) a control arms of health education, 2) hand washing education, or 3) hand washing and face mask use. Inclusion criteria including children 1 month to 15 years, illness onset <48 hours, not treated with antiviral medications, not at high risk of severe influenza complications, and rapid influenza test positive. Household member (HHM) inclusion criteria included having at least 2 other HHM, and no other HHM either vaccinated against influenza or with ILI in the previous 7 days. Households were followed with home visits on day 0/1, 3, 7, and 21 with respiratory and serum samples collected on all HHM. Observations included hand washing and

mask use behavior and specimens included nasal and throat swabs and blood. Diagnostic testing included rapid (QuickView) testing, rRT-PCR, quantitative RT-PCR, and hemagglutination inhibition (HI) serologies. Of 1543 children with influenza positive rapid tests, 642 were successfully enrolled and randomized. The control arm had 208 available for follow up and the corresponding numbers for the hand washing and hand washing plus mask arm were 205 and 203, respectively. The study was conducted over 3 influenza seasons (2008 through 2010) which included the 2009 pandemic. Forty-six percent of the children were 0-5 years old, 40% were 6-10 years old, and 14% 11-15 years old. Most households (68%) contained 3-4 people and 64% of households had no other children besides the index case. Ninety-two percent of children slept in the same room as their parents. At day 7, the number of reported times that HHM washed their hands per day was statistically significantly higher only in the hand washing plus face mask group relative to the education-only control group (4.8 times per day vs. 4.3 times per day in the control arm). The amount of liquid soap used did not differ statistically between the two intervention arms. Mask users averaged 11 masks per week. The secondary attack ratio (SAR) among all households on day 3 and 7 was 38% and the secondary attack ratio among all household *members* on those days was (21%). The median age of secondary cases was 34 years. In an analysis of households where the intervention(s) occurred with 48 hours of symptom onset, an intent-to-treat analysis revealed no statistically significant differences between the three groups either at the household level or at the individual level. Conclusions of this study were: 1) the interventions do not appear protective, 2) the power was diminished by the high proportion of households with secondary infections on day 1, 3) >90% of children sleep with their parents, and 4) the study was complicated by the 2009 H1N1 pandemic where general mask use and hand washing frequency may have increased across all study arm households.

Influenza virus contamination of common household surfaces during the 2009 influenza A (H1N1) pandemic in Bangkok, Thailand: Implications for contact transmission. Presented by Dr. Sonja Olsen, CDC, Thailand

This study has recently been published in Clinical Infectious Diseases (Simmerman et al, 2010). The major objective was to measure the prevalence of influenza virus contamination on common household surfaces and determine the effect of an intensive hand-washing intervention. Since study methods, results

and conclusions are published and readily available, this will be a brief summary. The study design was a cross-sectional, nested within a household-randomized controlled nonpharmaceutical intervention trial conducted in Bangkok in July and August of 2009 and is a substudy of the study described earlier by Dr. Olsen. For this substudy, two arms were included: 1) a control, health education arm and 2) a hand washing education arm. The study population included 90 Bangkok households with an influenza-positive child, inclusion criteria of which are reported earlier. The analysis was surface contamination in households from the two arms. On day 3 visits by study staff to participating homes, staff swabbed six different surfaces common to all households. In addition, finger pads of the dominant hand of the pediatric index case and any other household member with ILI symptoms were swabbed. The dew point and indoor air temperature were also measured. Sixteen households (18%) had at least one positive surface. The most common RT-PCR positive surfaces were the TV remote control and a frequently used plastic toy. Specimens were tested for influenza by RT-PCR and, to assess for viable influenza virus, they were also tested by viral culture. Households in the control arm were 2.2 times more likely to have an RT-PCR positive surface swabs than those in the hand washing arm, although this was not statistically significant. Households where the age of the index case was less than the median age of all index cases (8 years) were 4.05 times more likely to have a RT-PCR positive surface swab (CI 0.98-16.73, p=0.027). Households with secondary cases were more likely to have an RT-PCR positive surface swab (p=0.22); these households also had a lower absolute humidity (humidity values and p value for comparison not noted). However, there was no significant difference in rates of secondary household influenza infections between the two intervention arms. And, only one swab specimen, a specimen used to swab the finger of an index case, was positive for influenza by viral culture.

Impact of intensive hand hygiene campaigns on the incidence of laboratory-confirmed influenza and absenteeism in schoolchildren in Cairo: A randomized controlled trial. Dr. Maha Talaat, US Naval Research Unit, No. 3, Cairo, Egypt.

Dr. Talaat presented information from an published study conducted in Egypt. However, a manuscript on this study is in press as of January 2011. Dr. Talaat began by reminding the audience of the

tremendous world-wide health impact of acute respiratory infections (ARIs) and diarrheal diseases, with the majority of morbidity and mortality from these diseases occurring in the developing world. Hand hygiene can potentially play a role in reducing the toll of such diseases. Some studies have shown an impact of hand hygiene on ARIs and diarrheal diseases. However, changing such behaviors as hand washing is difficult and community-based intervention programs to promote hand hygiene are expensive and labor intensive. Still, school-based programs have been successful in many countries. The objectives of this study were to measure the effectiveness of an intensive hand hygiene intervention campaign on reducing: 1) incidence of absenteeism due to overall illness and illness due to ILI, diarrhea, and conjunctivitis, and 2) the incidence of laboratory- confirmed influenza. This was a randomized controlled trial in Cairo governorate public schools. Schools located in Cairo governorate were chosen based on the continuous availability of water in school settings. The majority of schools in Cairo have one large restroom with approximately ten units and sinks on the ground floor of the school. Additional sinks are always present in the playground area (8-10) to facilitate drinking and hand washing for students. No sinks are available in the classrooms.

A hand hygiene team was developed in each intervention school and they received intensive training. Six independent social workers were used to monitor the schools each week, check on data collection of the nurse and teacher teams, and inspect soap availability. The campaign was kicked off by a large communication campaign, with instructions on hand washing, making soap and towels available, posters, fliers, special song, drawing contest, plays, etc. The new behaviors were enforced at least weekly with songs, games, contests, etc.

Sixty elementary schools (30 intervention and 30 control) were randomly selected from a numbered list of 725 schools in the Cairo Governorate using a random number table. All children at the intervention schools were included in the general hand hygiene campaign activities, but absenteeism and illness data were only collected from children in the first three primary grades. The 60 schools were randomly assigned to either an intervention group that received an intensive hand hygiene promotional campaign or standard practice control group.

Data on absenteeism for illness were collected from schools for children in the first three primary grades (children about 6-10 years old). The ILI case definition was current fever or history of fever and

cough or sore throat. Swabs were obtained and a QuickVue rapid influenza test done for children who came to school with ILI. Parents of children who were absent were contacted and children with ILI were swabbed and the specimen was tested for influenza. Diarrhea was defined as loose or watery stools for more than three times a day. A student episode of absence due to illness was a student who was absent due to illness for any number of consecutive or non-consecutive days during a single calendar week with symptoms affecting the same organ system. The control and intervention schools were similar in their characteristics by age, gender and number of students. Over a 12 week period, the incidence of absence due to any illness, ILI, diarrhea, and conjunctivitis were lower in the intervention schools than in the control schools (reductions of 21%, 40%, 33% and 67%, respectively). These were all statistically significant at a $p<0.0001$ level. In addition, the intervention schools also had significantly fewer cases of laboratory confirmed influenza. Although encouraging, the study has several limitations including:

- study teams, schoolchildren, and parents were not blinded to the intervention;
- absence incidence may have been overestimated;
- the short duration of observation (12 weeks) may have led to an overestimation of the effect as participants may be more likely to adhere to new hand hygiene behaviors over a shorter period of time;
- the use of a rapid test for influenza (with 60% sensitivity) may have resulted in an underestimation of influenza-related illness in each group; and,
- the campaign was not started until the tail end of the winter season. and thus many influenza cases for the season may have been missed.

Dr. Talaat's conclusions were that 1) the intensive hand hygiene activities significantly reduced absenteeism due to any illness, ILI, diarrheal diseases, conjunctivitis, and laboratory-confirmed influenza, 2) the campaign was well-received by school principals, teachers, and students and their families, and 3) the campaign was difficult to sustain due to its expense, intensive labor requirements, and obtaining a continuous soap supply. She believes that alternate methods need to be developed which would be easy to implement and which will ensure the availability of soap and water.

Breakout Sessions

After these presentations, the participants were divided into three breakout sessions, organized according to mode of transmission [contact (which included direct and indirect contact), droplet spray, and aerosol transmission]. Each group was asked to discuss key questions/gaps in knowledge about that mode of transmission; each group was given a list of issues to consider; and each group was asked what study designs would be best for understanding the contribution of the particular mode of transmission assigned to their group, along with the pros and cons of different approaches. Each breakout group deliberated on these issues for 2 hours and 15 minutes in the morning. In the afternoon, each group presented a summary of its deliberations.

Prior to breaking into 3 groups, a discussion was led by Dr. David Weissman regarding what definitions of the 3 modes of transmission would be used for purposes of the discussions. Earlier in the meeting, concerns were expressed that a lack of common, clearly defined terminology that was well understood by participants from a wide range of disciplines was a barrier to discussing and making progress in understanding modes of transmission of influenza. Therefore, during the morning session, the entire group of participants discussed how modes of transmission should be defined for discussion during the breakout sessions. An important aspect of the discussion was dissatisfaction with the conventional terms “droplet transmission” and “airborne transmission.” As usually defined, these terms leave a gap on one hand between very large ballistic droplets generated by actions such as coughing that transmit disease by impacting on mucous membranes (“droplet transmission”) and on the other hand small respirable [< 10 micron aerodynamic equivalent diameter (AED)] particles capable of being inhaled into the terminal bronchioles and alveolar spaces of the lung (“airborne transmission”). Within the gap between these two extremes are particles < 100 microns AED and > 10 microns AED. Although they remain suspended in air for shorter periods of time than smaller particles in the respirable size range, these particles can be inhaled into the nose and mouth and those particles that are < 25 microns AED can even enter into the conducting airways of the lung. Also, interventions targeted toward eliminating aerosols, such as ventilation/filtration and upper air UV germicidal irradiation, would likely have far the greater impact on transmission mediated by particles in this size range than on transmission mediated by very large ballistic droplet sprays. In

industrial hygiene and aerosol science, the standard term used to describe particles less than 100 microns AED that can be inhaled into the nose or mouth is “inhalable.”

Based on these discussions, the group chose not to use the terms “droplet transmission” or “airborne transmission” in the breakout groups. Rather, the breakout groups addressed *contact transmission*, *droplet spray transmission*, and *aerosol transmission*. Definitions for each of these modes of transmission for purposes of break out discussions were as follows:

Contact transmission: There are two subgroups of contact transmission: direct and indirect. Direct transmission occurs when influenza is transferred by contact from an infected person to another person without a contaminated intermediate object. Indirect transmission involves the transfer of influenza by contact with a contaminated intermediate object. In both, contaminated hands play an important role in carrying influenza to mucous membranes.

Droplet spray transmission: person-to-person transmission of influenza through the air by droplet sprays. A key feature is deposition by impaction on exposed mucous membranes.

Aerosol transmission: person-to-person transmission of influenza through the air by aerosols in the inspirable (inhalable) size range or smaller. Particles are small enough to be inhaled into the oronasopharynx and distally into the trachea and lung.

Based on these definitions, the first breakout group was called “Best Methods for Studying Contact Transmission”, the second was called, “Best Methods for Studying Droplet Spray Transmission” and the third was called, “Best Methods for Studying Aerosol Transmission”. Results of the breakout group discussions are discussed below.

The Contact Transmission Breakout Group (Group 1) report was led by moderator Dr. Toby Merlin, CDC. Guidance materials provided to Group 1 at the start of the breakout session listed the following specific gaps as items for consideration:

- Level of documentation of influenza transmission via contact transmission
- Relative importance for transmission of influenza
- Dose-infection relationships for, and relative importance of, potential target mucosal surfaces
- Typical levels of surface contamination, how clean is clean enough to reduce spread via this route

- Impact of environmental factors (temperature, humidity, ambient UV, etc) on influenza spread among humans via this route
- Role of ventilation/air exchange via this route
- Interventions or exposures that may isolate the role of droplets in the spread of influenza

The remaining key questions and gaps noted by group participants included methodologic issues, particularly the use of RT-PCR identification and quantification versus culture to determine viability of viruses versus human infectivity. Other gaps included the lack of evidence for a contribution of contact transmission of human influenza, including information on the infectious dose for contact transmission and considering the impact of an individual's underlying immunity, information on the survival or viability of influenza viruses when on various surfaces, especially the hands, and what the relative roles were of the mucosal membranes of the eyes, lips, nose and/or mouth when exposed to influenza viruses. The group also mentioned the limited information on the environmental microbiology of influenza viruses, including the level of contamination of various surfaces and the contribution of humidity, temperature, ultraviolet light, and matrix on virus survival. They discussed the following study designs that may help to close these gaps and study design pros and cons:

1) Survival of virus on surfaces, especially hands, as a function of temperature, humidity, matrix, UV
    - Pros: Basic environmental microbiology needed to inform human studies
    - Cons: Does not prove role of contact transmission in humans
2) Human inoculation experiments, focusing on roles of lips, nose, mouth as entry points
    - Pros: Addresses major gaps
    - Cons: No demonstrated role in natural infection. Ethical concerns. Confounded by differences in strain, concentration, matrix. Expensive.
    - Suggestion: Do first in ferrets, especially eye inoculation
3) Determine environmental burden of influenza – laboratory and field studies
    - Pros: Informs human studies and models
    - Cons: Does not prove contact transmission
4) Human challenge studies

- Pros: Can selectively block different modes of transmission of human infections, can study interventions, able to control for confounders better than field studies
- Cons: Expensive, ethical issues, limited virus strains, not natural infections
- Suggestion: Use naturally infected donors, but logistically very problematic

5) Hand washing studies

- Pros: Best way of evaluating value of intervention

Cons: Does not effectively address role of contact transmission. Lots of confounders. Lots of conflicting results. Lack of positive result does not discard contact transmission.

Discussions for Breakout Group 2, droplet spray, were moderated by Dr. Carolyn Bridges and the summary of the discussions were presented to the workshop attendees by Drs. Andy Plummer and Lisa Grohskopf, both from CDC. Guidance materials provided to Group 2 at the start of the breakout session listed the following specific gaps as items for consideration:

- Level of documentation of transmission by large droplets; isolation of droplet transmission from transmission by droplet nuclei/aerosols
- Relative importance for transmission of influenza
- Typical numbers and levels of virus in droplets
- Dose-infection relationships for this route of transmission relative to other routes
- Relative importance of potential target mucosal surfaces via droplet transmission
- Distance over which droplet transmission may occur
- Impact of environmental factors (temperature, humidity, ambient UV, etc) on influenza spread among humans via this route
- Host factors that may increase or decrease transmission by droplet spread
- Interventions or exposures that isolated the role of large droplets in transmission

This group identified the following remaining key questions/gaps pertaining to droplet spray:

1) There are no data discriminating the relative contribution of droplet spray compared to other potential modes of transmission.
    - Use of masks versus respirators cannot distinguish between these modes of transmission because:
        - The conjunctiva are exposed, which may or may not be a route of infection
        - Masks differ widely in their ability to filter some smaller particles
        - People touch their faces many times in an hour and it is very difficult to exclude indirect contact transmission in non-experimental models or studies
2) The experimental models that mimic human generation of influenza droplets and aerosols may not be valid in natural infection
    - Methods that generate the propulsion of fluids/aerosols may a) alter the viability of the virus, b) not be designed to capture particles of all sizes, esp. large sizes, and/or c) use an artificial matrix or have missing cofactors that alter the viability of virus or the size distribution of particles.
3) The size distribution of infectious particles in natural infection is not well characterized.
    - The size distribution of these particles needs to be determined for various states, e.g. tidal breathing, talking, coughing.
    - More information is needed on person-to-person variability in particle size distribution.
4) There is a need to characterize the deposition of droplet spray on different sites of infection and the infectious dose by site of deposition
    - What sites of deposition of droplets (eyes, nose, lips, etc.) will most likely result in infection, i.e., what is the efficiency of infection by the site of deposition?
    - During droplet spray exposure, what proportion of droplets is distributed at different sites of the body that might lead to human infection?
    - If these data were available, they could be incorporated into one of the existing models which would then inform policy and further research.

5) There is need for a better understanding of the correlation between viral copies, as measured by RT-PCR, compared to live virus and how this relates to potential infectivity.
6) There is a need for a better understanding of the infectious viral dose by route of infection. In the published literature, human experimental models did not examine more than one route of transmission in the same experiment. The relatively low secondary attack rates and R_0 reported in the literature from outbreak investigations and community studies are inconsistent with the reports of a very small infectious dose by the aerosol route. This potential inconsistency needs to be reconciled.
7) The variability in viral shedding among individuals and the pre-existing cross-reactive immunity within households and populations makes it difficult to assess and control for these factors in population-based studies.

The droplet spray breakout group (Group 2) listed the following study designs as potential ways in which existing knowledge gaps could be addressed with their pros and cons:

1) Considerations for studying the relative contribution of droplet spray are:
    - Because spray implies a ballistic event, issues of temperature and humidity in the environment should be less of an issue than with other modes of transmission. However, humidity and temperature effects on the host may affect susceptibility by this route and others.
    - Study of droplet spray may be less complicated compared to aerosol because the virus from droplets is expected to be outside of the infected person for less than a second before it reaches the recipient.
2) Studies which assess the distribution of influenza virus in droplet sprays.
    - The infected person preferentially should be naturally infected. The person could then cough or sneeze toward an artificial target such as a mannequin, splash plates, or growth media. The distribution of virus on a mannequin could be assessed at various sites that may lead to infection, e.g., lips, conjunctiva, mouth, and nasal mucosa.

3) Human experimental studies which eliminate different modes of transmission. The risk of human to human transmission could be assessed by eliminating droplet spray, e.g., using a face shield to protect the eyes, nose, and mouth, using UV light in the room where the exposure occurs, and using a crosswind between the infected and exposed persons to eliminate the aerosol route.

4) Community studies of influenza transmission in which face shields, instead of face masks, are used for the intervention group compared to a control group. A prospective design would help eliminate any delay in the initiation the intervention and use of a face shield would prevent only droplet spray and would not prevent aerosol spread. It could, however, reduce face touching which may reduce indirect contact transmission.

5) Any experimental comparison of the relative efficiency of transmission by deposition on different parts of the face may not provide a good comparison to natural infection.

6) A non-experimental study design was discussed in which the index cases are naturally-infected hospitalized patients. Persons then exposed to the index case would be followed to assess the amount of time spent interacting with the index case, the amount of face-to-face time spent with the case, and the types of activities. The exposed persons would then be followed for development of any illness and specimens collected for detailed molecular assessment of influenza viruses identified. This would require intensive monitoring of staff and their activities, their use of PPE, and the amount of time spent with index cases (particularly face-to-face time that may have allowed for droplet spray). Genetic characterization of the viruses would be necessary to validate whether the virus from the index case was the likely source of infection for contacts.

7) Observation studies of healthcare workers was suggested to determine the amount of time they are in face-to-face contact with an index patient and where they may be likely exposed to droplet sprays through patient coughing, talking or sneezing.

8) The identified pros and cons of these various approaches included that:

    - A series of studies is likely needed to estimate the probability of exposure to droplet sprays and the types of exposures needed to cause infection.

- The cost of these studies would be high, especially for human experimental infection studies.
- There are ethical concerns for human exposure or inoculation studies.
- There may be variability in results due to differences between viruses, hosts (e.g. immunity), and routes of infection.
- There may be variability in the models and air samplers used in terms of their ability to capture different sizes of particles, particularly large particles, and the ability to detect live virus.
- The sample sizes required for human studies may be very high.
- There may be difficulty in generalizing study results.
- Compliance with interventions is a challenge as is the difficulty of controlling for multiple exposures in a community setting.

The aerosol transmission breakout group (Group 3) summary was led by the group discussion moderator, Dr. David Weissman, CDC**.** This group had the largest attendance and interest among workshop participants with the greatest amount of discussion and opinions expressed. Given the level of interest and intense discussion, the report from this breakout group is more detailed than the other two groups where discussion was less vigorous in comparison.

Guidance materials provided to Group 3 at the start of the breakout session listed the following specific gaps as items for consideration.

- Level of documentation of influenza transmission via small particle aerosol transmission.
- Influenza virus infectious dose for this route of transmission relative to other routes.
- Impact of environmental factors (temperature, humidity, ambient UV, etc.) on influenza airborne transmission.
- Duration of infectivity from aerosolized infectious particles.
- Evidence on distance(s) over which transmission by this route may occur
- Host factors that may increase/decrease transmission risk by aerosols
- How might changes in air exchange rates/ ventilation mitigate transmission via this route?

Group participants expressed that all of the above were important and that aerosol transmission as defined earlier for the purposes of the breakout group discussion played a role in human transmission of influenza. Details of discussions regarding the above list of considerations are described below. The key unanswered question was the contribution of aerosol transmission relative to the contact and droplet spray routes.

An interesting related question posed was whether there is a difference in disease induced by large droplets that deposit in the oronasopharynx vs. small droplets that deposit in the lung. The discussion definition of aerosol transmission includes a range of particle sizes that could deposit in either location. However, there is reason to believe that different size particles might cause different patterns of disease. Data from the 1940s suggests that inhaling an influenza aerosol into the lung is more likely to cause fever than instillation of influenza virus into the nose (Henle et al, 1946).

Group 3 reported that all the gaps noted were critical questions and had great practical importance. For example, without knowledge of typical exposure levels and infectious doses, normal approaches to recommendation of levels of respiratory protection (such as are used for chemical exposures) cannot be used for infectious agents, including influenza. Persistence of viability and infectivity in aerosols has very important implications for engineering controls such as HVAC system implementation.

An important issue for all of these questions is the need to improve methods for exposure assessment. Improved methodology is needed for detecting aerosolized influenza and measuring its viability and infectivity. Improved methodologies for exposure assessment are especially needed to assess dose-infection relationships for airborne influenza. Improved methods would also help researchers perform experiments to better understand factors that influence retention of viability and infectivity of airborne influenza virus. Improved exposure assessment would also help in documenting the impact of engineering controls used in intervention studies to understand modes of transmission.

There was some discussion about reasons for apparent differences between transmission of TB (long distances) and transmission by influenza (short distances). It was suggested that because TB is rare and has a low incidence in North America and Western Europe, it is easy to tell when disease is transmitted over a short or long distance. In case of influenza, which is often widespread in the community, it is often

hard to tell if transmission has occurred over a short or long distance. The infant study of Hall et al (2007) was suggested as an example of difficulty in perceiving influenza transmission over longer distances. Another such example might be an outbreak reported on a U.S. Navy ship (Earhart et al, 2001).

That being said, there are clearly major differences between the pathogens. It was noted that TB has a waxy coat and is hardier than influenza and the infectious dose is much smaller for TB. One comment was that it was not the best approach to compare viruses with TB; it would be better to compare influenza with measles or varicella if a comparison were to be made.

Host factors were felt to be a very important issue that should not be ignored. It was noted that some of the environmental factors that could affect influenza virus such as temperature and humidity might also affect hosts. For example, cold, dry air can trigger asthma attacks. The issue of how sources generate respiratory tract-derived aerosols is very important. Is the source of particles the upper or lower airway? Understanding and improving the ability to identify super-spreaders is also very important.

There was a fair amount of discussion about the potential role of air exchange rates/ventilation and mitigation of transmission via aerosols. The study presented earlier in the meeting that used computational fluid dynamics (CFD) to explain the pattern of secondary cases following noninvasive positive pressure ventilation of a COPD patient with influenza was mentioned as evidence that HVAC system characteristics could, under some circumstances, affect transmission (Wong et al, 2010).

Related to this discussion, it was noted that evaluating the impact of airflow was an important need. In the study noted above, directional airflows induced by the ventilation system from the infected patient into other rooms appeared to be an important issue. Impact of airflow directionality is related to another issue needing investigation, how long that influenza virus remains viable in aerosols. It was noted that after coughing in a room, cough–derived aerosol particles will distribute in minutes. Distribution is dependent on factors such as ventilation rate, direction, and how air is exhausted and returned. Computational fluid dynamic (CFD) modeling of airflow sometimes shows unexpected results. For example, even in negative pressure isolation rooms, sometimes based on high airflow and the location of the air supply and return, modeling shows contaminated airflow right into workers' breathing zones. In contrast, CFD modeling of ventilation in a regular patient room might sometimes document less worker exposure based on lower flow and the location of the air supply and return. Thus, simply supplying large volumes of airflow is not enough

– directionality, including exhaust ventilation for source control to limit the spread of contamination in a room is better than dilution alone. Question: Do optimally designed HVAC ventilation systems that not only provide dilution, but also provide directionality and filtration, decrease transmission?

The group then discussed "What study designs would be best for understanding the contribution of aerosol transmission relative to the other transmission routes? What are the pro's/con's?"

General Issues

As before, it was noted that definitions used in these studies must be clear. It is also important to have appropriate partnerships – for example, USAMRIID and the biodefense community. The need for multidisciplinary approaches was also noted.

In addition, it was noted that differentiation between aerosol and droplet spray transmission at close distances will be very challenging. At 1 or 2 meter proximity, exposures are to a large size range of droplets. Thus, it will be hard in this situation to tease out the relative roles of the two modes of transmission. There may be no single type of study that will be fully informative. Epidemiology alone cannot differentiate between short-distance aerosol and droplet spray transmission.

For purposes of organizing the discussion, approaches were categorized as animal research studies; human challenge studies; and intervention studies evaluating exposure and transmission in "real world" settings.

Animal Research Studies

Many participants expressed that animal models were useful for clarifying modes of transmission. However, a key issue is validation of relevance of animal studies to human influenza transmission. It is very important to validate animal models because we don't really understand how animal models relate to human disease. One participant expressed the opinion that animal studies will never convince the clinical community to change practice.

Ideally, animals with influenza used as infectors in animal models would produce respiratory aerosols similar to those produced by humans. Thus, animal studies that characterize the respiratory

aerosols generated by animals and compare them with the respiratory aerosols generated by humans would be important for validation of relevance to human transmission.

An important advantage of animal models is that it is really possible to control the study conditions. For example, there is the potential for great control over manipulations, such as air disinfection of ducts between animals. Thus, animal studies are good for studying the impacts of various interventions that are able to be applied. It was also suggested that animal models could be adapted to simulate clinical procedures in an effort to better understand the biology of aerosol-generating procedures. Some participants saw this as an especially high priority area for investigation. It was noted that animals could potentially be used as an *in vivo* system for detecting low amounts of airborne virus.

It was suggested that animal models should be refined to mimic normal transmission. The dose of influenza virus used to infect animals was noted to be important. For example, it was stated that when H5N1 was used as the pathogen and animals were infected with low doses, animals took longer to get sick, but ultimately most died. In contrast, when infected with higher doses of H5N1, animals get sick right away but some survive, perhaps due to more rapid clearance of influenza viruses that may be reflected by "cytokine storm"-like immune response.

It was also suggested that it was important to use low-passage virus that retained the genotype and phenotype found in patients instead of plaque-purifying a particular virus, because changes in the pathogen might lead to results that didn't reflect what occurs in nature. In addition, some suggested that studies should include broad representation of influenza viral strains. For example, some strains cause diarrhea and some don't, which could affect the contribution of contact transmission for a particular strain.

Some participants expressed that experimental conditions in animal studies could be established to differentiate between droplet spray and aerosol transmission. For example, in the biodefense community setting cages very far apart in a biobubble and at different heights can be a useful design overcoming the problem of differentiating between droplet spray and aerosol transmission associated with close cage placement. It was note that having a large area available is helpful in many experimental designs. Another participant noted that studying infector and recipient animals placed at varying distances apart, with varying levels of ventilation could really help to understand the impact of particle size.

Another participant also commented that it should be possible to design studies with guinea pigs or ferrets next to each other that were informative about routes of transmission. But it was important to be aware of the potential impact of coprophagy and urophagy resulting in fecal and oral transmission. It was noted that in animal H1N1 studies, 50% of fecal and urine samples were positive for influenza.

It was noted that guinea pigs don't get symptomatic and may not generate the same volume/intensity of exposure as other models (such as ferrets). In addition, ferrets don't cough – they sneeze. A participant noted that this is a difference from humans, where sneezing is often not a prominent symptom of influenza, even though that is asserted on most of the influenza guidance documents. We need to know how respiratory droplets are produced and how we translate animal data to humans

Human Challenge Studies Under Well-Controlled Conditions

One participant suggested that human challenge studies will be convincing to the clinical community and may be the only way to accomplish our goals. Another participant suggested that, in a way, human challenge studies are really just another type of animal model. Similar to animal models is the need to validate that human experimental influenza challenge studies are comparable to natural influenza. The type of virus, type of inoculation, and type and immune status of the host can all have important effects on results. Just as there is a need to validate that ferrets or guinea pigs generate aerosols similar to humans with natural infection, there is a need to validate that viral particles produced in human challenge models are similar to those in natural infection. Another participant suggested that these types of studies need to be viewed as hypothesis-generating, since they may or may not reflect natural conditions. It was noted that experimental infection in volunteers after high-dose nasal infection with egg-grown virus may cause a range of symptoms from a cold to a cough with some systemic symptoms. This participant also emphasized the need to really understand the types of particles generated in challenge studies. Both host factors and viral factors need to be considered. Not all influenza viruses have same level of transmissibility. Not all hosts are the same – if a group has the same exposure, not everyone has the same reaction. There are lots of factors to consider, whether in a human challenge or real world study. Changing temperature and humidity can affect both the host and the virus, for example. An issue was noted related to model systems for studying human influenza that included intentionally infected "donor subjects" and exposed "recipient

subjects". In such a system, nasal inoculation of "donor subjects" may produce a different pattern of infection than would occur naturally, so "donors" with experimental influenza should be examined to validate that the respiratory tract-derived aerosols they produce are similar to those produced by persons naturally infected with influenza. There was some discussion about the approach of using naturally-infected humans as donors in human challenge studies. Although this approach is theoretically appealing, the logistics and expense of lining up naturally-infected "donors" and sero-negative "recipients" to participate on short notice in these types of experiments were thought to be unfeasible. This problem would be magnified if large experimental groups were required to have adequate study power. A related suggestion was that doing environmental sampling as part of the experimental design for human challenge studies will be very important.

Sample size required for statistical significance of studies was raised as an important feasibility concern. Presentations earlier in the meeting of a specific approach to experimental influenza human challenge studies showed only 25% transmission, even with very intense exposure of uninfected volunteers to volunteers with experimental influenza infection. Thus, power is a very important concern in study design. Expense was noted as in issue in human challenge studies. These studies are very expensive. The problem is magnified if rates of transmission in models are low, requiring larger experimental groups.

It was suggested that the human challenge model might be especially helpful in studying the issue of presymptomatic transmission and minimally symptomatic infected people. There are many studies suggesting that these are potentially important issues. One such recent study was the comparison of N95 respirators and surgical masks reported by Loeb earlier in the meeting (Loeb et al, 2009). In that study, a large proportion of healthcare workers studied across an influenza season had incident infection as suggested by serological changes, but very few were symptomatic and fewer still had influenza documented by PCR evaluation of respiratory samples.

It was noted that to use human challenge studies to understand routes of infection, there is an important need to have experimental designs that cleanly include or exclude various routes of infection. It was suggested that there are lessons that can be learned from studies of experimental rhinovirus infection conducted by investigators in Wisconsin and Virginia. Some of the key issues are described in a supplement to a recently published paper assessing airborne rhinovirus in office environments (Myatt et al.

2004). In the Wisconsin studies, investigators caulked up all the cracks and tried to limit diluting ventilation where studies were performed. In the VA studies, there was a much larger air volume per patient and per infector, so the probability of detecting airborne infection was smaller. These methodological differences may have contributed to differing conclusions of these two groups about transmission of experimental rhinovirus infection. Also, in the cited study by Myatt et al, the amount of diluting ventilation indirectly assessed by indoor – outdoor carbon dioxide differences affected the ability to detect airborne rhinovirus, with higher indoor CO2 values (suggesting reduced ventilation) associated with increased chance of detecting airborne rhinovirus. It was suggested that, in experimental human influenza challenge studies, attention should be given to limiting unmeasured air dilution, and measuring air exchanges with tracer gases. It was also suggested to monitor indoor CO2 and indoor/outdoor CO2 differences continuously, as well as evaluating exhaled air.

However, another participant cautioned to be careful about using CO2 to assess ventilation, because CO2 would not be removed from recirculated air returned to a room, but it might have been treated in a way that removes infectious risk and contributes to effective ventilation (for example, by filtration or in-duct UV treatment). So there is a need to be careful about using CO_2 as an index of ventilation. Also, it was noted that indoor CO_2 levels can vary substantially depending on a variety of factors, such as whether air is coming from the outside of building or is recirculated or can vary from the front to rear of a building due to traffic.

It was suggested that, if details are attended to, upper room UV with good room air mixing may be a good intervention to help differentiate between droplet spray and aerosol transmission. However, it is critical to use fans that create good air mixing and prevent the development of high concentration aerosols near the donor. Such an intervention would be expected to reduce short-distance aerosol transmission, but have little effect on droplet spray transmission. It was noted that existing facilities can be retrofitted with upper room UVGI. There might be some effect on surfaces, but predominantly on airborne particles.

Other approaches to differentiating between aerosol and droplet spray transmission in volunteer challenge human studies might be designs like having the donor and recipient in rooms next door with the only connection being an air duct from one room to another. The problem is that there might be low rates of transmission. But, if rooms were small enough and volunteers were kept next door to each other long

enough, it might be possible to make the point that aerosol transmission occurs without contact other than through air. It would also be possible to apply other conditions, such as screens in the same room to prevent droplet spray transmission but not aerosol transmission.

There was less enthusiasm for study designs around respirators. They affect many routes of transmission, so if they were effective, it would be hard to say how they worked. Thus, there was more enthusiasm for using engineering controls, such as ventilation or air sanitation or barriers, that more cleanly impact on a specific route of transmission. That being said, a human challenge study design might consider using a full face respirator as a protective device vs. a face shield. A face shield would be better than a surgical mask because surgical masks provide some degree of aerosol filtration, which would complicate interpretation of results.

There was also discussion of ethical concerns in human experimental influenza challenge studies. Ethical concerns are a very important consideration, especially in view of the report noted earlier in the meeting of a case of myocarditis and findings of ECG changes in volunteers with experimental influenza infections (Ison et al, 2005). It is important to consider issues related to recruitment and who is volunteering to participate in studies. Participants are often vulnerable populations who are greatly in need of the compensation provided. It is very important to be careful about these vulnerable populations and compensation issues. Participants expressed that it is very important to assure that study volunteers are well informed about risks and inconveniences such as length of quarantine. Public or community engagement is important. Are there venues for public input over the course of the study? How and what is communicated to the public or community and when? It was recommended to be thoughtful about embedding some ethics research into the design as the study goes along. Consider using a pre and post survey to better understand who volunteered, why they volunteered, what the experience was like, what they expected it to be like, whether they were they informed about risks, and whether information provided about risks was well understood.

Intervention Studies of Exposure and Transmission in "Real World" Settings

Many participants expressed their opinions that these types of studies were very desirable and of the highest priority because of their potential both to answer scientific questions and to have impact on

public health prevention practices. It was noted that basic understanding will help in designing new hospitals or force protection through better design of battleships, etc. But, to the extent that the goal is to reduce transmission in a pandemic, then the most valuable studies will be those that show what interventions work in the real world. And, it was expressed that this type of research could provide information about routes of transmission. The Egyptian study presented earlier by Dr. Talaat showing benefits of hand washing by school children was raised as an example. Such studies might have the greatest chance to be informative about routes of transmission if they were well-controlled "efficacy" studies rather than studies of intervention effectiveness.

In order to plan the best studies of this type, the need was expressed to have surveillance information about which groups are at highest risk for acquiring influenza infection, e.g. pulmonary medicine providers, or elementary school teachers, etc. Knowing which workers and tasks are at higher risk can help with targeting study interventions.

In community interventions (and human challenge studies), there is a critical need to pay attention to risks for exposures outside of the site where the intervention is being provided. Otherwise, competing risks from outside of the study site have the potential to overwhelm any effects of interventions. To minimize this problem, it is best to study confined populations such as hospitalized patients, nursing home patients, or residents of boarding schools who will have less likelihood of encountering infections in the community outside of the study site.

It was suggested that one approach to assure that infections in study participants are being acquired at the study site (as opposed to in the community) is to use molecular techniques to characterize influenza strains. A problem raised with this approach was that, in many studies, exposures would occur to many strains of virus, complicating the use of molecular epidemiology techniques.

There was great enthusiasm for studies of engineering controls in general as a way to better understand the contribution of aerosol transmission. These are near the top of the hierarchy of controls, because individual adherence is not an issue. Another advantage of engineering controls such as upper-room UV irradiation is that it isn't necessary to know the exact infectious source for the intervention to have an effect. Also, use of engineering control interventions in confined populations would assure that these

populations were covered all the time and eliminate adherence as a confounder of results. One concern expressed about engineering controls was the ability to use them in homes.

There was much discussion and enthusiasm for use of upper-room UV germicidal irradiation (UVGI) with good air mixing as an approach to dissect out the role of aerosol transmission from droplet spray transmission. The approach is supported by findings of the Livermore VA study described earlier in the meeting, but that was never repeated as a study designed to evaluate impact on influenza transmission. UVGI would be expected to impact on aerosol transmission, but not on droplet spray transmission. One participant recommended intervention with UV air sanitation plus highly effective ventilation in excess of 20 air exchange equivalents per hour as a means of specifically knocking out aerosol transmission. Aggressive air mixing with ceiling fans was recommended to knock out local high concentrations of virus aerosol and allow differentiation between aerosol (affected by upper room UV) and droplet spray (not affected by upper room UV).

It was asked if UVGI might affect surfaces. In response, it was noted that upper room UV puts so little UV into the lower room that it wouldn't affect surfaces or ballistic droplets. A concern was that it might not be able to prevent aerosol transmission if there was a concentrated cloud near the source. To address this, the literature suggests that UV is more effective if there is good air mixing, such as with a ceiling fan creating downdraft or updraft to break up concentrations and bring them quickly up into the UV zone to be decontaminated. UV air decontamination can be very effective. With vaccinia virus, it is possible to get 1000 equivalent air exchanges per hour at low relative humidity in a test chamber. Achievable equivalent air exchanges for influenza will probably be less, because influenza is less sensitive to UV irradiation. It was suggested that 20 or 100 equivalent air exchanges per hour might be possible. Some have questioned if UV stimulates vitamin D production, which might be an alternative mechanism of effect. However, because levels of lower room UV are controlled to protect eyes, this should not be much of a factor.

A plea was made to keep low resource settings in mind when considering study designs, including healthcare or non-healthcare settings. Studies of upper room UVGI would provide useful scientific information about transmission and would also benefit such settings if found to be useful.

One participant discussed potential application of alternative approaches to air disinfection than upper room UVGI. Some technologies discussed were primarily point control. One example of this was to use inhaled antivirals to prevent transmission.

An alternative approach to UVGI for air disinfection mentioned by one participant was the use of triethylene glycol or propylene glycol vapor. These were studied in the 1940s and a paper titled, "The lethal effect of triethylene glycol vapor on air-borne bacteria and influenza virus" was published in 1943 (Robertson et al, 1943). The vapor works best in a dry environment. Humidity may also impact its effectiveness. It condenses a bit on surfaces, where it is also active. The participants remarked that 15-20 very good articles in the old literature evaluated this intervention, but interest was lost with development of polio and other vaccines, and the introduction of antibiotics for TB and other respiratory infections. From 1942 – 1952, the Army and Navy did a number of trials in barracks. A 1952 paper showed great reductions in airborne organisms (U.S. Navy, 1952). The ability to generate these vapors is much better now. The participant remarked that these approaches were not abandoned due to lack of germicidal effect or toxicity, but rather due to lack of interest. A contemporary report on the subject was recently provided to the FAA (Rudnick et al, 2009).

It was suggested also that the study of Loeb et al (2009) needs to be repeated but with larger study size, better exposure assessment, and better understanding of community/home exposures. However, concerns were also expressed that respirators are at the bottom of the hierarchy of controls. Also, because of the confounding effects of adherence and the impact of respirators and face masks on multiple modes of transmission, it was felt that respirator studies would not be as revealing about modes of transmission as intervention studies employing upper room UVGI or air filtration or directional airflow as interventions.

Others noted that point-source control is very important in controlling transmission of influenza in real-world settings and that interventions for point source control, such as masking ill patients, were key. This aspect should be considered in designing intervention studies in real-world settings.

Closure of the meeting

The meeting was closed by Dr. Bridges with thanks to the meeting participants, presenters, and CDC staff for their parts in contributing to a successful workshop and open discussion and suggestions.

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