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CONFIDENTIAL PROTOCOL

Virus shedding and environmental deposition of novel A(H1N1) pandemic influenza virus

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2. CONTENTS

3. SYNOPSIS

Title	Virus shedding and environmental deposition of novel A(H1N1) pandemic influenza virus
Short title	Virus shedding in novel influenza A(H1N1)
Chief Investigator	Professor Jonathan Van-Tam
Objectives	The objectives of the proposed study are: ***Primary:*** • To determine the quantity of infectious virus present in the nose, on surfaces, in the air and in stool, according to time from symptom onset, symptom constellation (e.g. presence of cough or sneeze), distance from source and particle size (in air); • To correlate serial virus shedding in pandemic influenza patients against data on near-patient environmental contamination (surfaces and air). *Secondary:* • To describe virus shedding (quantity of infectious virus) and duration according to important patient sub-groups, notably adults and children, those with mild illness (community patients) and those with more severe disease (hospitalised patients). • To determine if aerosol generating procedures (most likely to be performed on ITU) are associated with changes in the quantity of environmental contamination with live virus, either in relation to quantity or particle size, or distance from source. • To investigate the possibility of estimating the number of influenza-infected individuals in an area by the quantity of influenza virus recovered in sewage influent. *Policy related (to provide scientific data suitable for policy refinement on):* • 'Safety distances' around patients with pandemic and seasonal influenza. • Appropriate use of respiratory personal protective equipment (RPPE) and infection control practices for pandemic and seasonal influenza, according to patient type, illness severity and time since symptom onset. • Antiviral treatment duration for patients with pandemic influenza. • To develop an alternative surveillance strategy for quantifying influenza infections in a community.

Study Configuration	Multi-centre, observational + interventional
Setting	Community and Hospital
Sample size estimate	We will aim to recruit groups of about 25 patients with recent onset H1N1 influenza in each of the four main sub-groups identified under 'research methods'. Most statistical analysis will involve examining correlations between virus shedding and virus deposition in the environment. The figure below illustrates that sub-group sizes of 25, which also allow pooling of data by adults or children (50 per group) or the whole population gives high statistical power (>80%) to detect correlations of >0.55 in groups of size n=25, 0.4 in groups of size n=50, and 0.3 in groups of size n=100. As regards the duration of virus shedding, these data will be primarily descriptive but it will be important to be able to make formal statistical comparisons of the duration of shedding between adults and children. However by pooling data into adults vs. children (n=50 per group) differences of 5 days (adults) vs. 6 days (children) (two tailed-test) could be detected with >80% provided that the coefficient of variation in shedding was 0.3 or less. For larger differences e.g. 5 days vs.7 days or 5 days vs. 8 days, the study is well powered to coefficients of variation up to 0.6. We aim to recruit about 20 patients within the Nottingham patient group to participate in the viral shedding in stool sub-study. The patients will include roughly an equal mix of adults and children.
Number of participants	100
Eligibility criteria	Our clinical case definition of pandemic influenza (swine flu) is; • Fever (or recent history of) + any 1 of cough, sore throat, runny nose, fatigue or headache • Any 2 of cough, sore throat, runny nose, fatigue or headache **Planned Inclusion / Exclusion Criteria** <u>Inclusion criteria:</u> • Subject fulfils case definition • Informed consent obtained (from Parent/Guardian where appropriate) • Age >1 month • Near-patient test positive for influenza A or other substantive test positive for influenza A (including 'swine flu') • Willing to participate and agrees to allow both nasal and environmental samples to be taken

	Exclusion criteria: • Illness for >48h (community cases) • Illness for >96h (hospital cases) • A negative for swine flu (as part of NHS care) • Has taken part in influenza research involving an investigational medicinal product within the last 3 months
Description of interventions	Symptom assessment – At the first visit participants will be asked to complete a number of assessment forms that cover their medical history and current symptoms. Subsequently they will ask you to complete a diary of your symptoms. They will complete a simple chart which asks whether they are feeling certain symptoms and how severe they are. In addition to this we will take an oral temperature reading. These things will happen once a day. Nose swab – A large cotton bud will be used to take a swab from the inside of the nose. This will be collected every day. Surface sampling – A number of common household and hospital room surfaces will be swabbed. We will take swabs every other day when we visit. Air sampling – For a few patients we would like to conduct some air sampling in the room in which they spend most time. This involves running 2 small machines that suck in air and collect air particles. The machines will stand in a room and run for a maximum of 3 hours. This will be done every other day during the study. Stool sampling – We will ask patients to submit a stool sample each day
Duration of study	Total duration = 21 months Maximum for a participant (days); Adult = 10, Child = 12 Start date = 25th August 2009
Outcome measures	• Virus shedding and deposition as measured by virus culture and quantitative PCR. • (Quantitative PCR and plaque assay of respiratory virus specimens (nasal swabs) from patients and surfaces and air around them).Virus shedding and deposition as measured by virus culture and quantitative PCR. • Daily symptom scores and patient temperature readings • Medication logs • Household/ward daily temperature and humidity logs
Statistical methods	We will perform a detailed descriptive analysis of the data. The symptom constellation of patients in the different groups will be presented. The mean (standard deviation, range) of the quantity of infectious virus in the patient, on surfaces and in the air will be plotted for each patient group and as a function of time since onset, symptom constellation and distance from source (when relevant). The mean (standard deviation,

	range) duration of shedding will also be plotted for each patient group and as a function of symptom constellation. For a better representation of inter-individual variation (which is expected to be important), we will also plot individual trajectories. In a second stage, formal tests will be used to determine which outcomes are significantly associated / correlated. Statistical tests will also be implemented to compare the mean duration of shedding among children and adults as well as among mild and severe cases. In a third stage, a Generalized Linear Model with random effects will be used to determine the key predictors for the quantity of infectious virus in surfaces and in the air. A survival analysis will also be implemented to assess the key predictors for the duration of viral shedding.

4. ABBREVIATIONS

AGP	Aerosol Generating Procedures
CI	Chief Investigator
CRF	Case Report Form
GCP	Good Clinical Practice
ICF	Informed Consent Form
ILI	Influenza Like Illness
PIS	Participant Information Sheet
REC	Research Ethics Committee

5. Background Information and Rationale

As pandemic preparedness activities in the UK and worldwide have gathered pace over the last 5 years, it has become very clear that influenza transmission is one area that is very poorly understood. In particular it has not been conclusively established to what extent influenza transmission occurs via direct and indirect contact (contact with contaminated surfaces), by large droplets (typically >5 microns in size that settle at short range (with 3-4 feet) or by smaller particles (aerosols) that can remain suspended for longer periods of time and travel longer distances. Distinguishing the relative importance of these modes of transmission is critical for the development of infection control precautions in healthcare settings and in the home. For example, if contact transmission is dominant then hand hygiene is the most critical intervention. However, if droplet transmission is important, surgical face masks may be important and the safe distance away from an infected patient might be as great as 4 feet. Such issues are highly relevant to seasonal influenza, but have been brought into sharper focus by the emerging novel A/H1N1 pandemic virus, which is expected to produce widespread UK activity in autumn 2009. At present opinions are sharply divided on the importance of aerosol versus droplet transmission [1, 2]. Currently the UK recommends droplet as opposed to aerosol precautions (surgical masks rather than respirators) for most forms of contact with pandemic flu patients; however, this is contested by some frontline healthcare workers who believe these safeguards are inadequate and there is little evidence with which to reassure them.

In parallel, the kinetics of nasopharyngeal and faecal virus shedding (duration) in relation to symptom onset and severity are both unknown for the novel A/H1N1 virus, but highly relevant in relation to estimation of the likely period of infectivity and in relation to virus replication and therapeutic management (in particular, optimal duration of antiviral drug therapy). In all previous research on influenza virus excretion, shedding has been determined by measurement of the quantity of virus recoverable from the patient's nasopharynx by the deliberate insertion of a cotton swab, nasopharyngeal aspiration or the performance of a nasal wash; i.e. virus has been recovered by a deliberately performed invasive technique. Whilst this data is useful, we propose that these data should be linked to near-patient environmental sampling which would determine the extent to which infectious virus has been deposited onto surfaces and into the air in the patient's immediate vicinity (thus allowing an estimation of the potential for contact transmission) and the measurement of infectious virus in air according to particle size and distance from the patient. We believe that the correlation of virus shedding data against environmental contamination via linked data is critical translational research that will assist policy development far more effectively than virus shedding data obtained in isolation.

Our consortium already has experience in performing virus shedding studies in experimentally infected patients with influenza and virus sampling and virus survival work in relation to contaminated surfaces. It also has air sampling equipment provided on loan from the US Centers for Disease Control (CDC) and the National Institute for Occupational Safety and Health (NIOSH) which has been validated for use with patients with confirmed influenza infection. Although the findings of this research will clearly have long-term relevance to influenza infection control practices, given the strong likelihood of significant pandemic activity by mid-late autumn 2009, the emphasis will be on gaining early data from pandemic influenza patients in August and September 2009, with the intention of providing an early 'policy steer' as well as a longer-term answer.

The Centre for Ecology & Hydrology will be dedicated to using the viral shedding data from stools to inform a model which is being generated to predict the number of influenza-infected people within a geographic area based on the quantity of influenza virus recovered in sewage

influent. This generic approach is already in use by the WHO to assess polio infections/immunizations in an area. It is our aim to test whether sewage influent can serve as a medium for estimating (pandemic) influenza-infected individuals within a region. We believe that if this study can demonstrate that there is a predictable amount of viral shedding in the stool of influenza-infected patients, the sewage-based epidemiology screening approach could be used as an early detection tool for the spread of pandemic influenza within an area.

Existing Research:

Virus Shedding:

To our knowledge no data are yet available publicly on the kinetics of virus shedding in patients with novel influenza A (H1N1). However, confidential data obtained from diagnostic specimens by the Health Protection Agency suggest that the duration of shedding may be slightly longer than with seasonal influenza and up to 8 days in some patients. However these data derive from semi-quantitative PCR readings and so relate to the detection of swine virus specific nucleic acid but not to the presence of infectious virus. In addition, the data are cross-sectional, i.e. pooled from single samples taken from individuals at different time points in their illnesses as opposed to serial measurements from the same individuals (*M.Zambon: personal communication; confidential unpublished data*). Most data are from schoolchildren in whom the duration of shedding tends to be longer than in adults in any case. Until data become available from studies such as the one we propose, the estimated duration of influenza virus shedding is based upon previous experience with seasonal influenza virus infection.

The period of viral shedding can be inferred from the length of time that virus can be recovered from respiratory secretions and is influenced by age of the person infected, level of immune competence and treatment with antiviral agents. It may also be influenced by symptom severity and fever (both proxies for virus replication and viral load) or other unknown factors.

Adults;

Older data suggest that virus shedding is proportional to symptom severity and that virus shedding in adults declines markedly on the third day after symptom onset. Contemporary data on virus shedding in healthy adults derives from studies performed for the licensure of antiviral drugs [3, 4]. It is normally quoted that the shedding of infectious virus (as opposed to PCR detectable virus) is 5 days in adults and CDC infection control guidance reflects this. PCR can detect virus after this time but culture is usually negative. A recent study found that adult patients could shed virus (detected by polymerase chain reaction (PCR) and culture) beyond this traditional period, though patients were elderly and nearly all had underlying medical conditions [5]. Indeed, it is well documented that older patients, those with chronic illnesses and those with immunocompromise can shed live virus for longer periods because virus replication is less inhibited [6]. In the current pandemic however, this may not apply to the elderly because there is already (unpublished) evidence that the level of cross-protective immunity in elderly subjects to the novel A/H1N1 virus is higher than in younger adults and children.

It should be remembered that approximately 50% of all influenza infections are asymptomatic [7] and that infected people (typically adults) can shed influenza virus without any evidence of respiratory symptoms [8]. However, the importance of transmission from infected people during the incubation period or from those with asymptomatic infection is uncertain and is probably substantially less than from symptomatic people.

Children:
CDC guidelines state that children shed virus for up to 10 days (http://www.cdc.gov/flu/professionals/infectioncontrol/healthcarefacilities.htm). Studies of naturally occurring influenza B infection in children have shown that 93% shed detectable virus during the first three days of symptomatic illness, 74% on day four and roughly 25% on day six and that viral shedding is proportional to severity of illness and temperature elevation [9]. In general, children cease shedding influenza virus seven to eight days after onset of symptoms, but they can shed infectious virus several days before onset of illness [10,11]

Other virus shedding work:
The applicants are already involved in work which is similar to the current proposal, studying A/H3N2 experimental virus infection in health volunteers (ITSDG-01 Proof of Concept study; funder - Department of Health England; sponsor - University of Nottingham). The primary aim of this study is to establish that an experimental influenza infection induced by means of viral challenge is transmissible to other individuals. Healthy young adult subjects (Donors) were inoculated with Influenza A/H3N2/Wisconsin/67/2005. At the onset of symptoms consistent with an influenza-like illness (ILI), a second group of healthy young adult volunteers (Recipients) were exposed to Donors by occupying the same living space and performing certain tasks, consistent with close social mixing, as in a household setting. After 48 hours the two groups were separated into different quarantine areas. Use of symptom diaries and diagnostic tests for influenza allowed the presence of subsequent illness to be identified. Additionally, during the study serial nasal washes were obtained from donors and recipients to study virus shedding and environmental sampling (fomites and air) was performed using validated equipment from CDC/NIOSH, with the aim of detecting environmentally shed influenza virus by PCR and infectious virus by plaque assay. The laboratory assays are currently awaited but there are several uncertainties about extrapolating data from a seasonal influenza challenge model in healthy volunteers to wild type infection with a novel virus in a wider range of patient groups including children.

Influenza in the near-patient environment:

Fomites:
The role of fomites and surfaces in the transmission of influenza A is unclear and studies assessing the presence of virus on fomites are lacking. Similarly there is a paucity of scientific data on virus survival on surfaces and no studies looking at viable virus in the vicinity or homes of infected individuals. Limited data are available to support the possibility of indirect contact transmission of influenza; Morens et al concluded that influenza transmission may have been mediated by staff via either contaminated hands or fomites during an outbreak of influenza in a nursing home [12] while Bean et al. indicated that spread of infection by contact with contaminated fomites is possible. They showed that human influenza viruses could survive on a variety of surfaces at 35%–49% humidity and a temperature of 28^0C. Both influenza A and B viruses were cultured from experimentally contaminated, nonporous surfaces, such as steel and plastic, up to 24–48 h after inoculation, and from cloth, paper, and tissues up to 8–12 h after inoculation. However, viruses could be recovered from hands for only 5 min and only if the hands were contaminated with a high viral titer. Viable virus could be transferred from nonporous surfaces to hands for 24 h and from tissues to hands for 15 min [13].

Air:

If influenza virus can transmit via aerosols then we would expect to be able to detect virus in such aerosols and such evidence is now emerging. Studies performed over 30 years ago showed that artificially aerosolised influenza could be detected for up to 24 hours after release and that aerosolized virus is able to infect some animals [14, 15]. More recently influenza virus was detected in aerosol samples taken from medical facilities. Air sampled in an emergency department during an influenza season showed virus to be present [16] and during the 2009 influenza season, air sampling for aerosol particles containing influenza and RSV viruses was conducted at an urgent care walk-in medical clinic. During each of 11 sessions, healthcare workers wore personal aerosol samplers and tripods holding two stationary samplers were placed in six examination rooms, two procedure rooms, and next to the patient scale in the connecting corridor. Three tripods were also placed in the patient waiting room. Preliminary results indicate that 46 of the stationary samplers (17%) and 4 of the personal samplers (19%) captured influenza A RNA and 84 stationary samplers (32%) and 8 personal samplers (38%) contained RSV RNA. During the peak session with 4 confirmed influenza patients, 79% of the stationary samplers collected influenza A viral RNA (*D. Beezhold & W. Lindsley: personal communication;* ***confidential unpublished data***).

Despite the above, the detection of *viable* virus in aerosols generated by humans has not been shown before (as far as we know). The generation of information about the presence of viable influenza virus in the environment will be fundamental to our understanding of the routes of transmission. With this in mind, we have recently attempted to demonstrate that viable influenza virus can be found in aerosols as part of the Proof of Concept study (ITSDG-01) mentioned earlier. In preparation for this, the University of Nottingham received sampling equipment from CDC/NIOSH (identical to that used by Beezhold et al above). Prior to its use we approached the Health and Safety Laboratory in England to pilot setting-up of the sampling equipment, calibration and evaluating the utility of the sampler for capturing live influenza. Following experiments that involved aerosolizing influenza virus in a laboratory, live virus could be detected by an air sampler using the virus plaque assay technique (and PCR results were concordant). Following on from this, air sampling recently took place during the proof of concept study – results are awaited. Thus the technique of air sampling using the CDC/NIOSH equipment has been validated in the UK at the Health and Safety Laboratory and (pending results) during a quarantine based challenge study.

Research at CEH has already demonstrated in preliminary research the capacity of the influenza virus to persist in sewage influent for over 2 hours with only a 60% loss in total counts (quantitative PCR). Given that our ability to detect the virus spans >8 orders of magnitude using quantitative PCR, a 60% decline is negligible (e.g., lowering virus counts from 5.0×10^6 to 2.0×10^6). Hence, there is every reason to expect that if the virus is being shed by influenza-infected patients, the virus should be recoverable in the sewage influent.

6. Research Objectives

The objectives of the proposed study are:

Primary:

i) To determine the quantity of infectious virus present in the nose, on surfaces, in air and in stools, according to time from symptom onset, symptom constellation (e.g. presence of cough or sneeze), distance from source and particle size (in air);
ii) To correlate serial virus shedding in pandemic influenza patients against data on near-patient environmental contamination (surfaces and air).

Secondary:

iii) To describe virus shedding (quantity of infectious virus) and duration according to important patient sub-groups, notably adults and children, those with mild illness (community patients) and those with more severe disease (hospitalised patients)
iv) To determine if aerosol generating procedures (most likely to be performed on ITU) are associated with changes in the quantity of environmental contamination with live virus, either in relation to quantity or particle size, or distance from source.
v) To investigate the possibility of estimating the number of influenza-infected individuals in an area by the quantity o influenza virus recovered in sewage influent.

Policy related (to provide scientific data suitable for policy refinement on):

vi) 'Safety distances' around patients with pandemic and seasonal influenza
vii) Appropriate use of respiratory personal protective equipment (RPPE) and infection control practices for pandemic and seasonal influenza, according to patient type, illness severity and time since symptom onset
viii) Antiviral treatment duration for patients with pandemic influenza
ix) To develop an alternative surveillance strategy for quantifying influenza infections in a community.

The primary objective of this study is to correlate the amount of virus detected in a patient's nose with that found in the environment around them and with the time since illness onset and symptom severity. The point being that so called 'virus shedding' studies that measure virus recovered from the nose do not actually define environmental contamination and hazard to others. To the best of our knowledge such work has not been done before. The study has the potential to address the issues of how, when and where in relation to virus transmission, all of which we believe could inform policy. By collecting stools, we can also correlate influenza shedding in the nose with the stool and thereby provide a mechanism for generating estimates of influenza in the stool to populate the sewage-based epidemiology model.

How – Are touched surfaces important in virus transmission and does respired air present a significant transmission route?

A virus can get on to a surface in a number of ways (e.g. indirectly via touch and droplets of any size settling out), but which surfaces (both in terms of proximity to the patient and physical nature) are commonly contaminated and how long virus remains viable for are uncertain. A virus can also become airborne and transmit through this route (inhalation and direct impaction of droplet nuclei on mucous membranes). The proposed research will evaluate the relative hazard of the touched environment versus the respired environment. In doing this it will provide a policy steer towards interventions that are likely to be important in reducing transmission. For example; if the touched environment is associated with much higher quantities of viable virus than the respired environment then hand hygiene and surface cleaning advice needs greater emphasis; but conversely if the respired environment is more important, strengthening PPE guidance (particularly around face masks and respirators) or applying 'distance or proximity rules' would be of greater importance.

Where - 'Safety distances' around patients with pandemic and seasonal influenza;

The devices we propose to use for air sampling are not only portable but are also validated and capable of separating out particles into three size ranges. Sampling air within 3 feet and >7 feet away from a patient will inform safety distances. For example;

Healthcare settings;

- If air sampling detects virus only within 3 feet of a patient then we can be confident about need for PPE within 3 feet. If viable virus is detected in the air at greater distances then the standard 3 feet safety distance should be revised; but the need for respirators would depend on the size of particles from which we detect viable virus.
- This may have a significant impact on the care of patients in NHS facilities and the advice given to HCWs regarding the implementation of infection control procedures.

Community;

- When a person with a high risk condition (for complications of influenza) resides in a household with an index case, then safety distances around an infected case could be important, potentially helping co-habitees to protect themselves. At the height of the pandemic, it is almost certain that families will have to care for each other as hospital capacity will be saturated. Families need to know the safest procedures to adopt and the government needs to issue this advice.

When - Appropriate use of respiratory personal protective equipment (RPPE) and infection control practices for pandemic and seasonal influenza

Several variables may impact on 'viral shedding' from patients; adult v child, illness severity, time since symptom onset and the effect of antivirals. Knowledge about how long PPE is needed for when caring for patients is important, especially when considering the need to preserve stockpiles of PPE. For example;

Healthcare settings;

- If viable virus can only be recovered from patients for example, up to 3 days after symptom onset, isolation precautions, including use of PPE would not be needed for longer than this, especially if there were shortages.

Community:

- Information about how long patients are infectious for could inform guidance around how long patients need to isolate themselves e.g. avoid caring for children, staying off work / school.

7. Research Team

Expertise

The consortium making this application has several key strengths:

1. Prof Van-Tam, Drs Hayward, Killingley, Greatorex and Cauchemez and Mrs Enstone have worked closely together on the recent influenza virus challenge study, ITSDG-01.
2. Profs Van-Tam and Nicholson are recognised global experts on influenza; both are members of the UK Scientific Pandemic Influenza Advisory Committee (SPI) and the UK Scientific Advisory Group for Emergencies (SAGE). They have worked together for almost 20 years.
3. Dr. Lim was responsible for the creation of the UK national pandemic influenza clinical management guidance.
4. Profs Van-Tam, Nicholson and Read, and Dr Lim are FLU-CIN co-participants.
5. The group has recent experience of conducting virus shedding studies and has validated techniques for this purpose (DH funded study: ITSDG-01).
6. The group has recent experience of conducting virus survival studies using commonly touched household materials and has extensively validated protocols for virus recovery, RT-PCR and plaque assay (HPA funded study).
7. The group has access to BSL Level 3 facilities in Cambridge for its virology work.
8. Dr Hayward is the leader of MRC FluWatch and its subsequent proposed extension. Prof Van-Tam is a FluWatch co-applicant.
9. Dr Singer is a leader in the effort to understand the environmental implications of pharmaceutical use during an influenza pandemic and is a member of the UK Scientific Pandemic Influenza Advisory Committee (SPI).
10. Dr Singer and Dr. Hussey are experienced in molecular virology techniques and have access to the BSL Level 3 facilities at the Centre for Ecology & Hydrology, Oxford.
11. Dr. Andrew Johnson is a world leader in the field of modelling of pollutants in the environment and has significant experience working within sewage works—a necessary component of the epidemiology model.

We have asked members of a team at the Health and Safety Laboratory in Buxton to collaborate with us on this study. HSL is the UK's premier health and safety facility with over thirty years experience in understanding the causes of ill-health and major incidents in UK workplaces. It has specialists from a diverse range of disciplines all under one roof, working to help control hazards and assist in the management of occupational health.
HSL also has a strong track record in healthcare related research and consultancy, in the public, private and charity sectors with a range of clients including the Department of Health, NHS Estates, Hospital Infection Society, Care Quality Commission and BUPA. Therefore, HSL is well placed to offer specialist technical support and has expert scientists specialising in the

areas of virology, aerobiology, environmental microbiology and ventilation in-house;

- Dr Brian Crook: Microbiology Team Leader; expertise in environmental microbiology and aerobiology
- Dr John Saunders: Ventilation and Aerosols team leader; expertise in ventilation systems, air movement
  measurement and control of aerosol hazards
- Dr Jonathan Gawn: Virology Team Leader; expertise in virology, including the extraction of live viruses from the air
- Steve Stagg: General microbiology field scientist; expertise in all aspects of microbiological workplace sampling

HSL is active in Pandemic Flu research and they have recently completed a large study for the Department of Health to evaluate the efficacy of fumigation devices for hospital acquired infections (including influenza) and are developing proposals to assess the efficacy of surgical facemasks and respirators in relation to the transmission of influenza.
We propose to conduct 3 face to face meetings with this team over the course of the study to discuss the design, methods and ultimately outcomes of the environmental sampling work. One meeting should happen as soon as possible to inform our final protocol, the second should take place prior to study start and a third after the study ends.

Collaborators:
Dr David Thomas – Consultant Paediatrician, Nottingham University Hospitals NHS trust.
Dr Paul Digard – Senior University Lecturer, Virology Department, University of Cambridge.
Dr William Lindsley – National Institute for Occupational Safety and Health, USA
Dr Donald Beezhold - National Institute for Occupational Safety and Health, USA

Clinical Team:
A team of nurses will be covering the 3 different sites (Nottingham, Leicester and Sheffield). These nurses will work under the clinical direction of Dr Killingley and the administrative control of the Support Worker who will coordinate daily patient tracking and maintain deployment logs. In each location the nurses will be supported by a consultant physician / paediatrician. Regarding laboratory work, Dr Greatorex (Post Doc Scientist at the HPA laboratory in Cambridge) will be responsible with assistance from a laboratory scientist.

8. Research Methods

Study Design – Multi Centre, Observational + Interventional

When performing studies of virus shedding, certain principles are important:

1. Because serial virus shedding is labour intensive to measure and costly to analyse in the laboratory, there must be a strong likelihood that subjects who are recruited have the disease in question, i.e. the predictive value of screening procedures applied to potential participants must be high. This can be achieved by careful selection criteria and application of a near-patient test.
2. Virus shedding needs to be monitored by taking daily measurements over at least one week during which shedding would be expected to decline; thus it is desirable to recruit ‘fresh’ patients as soon as practically possible after symptom onset. Nevertheless it is

important to recognise that patients will be recruited to any such study at different intervals after symptom onset; and that patients admitted to and recruited in hospital, may well have been ill for several days when sampling starts. An 'ideal study' would choose hospitalised patients by choosing only those which were followed from community onset into hospital; however achieving this in practice would require following hundreds of patients to identify that subset of 5% who are admitted, and would be wholly impractical. Nevertheless, selection criteria can be used to avoid patients who have already been ill for an excessively large number of days.

3. Single index cases in households or patients housed in single rooms on wards should be recruited whenever possible because these offer the best chance of providing data that are easy to interpret in the context of environmental sampling. For example, if two brothers shared a bedroom and both had symptoms, it would be easy to perform the virus shedding work on both, but impossible to deduce which of the two cases had contaminated the environment.

It is anticipated that this particular study will be performed mainly in August and September 2009 in order that sufficient preliminary data are available to give a policy steer to the Department of Health, England by early October 2009 in advance of a large second wave. Since the daily number of pandemic influenza cases is growing at the present time, but the trajectory of the epidemic curve still contains a high degree of uncertainty, it is impossible to predict precisely how many cases of pandemic influenza will be occurring by study start.

Our study design will therefore be based around the following principles:

1. Based on confidential unpublished HPA data from the FF100 database of confirmed swine flu patients, we already know that the most commonly experienced symptoms are: fever (91%), fatigue (79%), cough (76%) and sore throat (75%). We will select a clinical case definition based on the most common symptoms. We would alter the case definition if new epidemiological data suggested this was warranted.
2. In addition, patients who fit the clinical case definition will be tested with a Quidel QuickVue ® near patient test before proceeding to the next stage of the protocol and only those with a positive test would proceed to sampling. We recognise that patients who pass a near patient test clearly have measurable virus and this might bias the sample towards patients with a higher viral load. However the alternative of over-sampling and later discarding 'non-flu' patients would be too labour intensive and wasteful of resources. However, if we found in practice that most patients recruited on symptoms alone were also positive on near-patient testing, this stage could be amended (omitted) via a protocol modification.
3. We have a limited number of air sampler units available (n=6). Thus we will only sample the environment where it will be possible to interpret the results clearly (patients in side rooms or single (index) cases in households).
4. In order to ensure that patients with relatively recent onset of symptoms are recruited we will set exclusion criteria of >48h after symptom onset for community cases (but aim for recruitment of cases who are within 24h of symptom onset); and > 96h after symptom onset for hospitalised cases (but aim for recruitment within 48h).

Study Management

The study will be managed from a central coordinating site (Nottingham University) by a project manager and administrator. Data will be collected on to source documents and CRFs by the clinical team. Data will subsequently be entered onto a database. All data will be stored at the

University of Nottingham and they will act as custodian of it. Data generated from CEH will be shared with the project team and stored along with the rest of the virus shedding data.

Duration of the study and participant involvement
Each participant's involvement with the study will last for up to 2 weeks. No further follow up of participants is planned. Enrolment will begin in August 2009 and will cease in October 2009. Processing of samples collected and data extraction will continue until February 2010

End of the Study
The end of the trial will follow the completion of the laboratory analysis of samples and subsequent data analysis and presentation.

9. Selection and withdrawal of participants

See Appendix 1 for study outline

Cases
We propose the study of small numbers of symptomatic pandemic influenza patients from four groups:

i) Hospitalised adults
ii) Hospitalised children (up to the age of 16 years)
iii) Adults in their own homes
iv) Children in their own homes (up to the age of 16 years)

We regard these four groups as the minimum desirable based on known differences in virus shedding and respiratory etiquette between adults and children and likely differences in symptom severity between patients managed in the community and those who require hospital admission.

Hospital cases once discharged will be followed up and further sampling will take place in the patient's own home with consent. Similarly if a community patient is admitted to hospital mid-way through sampling we would attempt to follow them up in hospital.

Recruitment

HOSPITAL CASES:

Hospital cases will be identified through the clinical teams looking after patients in the participating centres.. We will not receive personal information about patients or approach them until their consent for us to do so has been granted.

FLU-CIN is an acronym for the newly formed Influenza (flu) Clinical Information Network funded by the Department of Health, England. When the swine influenza crisis began, the Department of Health and the Scientific Advisory Group for Emergencies considered it essential that a system was put in place rapidly to gain as full an understanding as possible of the most serious effects of the virus, and the effectiveness of different methods of treatment for those effects.

This means collecting information rapidly on the clinical condition and treatment of any patients hospitalised as a result of pandemic influenza. Cases are likely to appear in four main areas – adult medicine including infectious diseases and respiratory medicine; children's services; maternity services; and intensive care. Provisional guidelines for the clinical management of patients with an influenza-like illness during an influenza pandemic have been drawn up by the British Infection society, the British Thoracic Society and the Health Protection Agency in collaboration with the Department of Health. FLU-CIN will provide data which will allow revision of those guidelines in the light of emerging information specific to swine influenza.

Hospital cases will be identified from participating FLU-CIN centres in the East Midlands (Nottingham and Leicester) and South Yorkshire (Sheffield). These hospitals form three of five pilot centres for the network. They have the advantage of being close to the co-ordinating centre for this proposal, and will be staffed by DH funded Support Nurses whose job it will be to identify early, patients admitted with pandemic influenza.

Recruitment targets at these sites;
Nottingham - 9 adults and 25 children
Sheffield – 8 adults
Leicester – 8 adults

We recognise that some patients are likely to have been ill for a period of time before being admitted to hospital and therefore may have passed their peak of viral shedding. Nevertheless some patients may well have deteriorated relatively quickly and patients requiring hospital admission usually have more severe disease. In all probability this may lead to a higher viral load and slower decline in virus shedding than in community patients and healthcare workers will be heavily and closely exposed to such patients. Thus we are firmly of the opinion that viral shedding data in this group of patients will still be of significant value.

<u>COMMUNITY CASES:</u>
We plan to recruit via 3 sources;

1. Local Media

We will advertise in the local press for volunteers with flu like symptoms to take part in the study. The advert will invite people who have or who develop a flu-like illness to participate in a research study that aims to improve our understanding of how swine flu is transmitted between people. We will ask people who are interested in helping to call our research office. Preliminary details will be obtained to establish their potential eligibility and an appointment will then be made for a member of the research team to visit the patient at home. Advertising in this way should enable us to pick up patients early in their illness. Adverts will run once a week for 4 weeks depending on recruitments rates.

2. Antiviral Collection Points

A back up to our planned recruitment via the local media will be to recruit patients who have been diagnosed with swine flu and who have been issued with a 'prescription' for oseltamivir. When a patient's family member or 'flu friend' collects the medicine from a designated collection point, a leaflet will be given out that describes our study and invites people to take part. Interested patients will be asked to ring our research office for further information and we can then establish their eligibility.

This method of recruitment gives us access to a significant number of people already clinically confirmed to have swine flu. A drawback is that we would only be able to recruit patients taking

oseltamivir, i.e. we would not be study the natural course of infection in this group. Furthermore, by using this approach it may be that some cases have had symptoms for some time before we make contact with them.

We have the support of the director of Public Health for Nottingham PCT (Dr Chris Packham) for this recruitment mechanism.

3. University of Nottingham
We will make contact with both well and unwell (symptoms of acute respiratory infection) students at the University of Nottingham through the student health centre and university accommodation / halls of residence.

- Unwell students would be asked to take part and then assessed for study eligibility
- Contacts of unwell students would be sought and asked to take part if they become unwell
- Well students would be asked to contact us should they become unwell

Case definitions:
There are a number of options available to us in defining the patients we wish to recruit;

1. Formal virological diagnosis of novel influenza A or novel A(H1N1) swine flu
2. Symptomatic and influenza antigen rapid test positive i.e. confirmed Influenza A/B
3. Symptomatic and a close contact of a case of confirmed swine flu
4. Symptomatic and fulfils a clinical case definition

It is likely that our case definition may change as the epidemic in the UK progresses. For example, before case numbers escalate the positive predictive value (PPV) of symptoms of ILI being swine flu may not be high and in this instance we will want to conduct a rapid test. However, as the PPV of symptoms being caused by swine flu rises, a rapid test may not be needed. So, our initial method of case selection will be number 2 above (symptomatic definition + rapid test), possibly followed by number 4 (symptoms alone). Some patients may already have a confirmed diagnosis by PCR at the point of recruitment (1). However, we recognise that at the present time there is a significant delay between symptom onset and formal diagnosis in the majority of patients. We therefore do not feel confident that relying on formal PCR diagnosis alone will ensure that a large enough number of patients will be detected with ‘fresh’ symptoms. In addition, as the pandemic progresses it is likely that diagnostic testing will not be performed routinely. Option 3 is also unsuitable for our purposes because we cannot perform environmental sampling if there are two possible patient sources as the data would not be easily interpretable at individual level.

Clinical case definition:
Symptom data are beginning to emerge from swine flu patients in the UK via the unpublished HPA FF100 dataset (**confidential**) and from US patients via online sources;

Symptom	Symptom Frequency	
	UK	US
Fever	91%	94% (371 / 394)
Cough	76%	92% (365 / 397)
Sore Throat	75%	66% (242 / 367)
Fatigue	79%	-
Headache	74%	-
Runny Nose	69%	-
Sneezing	60%	-

US data;
http://www.cidrap.umn.edu/cidrap/content/influenza/swineflu/biofacts/swinefluoverview.html

Our clinical case definition of pandemic influenza (swine flu) is;

- Fever (or recent history of) + any 1 of cough, sore throat, runny nose, fatigue or headache
- Any 2 of cough, sore throat, runny nose, fatigue or headache

Planned Inclusion / Exclusion Criteria

Inclusion criteria:

- Subject fulfils case definition
- Informed consent obtained (from Parent/Guardian where appropriate)
- Age >1 month
- Near-patient test positive for influenza A or other substantive test positive for influenza A (including 'swine flu')
- Willing to participate and agrees to allow both nasal and environmental samples to be taken

Exclusion criteria:

- Illness for >48h (community cases)
- Illness for >96h (hospital cases)
- A negative for swine flu (as part of NHS care)
- Has taken part in influenza research involving an investigational medicinal product within the last 3 months

Randomization
Randomisation to the days of surface swabbing will occur. 50% of participants will have surface swabbing done on alternate days from the first visit whilst the other 50% will have swabbing done on alternate days from the second visit. Envelopes will contain instructions to 'swab from Day 1' or 'swab from Day 2' in a 1:1 ratio. The envelopes will be identical and number of them will be given to each study nurse who will open an envelope following enrolment of a participant.

Participant Withdrawal
Participation in this study may be discontinued for any of the following reasons:

1. The wish of the subject. A subject can withdraw from the study at any time, for any reason, without prejudice to their future medical care. Participants will be made aware (via the information sheet and consent form) that should they withdraw the data collected to date cannot be erased and may still be used in the final analysis.
2. Non compliance with study procedures.
3. If a patient has a virological test that is negative for swine flu as part of NHS care.
4. Investigator's decision that withdrawal from further participation would be in the subject's best interest.
5. Termination of the study by the Investigator or Sponsor.

Data will be collected on participants who are withdrawn with outlining the reason(s) for discontinuation.

Criteria for terminating the study
Termination of the study as a whole may result from new information regarding H1N1 or issues with study conduct (e.g. poor recruitment, loss of resources).

Informed consent
All participants will provide written informed consent or in the case of a child a parent / guardian will be asked to provide consent. The Consent Form will be signed and dated by the participant before they enter the study. The Investigator will explain the details of the study and provide a Participant Information Sheet, ensuring that the participant has sufficient time to consider participating or not. The Investigator will answer any questions that the participant has concerning study participation.

Informed consent will be collected from each participant before they undergo any interventions (including physical examination and history taking) related to the study. One copy of this will be kept by the participant, one will be kept by the Investigator, and a third will be retained in the patient's hospital records (where appropriate).

In the event that a patient loses the capacity to consent during the study e.g. sedated ventilated patients, we would wish to retain them in the study. Within the consent form there will be a section seeking agreement to continue to sample patients if they do become incapacitated. In this instance we will also seek consent to continue from a relative (to whom an information sheet will be provided). We will not recruit patients who lack capacity to consent at the outset.

Should there be any subsequent amendment to the final protocol, which might affect a participant's participation in the study, continuing consent will be obtained using an amended Consent Form which will be signed by the participant.

Study Sites

Nottingham – Nottingham University Hospitals. Contact Dr Wei Shen Lim
City Hospital Campus, Hucknall Road, Nottingham, NG5 1PB
Queens Medical Centre Campus, Derby Road, Nottingham, NG7 2UH

Nottingham – Univeristy of Nottingham health service (Cripps health centre), University Park, Nottingham NG7 2QW.

Sheffield – Sheffield Teaching Hospitals. Contact Prof Robert Reid
The Royal Hallamshire Hospital, Glossop Road, Sheffield, South Yorkshire, S10 2JF

Leicester – Leicester University Hospitals. Contact Prof Karl Nicholson
Leicester Royal Infirmary, Infirmary Square, Leicester LE1 5WW

10. Study Procedures

See Appendix 2 for a sample patient schedule

Collection of data (Hospital and Home):
In addition to collecting initial symptom data to confirm a patient's eligibility, ongoing data collection will be needed to achieve our primary and secondary objectives. These will include;

- Daily symptom diary cards – This will allow a correlation of illness and viral shedding to be made. It will be completed by the patient on each researcher visit. A sample is attached as appendix 3; this scale has been previously validated in numerous live challenge studies.
- Daily temperature readings. Patients at home will be supplied with a digital thermometer and asked to take twice daily readings and additional readings whenever feeling feverish.
- A record of all medication taken during the follow up period will be kept. This would include paracetamol, aspirin, antivirals and antibiotics.
- Whilst in hospital a log documenting the performance of any aerosol generating procedures will be kept (e.g. aspiration of respiratory tract, intubation, resuscitation, bronchoscopy)
- A log will also be kept of the use of nebulisers as it is possible that the use of these generates aerosols [17] .
- Room temperature and humidity records will be kept by the visiting researcher. Recordings will be taken at the beginning of any sample collection.

Sample Collection
We will be collecting the following samples;

1. Upper respiratory tract specimens from patients.
2. Surface swabs to detect virus on commonly touched surfaces near the patient.
3. Air particles to detect virus in room air around a patient.
4. Stool samples from patients.

1. Upper respiratory tract specimens;
Consideration has been given to what specimens should be collected for influenza tests from persons with suspected influenza. A number of papers compare the utility of nasal swabs (NS) versus nasopharyngeal aspirates (NPA) in the diagnosis of respiratory viral infections, mostly in children. Whilst the sensitivity of viral detection is slightly higher with NPA (with both PCR and culture diagnostic techniques) NS are regarded as adequate by many, especially for collection done at home where less equipment is needed [18,19,20,21,22]. In addition NS will be easier to manage in terms of staff training and consistency of specimen collection. It is for these reasons that NS will be preferred method of specimen collection. However, we recognise that children may also have NPAs done for therapeutic reasons as part of their normal medical care. In this instance we would still perform a nasal swab.

Patients will undergo daily nasal swabbing (dry cotton swab passed around the anterior nares and then immersed in viral transport medium (VTM). As discussed earlier, seasonal influenza virus is generally shed by adults for up to 5 days and young children for up to 10 days. There is some early evidence to suggest that viral shedding with H1N1 swine flu is occurring over a slightly extended time. In light of this we will attempt to undertake sampling daily for up to 10 days from the start of symptoms in adults and children ≥13 years of age and up to 12 days in children <13 years. In practice this will likely mean performing swabs daily on average 8 days in adults and 10 days in children <13 years.

We expect to collect 950 samples in total:

- Hospitalised adults: 25 patients, 1 sample a day for (on average) 8 days = 200
- Hospitalised children: 25 patients, 1 sample a day for (on average) 11 days = 275
- Adults in their own homes: 25 patients, 1 sample a day for(on average) 8 days = 200
- Children in their own homes: 25 patients, 1 sample a day for (on average) 11 days = 275

2. Surface Swabbing

The purpose of this is to establish the relationship between viral shedding and contamination of the environment with viable virus. The consortium is already heavily involved in HPA funded work concerned with virus survival, which is specifically looking at virus survival on fomites and the efficacy of household cleaning agents. The consortium therefore has particular expertise in this area and has already validated methods of environmental sampling.

To analyse such a relationship between viral shedding and environmental contamination, it will be necessary to ensure that only one person (the index case) is contributing to environmental shedding. Therefore it will be necessary to limit our sampling to those hospital patients who are in side rooms and those patients at home who are the only symptomatic members of that household. However, we recognise that over a period of sampling time (up to 10 days in adults, 12 in children) other members of a household may well develop symptoms. In this instance we would continue sampling (index case and surfaces) but would record the symptoms of all symptomatic individuals.

It will be necessary to clean down surfaces following swabbing each day to remove viral genomic material, so that the following days swabs reflect the deposition of new material. This will preferably be done with a chlorine based agent but will depend on the surface. It may then be necessary to was the wash the cleaned surface with distilled water to remove any residue of cleaning agent that may affect virus that is subsequently shed upon it.

Samples will be taken every other day during the period of follow up, i.e. nasal swab one day, nasal swab + surface swabs on the next day. We will randomly allocate patients to have surface swabbing done on either days 1, 3, 5, 7, 9 and 11 or 2, 4, 6, 8, 10 and 12.

Samples will be taken by swabbing 2 cm^2 areas on selected surfaces from within the rooms housing patients. For consistency we have chosen the following surfaces though we will allow some discretion to be exercised by the researcher;

Hospital;

- Patient table (mid-point or nearest to midpoint)
- Patient line console (e.g. on/off button) / Nurse call button – depending on circumstances
- Door handle
- Patient drinking receptacle (cup / glass)

Home;

- Kitchen –Fridge door handle+ kettle handle
- Lounge – TV remote control (mid point on the back of the device), light switch
- Bathroom – Tap + door handle
- Mobile phone

We expect to obtain 1875 samples in total:

- Hospitalised adults: 12.5 patients (we estimate that 50% of hospital patients will be in side rooms), 12 samples (3 samples every other day for 8 days) = 150
- Hospitalised children: 12.5 patients (we estimate that 50% of hospital patients will be in side rooms), 18 samples (3 samples every other day for 12 days) = 225
- Adults in their own homes: 25 patients, 24 samples (6 samples every other day for 8 days) = 600

- Children in their own homes: 25 patients, 36 samples (6 samples every other day for 12 days) = 900

Method of sampling:
Cotton / fibre tipped swabs to be dipped in tube containing 1.5 ml viral transport medium and then rubbed across 2 cm^2 area of surface in 6 different directions, applying even pressure. Swab to be broken off into tube containing SFM.

3. Air Particle Collection

A two-stage bio aerosol cyclone sampler will be used to i) measure the quantity of influenza virus and ii) look for live virus in aerosol particles around patients. The sampling devices and accessory equipment have been loaned by NiOSH as previously mentioned and have been validated both in the UK and the US (see picture at appendix 2). The sampler draws in air at 3.5 litres/min and separates particles into three size fractions (>4, 1-4 and <1 micrometers). The particles are collected in falcon conical tubes containing VTM or on filter paper These fraction sizes are important because particles of less than 4 micrometers in diameter (aerosols) are capable of being inhaled and reaching the lower respiratory tract, where as particles >4 micrometers behave as droplets. It would therefore be interesting to know whether influenza, particularly viable influenza can be found in such particles as this would weight to premise that influenza can be transmitted by aerosols. In addition, by placing samplers at specified or consistent distances away from patients we can assess whether larger particles (droplets) can travel more than commonly accepted 4ft distance.
The samplers will run for 3 hours for each collection. They are powered by an air pump which does generate some noise but this is not excessively intrusive. They will be positioned in the following places;

- Hospital setting: One sampler will be placed within 4ft of the patient's bed, at chest height and within a 180 degree angle of the patients face. A further sampler will be placed at a distance of >7ft from the patients bed ideally against the wall opposite the patient, 150cm off the ground. Samplers will be mounted on drip stands.
  If a patient moves out of a side room we will continue nasal swabbing but will stop environmental sampling.

- Household setting: We will only collect samples if we know that a patient will be relatively stationary for the duration of sampling, e.g. in bed. Samplers will be placed as above.

Samples will be taken every other day during a patients follow up from the first day. We expect to have the use of 6 sampling devices but because of equipment and time constraints we will

not be able to perform air sampling around every patient. Over the course of the study we will aim to follow 16 patients.

Based on this we expect to obtain 480 samples in total:

- Hospitalised adults: 4 patients, 4 sampling days (sampling every other day for 8 days), 6 samples each time (3 from each sampler) = 96
- Hospitalised children: 4 patients, 6 sampling days (sampling every other day for 12 days), 6 samples each time (3 from each sampler) = 144
- Adults in their own homes: 4 patients, 4 sampling days (sampling every other day for 8 days), 6 samples each time (3 from each sampler) = 96
- Children in their own homes: 4 patients, 6 sampling days (sampling every other day for 12 days), 6 samples each time (3 from each sampler) = 144

A sample patient schedule can be seen at appendix 2.

4. Stool Sample Collection

Detailed instructions will be provided explaining how to obtain a sample. We will ask the patient to empty their bladder first if possible. They will then place a collecting plate in the toilet bowl which will catch the stool. A sample can then be taken and put in the container. The remaining stool is then tipped into the toilet and flushed away. The plate is disposed of in a rubbish bag. Once the sample container has been securely capped, it should be placed in a specimen bag and kept in a small cooler box (which will be provided). Hand washing / hygiene measures will be stressed. Stool samples will be collected daily along with the other samples.

Sample Processing (stool samples dealt with separately – see below)

The generation of ≈ 3,300 samples for both PCR and PA is a considerable amount of work requiring not just expertise but also significant laboratory resources, including time. Thus, it is not possible to generate results on all samples collected in a short period. We therefore propose to define a sample processing protocol based on results from the first few cases. It could include the following;

- If a patient tests negative for swine flu (as part of NHS care) we will exclude them from further study.
- Environmental swabs will not be processed if nasal swabs from a case are PCR negative.
- Environmental swabs will only be processed if nasal swabs from a case show a high viral load.
- Environmental swabs will not be processed for PA if nasal swabs from a case are PA negative.

Note; samples that are not processed rapidly will be retained for analysis in the future should this be of interest.

Transport and storage of participant samples

Transport
Collected samples will be placed into viral transport medium and kept on 'wet' ice until being frozen at -80^{0}C. For hospital samples freezing would likely happen within 4 hours and community samples within 9 hours. Samples will be carried / transported locally by researchers in dedicated equipment. Samples will be sent to the Cambridge laboratory once each week from each of the 3 centres and will be transported by a professional delivery company.

Storage
Samples will be kept frozen until analysis at the HPA microbiology laboratories, Addenbrookes Hospital, Cambridge. They will be identifiable through participant study codes, participant initials and date of birth. Following analysis all samples will be destroyed. Analysis is expected to be complete by February 2010

Laboratory analyses

Sample Processing
The generation of ≈ 3,300 samples for both PCR and PA is a considerable amount of work requiring not just expertise but also significant laboratory resources, including time. Thus, it is not possible to generate results on all samples collected in a short period. We therefore propose to define a sample processing protocol based on results from the first few cases. It could include the following;

- If a patient tests negative for swine flu (as part of NHS care) we will exclude them from further study.
- Environmental swabs will not be processed if nasal swabs from a case are PCR negative.
- Environmental swabs will only be processed if nasal swabs from a case show a high viral load.
- Environmental swabs will not be processed for PA if nasal swabs from a case are PA negative.

Note; samples that are not processed rapidly will be retained for analysis in the future should this be of interest (note this will happen within the study timecourse).

Laboratory Testing

- HPA Laboratory, Addenbrookes Hospital, Cambridge will be process samples by PCR methods. The contact person is Dr Jane Greatorex.
- University of Cambridge department of pathology, virology laboratory, Addenbrookes Hospital, Cambridge will process samples for virus culture. The contact person is Dr Jane Greatorex.

Samples will be analysed using real-time quantitative PCR and/or plaque assay (PA - quantification of infectious virus present in the sample). Upon defrosting prior to testing, samples will be split for PCR (refrozen) to detect genome and culture to detect viable virus. The PCR assay is a modification of the real-time quadriplex PCR assay for the detection of influenza (VSOP 25) issued by the Standards Unit, Health Protection Agency, Centre for Infections, Colindale, London. The assay will be performed following good laboratory practice, by trained individuals. Appropriate controls, both negative and positive will be included in each run. All machinery and laboratory equipment is maintained to clinical standards by the East of England Regional Health Protection Laboratory.

The plaque assays are performed in the Division of Virology, Department of Pathology, University of Cambridge, following a risk assessed procedure. The laboratories are maintained by the University and are regularly inspected. Both PCR and plaque assays will be performed by trained biomedical scientists.

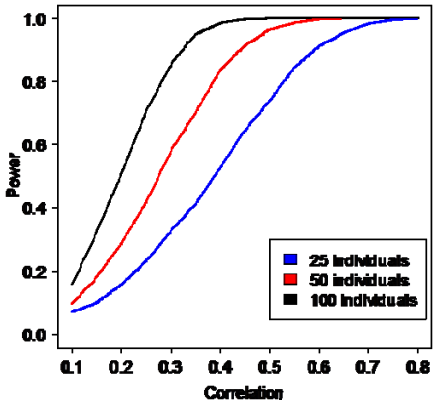
Stool sample processing

Centre for Ecology & Hydrology will process samples using the same PCR methods as determined by Dr. Jane Greatorex. Stool samples will be stored in a -80C freezer located at the Centre for Ecology & Hydrology, Oxford, Mansfield Rd., Oxford, OX1 3SR. Samples will be identifiable through participant study codes and date of birth, as per the nasal swab samples. Following analysis, all samples will be destroyed. Analysis is expected to be complete by February 2010.

11. STATISTICS

Proposed Sample Size

We will aim to recruit groups of about 25 patients with recent onset H1N1 influenza in each of the four main sub-groups identified under 'research methods'. Most statistical analysis will involve examining correlations between virus shedding and virus deposition in the environment. The figure below illustrates that sub-group sizes of 25, which also allow pooling of data by adults or children (50 per group) or the whole population gives high statistical power (>80%) to detect correlations of >0.55 in groups of size n=25, 0.4 in groups of size n=50, and 0.3 in groups of size n=100.



As regards the duration of virus shedding, these data will be primarily descriptive but it will be important to be able to make formal statistical comparisons of the duration of shedding between adults and children. However by pooling data into adults vs. children (n=50 per group) differences of 5 days (adults) vs. 6 days (children) (two tailed-test) could be detected with >80% provided that the coefficient of variation in shedding was 0.3 or less. For larger differences e.g. 5 days vs.7 days or 5 days vs. 8 days, the study is well powered to coefficients of variation up to 0.6.

Statistical Analysis

We will perform a detailed descriptive analysis of the data. The symptom constellation of patients in the different groups will be presented. The mean (standard deviation, range) of the quantity of infectious virus in the patient, on surfaces and in the air will be plotted for each patient group and as a function of time since onset, symptom constellation and distance from source (when relevant). The mean (standard deviation, range) duration of shedding will also be plotted for each patient group and as a function of symptom constellation. For a better representation of inter-individual variation (which is expected to be important), we will also plot individual trajectories.
In a second stage, formal tests will be used to determine which outcomes are significantly associated / correlated. Statistical tests will also be implemented to compare the mean duration of shedding among children and adults as well as among mild and severe cases.
In a third stage, a Generalized Linear Model with random effects will be used to determine the key predictors for the quantity of infectious virus in surfaces and in the air. A survival analysis will also be implemented to assess the key predictors for the duration of viral shedding.

Outcome Measures

1. Virus shedding and deposition as measured by virus culture and quantitative PCR. (Quantitative PCR and plaque assay of respiratory virus specimens (nasal swabs) from patients and surfaces and air around them).Virus shedding and deposition as measured by virus culture and quantitative PCR.
2. Daily symptom scores and patient temperature readings
3. Medication logs
4. Household/ward daily temperature and humidity logs

12. ADVERSE EVENTS

The occurrence of adverse as a result of participation within this study is not expected and no adverse event data will be collected routinely.

13. ETHICAL AND REGULATORY ASPECTS

The study does not raise particular ethical issues as it will not impinge upon normal care provided by the NHS. No personal or sensitive information will be disclosed.

Risks / Benefits

There is no specific treatment benefit as we will not influence participants normal care. The work as a whole is seeking to provide information on swine flu infection that could improve the way we deal with it, particularly from an infection control point of view and the public will benefit from this. Participants may gain some reassurance from the fact that a member of the research team will be visiting each day. However, as stated above they would not interfere directly with normal medical care. Of course, should there be any concerns they will raise them with the participant or their family so they can contact a GP or other responsible medical professional.

The study will not be initiated before the protocol, consent forms and participant and GP information sheets have received approval / favourable opinion from the Research Ethics Committee (REC), and the respective National Health Service (NHS) Research & Development (R&D) department. Should a protocol amendment be made that requires REC approval, the changes in the protocol will not be instituted until the amendment and revised informed consent

forms and participant and GP information sheets (if appropriate) have been reviewed and received approval / favourable opinion from the REC and R&D departments. A protocol amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately providing that the REC are notified as soon as possible and an approval is requested. Minor protocol amendments only for logistical or administrative changes may be implemented immediately; and the REC will be informed.

The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, 1996; the principles of Good Clinical Practice, and the Department of Health Research Governance Framework for Health and Social care, 2005.

Informed consent and participant information

The process for obtaining participant informed consent or assent and parent / guardian informed consent will be in accordance with the REC guidance, and Good Clinical Practice (GCP) and any other regulatory requirements that might be introduced. The investigator or their nominee and the participant or other legally authorised representative shall both sign and date the Consent Form before the person can participate in the study.

The participant will receive a copy of the signed and dated forms and the original will be retained in the Study records. A second copy will be filed in the participant's medical notes (when available) and a signed and dated note made in the notes that informed consent was obtained for the study.

The decision regarding participation in the study is entirely voluntary. The investigator or their nominee shall emphasize to them that consent regarding study participation may be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care, or loss of benefits to which the participant is otherwise entitled. No study-specific interventions will be done before informed consent has been obtained.

The investigator will inform the participant of any relevant information that becomes available during the course of the study, and will discuss with them, whether they wish to continue with the study. If applicable they will be asked to sign revised consent forms.

If the Consent Form is amended during the study, the investigator shall follow all applicable regulatory requirements pertaining to approval of the amended Consent Form by the REC and use of the amended form (including for ongoing participants).

Records

Case Report Forms;

Each participant will be assigned a study identity code number, for use on CRFs, other study documents and the electronic database. The documents and database will also use their initials (of first and last names separated by a hyphen or a middle name initial when available) and date of birth (dd/mm/yy). CRFs will be treated as confidential documents and held securely in accordance with regulations. The investigator will make a separate confidential record of the participant's name, date of birth, local hospital number or NHS number and participant study number, to permit identification of all participants enrolled in the study. CRFs shall be restricted to those personnel approved by the Chief or local Investigator and recorded as such in the study records.' All paper forms shall be filled in using black ballpoint pen. Errors shall be lined out but not obliterated by using correction fluid and the correction inserted, initialled and dated.
The Chief or local Investigator shall sign a declaration ensuring accuracy of data recorded in the CRF.

Source documents;
Source documents shall be filed at the investigator's site and may include but are not limited to, consent forms, study records, field notes, interview transcriptions and audio records. A CRF may also completely serve as its own source data. Only study staff shall have access to study documentation other than the regulatory requirements listed below.

Direct access to source data / documents;
The CRF and all source documents shall made be available at all times for review by the Chief Investigator, Sponsor's designee and inspection by relevant regulatory authorities.

Data protection
All study staff and investigators will endeavour to protect the rights of the study's participants to privacy and informed consent, and will adhere to the Data Protection Act, 1998. The CRF will only collect the minimum required information for the purposes of the trial. CRFs will be held securely, in a locked room, or locked cupboard or cabinet. Access to the information will be limited to the trial staff and investigators and any relevant regulatory authorities (see above). Computer held data including the study database will be held securely and password protected. Access will be restricted by user identifiers and passwords. Information about the study in the participant's medical records / hospital notes will be treated confidentially in the same way as all other confidential medical information. Electronic data will be backed up every 24 hours to both local and remote media in encrypted format.

14. QUALITY ASSURANCE & AUDIT

Insurance and indemnity
Insurance and indemnity for clinical study participants and study staff is covered within the NHS Indemnity Arrangements for clinical negligence claims in the NHS, issued under cover of HSG (96)48. There are no special compensation arrangements, but study participants may have recourse through the NHS complaints procedures.

The University of Nottingham has taken out an insurance policy to provide indemnity in the event of a successful litigious claim for proven non-negligent harm.

Study conduct
Study conduct will be subject to systems audit of the Trial Master File for inclusion of essential documents; permissions to conduct the trial; Study Delegation Log; CVs of study staff and training received; local document control procedures; consent procedures and recruitment logs; adherence to procedures defined in the protocol (e.g. inclusion / exclusion criteria, correct randomisation, timeliness of visits); accountability of study materials and equipment calibration logs.

Study data
Monitoring of study data shall include confirmation of informed consent; source data verification; data storage and data transfer procedures; local quality control checks and procedures, back-up and disaster recovery of any local databases and validation of data manipulation. The Study Coordinator, or where required, a nominated designee of the Sponsor, shall carry out monitoring of study data as an ongoing activity.
Entries on CRFs will be verified by inspection against the source data. A sample of CRFs (10%) will be checked on a regular basis for verification of all entries made. In addition the subsequent

capture of the data on the study database will be checked. Where corrections are required these will carry a full audit trail and justification.
Study data and evidence of monitoring and systems audits will be made available for inspection by the REC as required.

Record retention and archiving
In compliance with the ICH/GCP guidelines, regulations and in accordance with the University of Nottingham Research Code of Conduct, the Chief or local Principal Investigator will maintain all records and documents regarding the conduct of the study. These will be retained for at least 7 years or for longer if required. If the responsible investigator is no longer able to maintain the study records, a second person will be nominated to take over this responsibility.
The study documents held by the Chief Investigator on behalf of the Sponsor shall be finally archived at secure archive facilities at the University of Nottingham. This archive shall include all study databases and associated meta-data encryption codes.

Discontinuation of the trial by the sponsor
The Sponsor reserves the right to discontinue this study at any time for failure to meet expected enrolment goals, for safety or any other administrative reasons. The Sponsor shall take advice as appropriate in making this decision.

Statement of confidentiality
Individual participant medical or personal information obtained as a result of this study are considered confidential and disclosure to third parties is prohibited with the exceptions noted above. Participant confidentiality will be further ensured by utilising identification code numbers to correspond to data in the computer files. Such medical information may be given to the participant's medical team and all appropriate medical personnel responsible for the participant's welfare. Data generated as a result of this study will be available for inspection on request by the participating physicians, the University of Nottingham representatives, the REC, local R&D Departments and the regulatory authorities.

15. PUBLICATION AND DISSEMINATION POLICY

The Department of Health as funder would be involved in the dissemination of any key findings. They have responsibility for public health issues and are tasked with communicating health related messages to the public. It is envisaged that they may find the results of this study critical in underpinning guidance given to the public about minimising influenza transmission. If there was a desire to publicise such information to the media or other organisations in a timely fashion, perhaps in advance of the Department of Health's own comprehensive campaign, the UoN communications office would be in a position to liaise with the Department of Health (or other appropriate agencies) to facilitate this. The UoN has a communications office with extensive experience of disseminating research findings. In addition to liaising with the national and international media and publications industry they are used to working closely with funding bodies and government departments. Prof Van-Tam retains strong links with the Health Protection Agency and its Press Office who have considerable experience in relation to public communication on avian and pandemic influenza. Confidentiality of participants in the study will be maintained and they will not be identified in any publications.

16. USER AND PUBLIC INVOLVEMENT

N/A

17. STUDY FINANCES

This study is funded by HTA programme within the NIHR
Participants will not be paid to participate in the study

18. CHIEF INVESTIGATOR'S SIGNATURE

The Investigators and the Sponsor have discussed and agreed upon the content of this protocol. The Investigators agree to perform this investigation according to protocol and in conformance with GCP, and to abide by this protocol except in the case of medical emergencies or where departures from the protocol are necessary in the interest of subject safety. They agree to give access to all relevant data and records to the monitors, auditors, Clinical Quality Assurance representatives, and regulatory authorities as required.

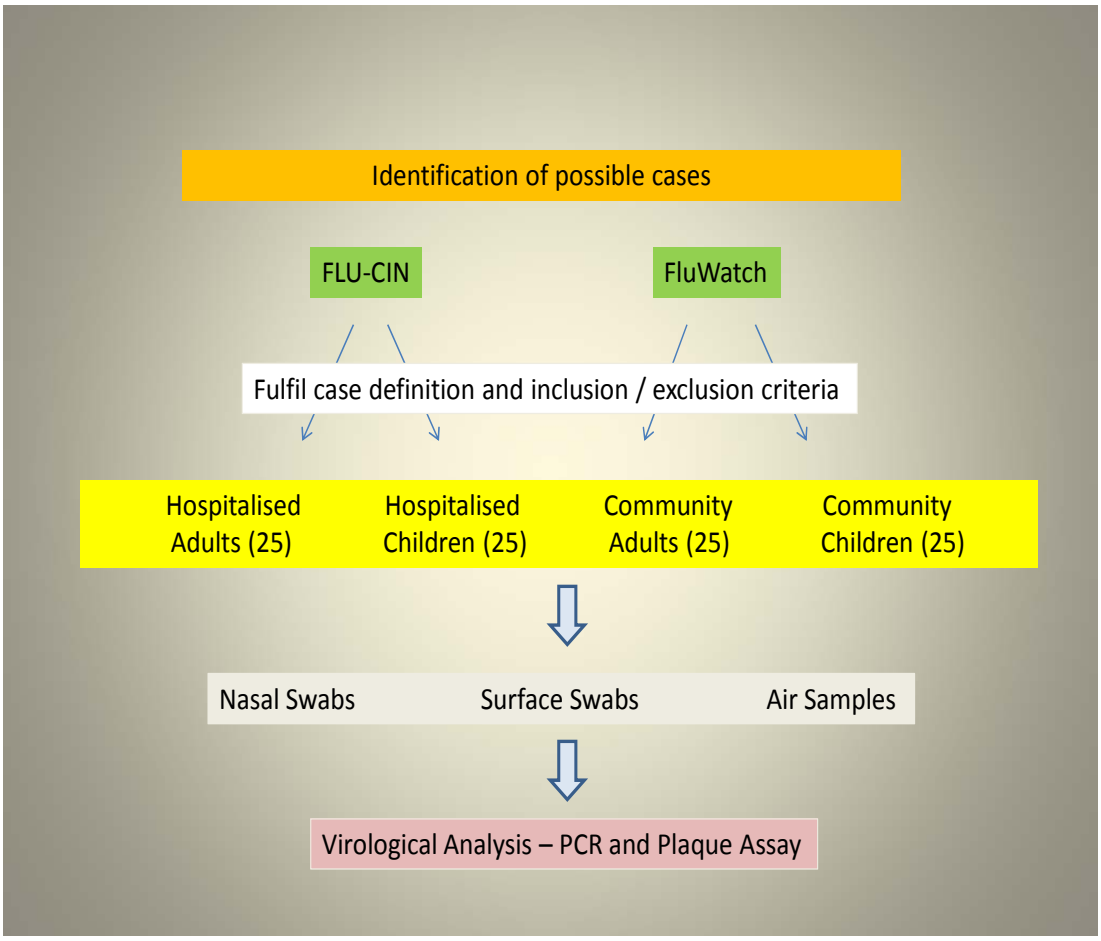
Chief Investigator, Professor Jonathan Van-Tam Date: 27 Aug 2010
MBE, BMedSci, BMBS, DM, FFPH, FRIPH
GMC No. 3241998

19. REFERENCES

1. Brankston, G., L. Gitterman, Z. Hirji, C. Lemieux, and M. Gardam, *Transmission of influenza A in human beings.* Lancet Infect Dis, 2007. 7(4): p. 257-65.
2. Tellier, R., *Review of aerosol transmission of influenza A virus.* Emerg Infect Dis, 2006. 12(11): p. 1657-62.
3. Treanor, J.J., F.G. Hayden, P.S. Vrooman, R. Barbarash, R. Bettis, D. Riff, S. Singh, N. Kinnersley, P. Ward, and R.G. Mills, *Efficacy and safety of the oral neuraminidase inhibitor oseltamivir in treating acute influenza: a randomized controlled trial. US Oral Neuraminidase Study Group.* JAMA, 2000. 283(8): p. 1016-24.
4. Boivin, G., N. Goyette, I. Hardy, F. Aoki, A. Wagner, and S. Trottier, *Rapid antiviral effect of inhaled zanamivir in the treatment of naturally occurring influenza in otherwise healthy adults.* J Infect Dis, 2000. 181(4): p. 1471-4.
5. Leekha, S., N.L. Zitterkopf, M.J. Espy, T.F. Smith, R.L. Thompson, and P. Sampathkumar, *Duration of influenza A virus shedding in hospitalized patients and implications for infection control.* Infect Control Hosp Epidemiol, 2007. 28(9): p. 1071-6.
6. Hayden, F.G., *Prevention and treatment of influenza in immunocompromised patients.* Am J Med, 1997. 102(3A): p. 55-60; discussion 75-6.
7. Bridges, C.B., M.J. Kuehnert, and C.B. Hall, *Transmission of influenza: implications for control in health care settings.* Clin Infect Dis, 2003. 37(8): p. 1094-101.
8. Foy, H.M., M.K. Cooney, I.D. Allan, and J.K. Albrecht, *Influenza B in households: virus shedding without symptoms or antibody response.* Am J Epidemiol, 1987. 126(3): p. 506-15.
9. Hall, C.B., R.G. Douglas, Jr., J.M. Geiman, and M.P. Meagher, *Viral shedding patterns of children with influenza B infection.* J Infect Dis, 1979. 140(4): p. 610-3.

10. Frank, A.L., L.H. Taber, C.R. Wells, J.M. Wells, W.P. Glezen, and A. Paredes, *Patterns of shedding of myxoviruses and paramyxoviruses in children.* J Infect Dis, 1981. 144(5): p. 433-41.
11. Sato, M., M. Hosoya, K. Kato, and H. Suzuki, *Viral shedding in children with influenza virus infections treated with neuraminidase inhibitors.* Pediatr Infect Dis J, 2005. 24(10): p. 931-2.
12. Morens, D.M. and V.M. Rash, *Lessons from a nursing home outbreak of influenza A.* Infect Control Hosp Epidemiol, 1995. 16(5): p. 275-80.
13. Bean, B., B.M. Moore, B. Sterner, L.R. Peterson, D.N. Gerding, and H.H. Balfour, Jr., *Survival of influenza viruses on environmental surfaces.* J Infect Dis, 1982. 146(1): p. 47-51.
14. Loosli, C.G., H.M. Lemon, O.H. Robertson, and E. Appel, *Experimental airborne influenza infection.I. Influence of humidity on survival of virus in air.* Proceedings of the Society of Experimental Biology and Medicine, 1943. 53: p. 205-206.
15. Wells, W.F. and H.W. Brown, *Recovery of Influenza Virus Suspended in Air.* Science, 1936. 84(2168): p. 68-69.
16. Blachere, F.M., W.G. Lindsley, T.A. Pearce, S.E. Anderson, M. Fisher, R. Khakoo, B.J. Meade, O. Lander, S. Davis, R.E. Thewlis, I. Celik, B.T. Chen, and D.H. Beezhold, *Measurement of airborne influenza in a hospital emergency department.* Clinical Infectious Diseases, 2009. 48: p. 438-40.
17. Lee, N., D. Hui, A. Wu, P. Chan, P. Cameron, G.M. Joynt, A. Ahuja, M.Y. Yung, C.B. Leung, K.F. To, S.F. Lui, C.C. Szeto, S. Chung, and J.J. Sung, *A major outbreak of severe acute respiratory syndrome in Hong Kong.* N Engl J Med, 2003. 348(20): p. 1986-94.
18. Lambert, S.B., D.M. Whiley, N.T. O'Neill, E.C. Andrews, F.M. Canavan, C. Bletchly, D.J. Siebert, T.P. Sloots, and M.D. Nissen, *Comparing nose-throat swabs and nasopharyngeal aspirates collected from children with symptoms for respiratory virus identification using real-time polymerase chain reaction.* Pediatrics, 2008. 122(3): p. e615-20.
19. Sung, R.Y., P.K. Chan, K.C. Choi, A.C. Yeung, A.M. Li, J.W. Tang, M. Ip, T. Tsen, and E.A. Nelson, *Comparative study of nasopharyngeal aspirate and nasal swab specimens for diagnosis of acute viral respiratory infection.* J Clin Microbiol, 2008. 46(9): p. 3073-6.
20. Spyridaki, I.S., I. Christodoulou, L. de Beer, V. Hovland, M. Kurowski, A. Olszewska-Ziaber, K.H. Carlsen, K. Lodrup-Carlsen, C.M. van Drunen, M.L. Kowalski, R. Molenkamp, and N.G. Papadopoulos, *Comparison of four nasal sampling methods for the detection of viral pathogens by RT-PCR-A GA(2)LEN project.* J Virol Methods, 2009. 156(1-2): p. 102-6.
21. Heikkinen, T., A.A. Salmi, and O. Ruuskanen, *Comparative study of nasopharyngeal aspirate and nasal swab specimens for detection of influenza.* BMJ, 2001. 322(7279): p. 138.
22. Harper, S.A., J.S. Bradley, J.A. Englund, T.M. File, S. Gravenstein, F.G. Hayden, A.J. McGeer, K.M. Neuzil, A.T. Pavia, M.L. Tapper, T.M. Uyeki, and R.K. Zimmerman, *Seasonal influenza in adults and children--diagnosis, treatment, chemoprophylaxis, and institutional outbreak management: clinical practice guidelines of the Infectious Diseases Society of America.* Clin Infect Dis, 2009. 48(8): p. 1003-32.

Appendix 1 – Study outline



Appendix 2 – Sample patient schedule

Procedures		Day1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
Meets case definition		x											
Fulfils entry criteria		x											
Consent		x											
Symptom diary card		x	x	x	x	x	x	x	x	x	x	x	x
Oral temperature		x	x	x	x	x	x	x	x	x	x	x	x
Room temperature and humidity		x		x		x		x		x		x	
Concomitant meds?		x	x	x	x	x	x	x	x	x	x	x	x
Adverse event?		x	x	x	x	x	x	x	x	x	x	x	x
Nasal swab		x	x	x	x	x	x	x	x	x	x	x	x
Surface swabs		x		x		x		x		x		x	
Air sampling		x		x		x		x		x		x	
Stool sample (optional)		x	x	x	x	x	x	x	x	x	x	x	x

Appendix 3 - Symptom Diary Card

Subject Number	Subject Initials	Date
	F M L	D D M M Y Y Y Y

Time (24 hour) _____ Study Day: ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10 ☐11 ☐12

Place an "X" in the box in each symptom row that best describes how you have felt since completing your last diary card. Grade your symptoms based on the descriptions provided. Use the space to the right to note down any other symptoms you have.

Level	0	1	2	3	Other Symptoms:
Symptoms:	I have NO symptoms	Just noticeable	It's clearly bothersome from time to time, but it doesn't stop me from participating in activities	It's quite bothersome most or all of the time, and it stops me from participating in activities	
Runny Nose					
Stuffy Nose					
Sneezing					
Sore Throat					
Earache					
Sinus Tenderness					
Malaise (tiredness)					
Cough					
Shortness of breath					
Wheezing					
Headache					
Muscles and/or joint ache					