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

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Abstract

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Background: The relative importance of different routes of influenza transmission, including the role of bioaerosols, and ability of masks and/or hand hygiene to prevent transmission, remains poorly understood. Current evidence suggests that infectious virus is not typically released from adults after 5 days of illness, however, little is known about the extent to which virus is deposited by infected individuals into the environment and whether deposited virus has the ability to infect new hosts. Further information about the deposition of viable influenza virus in the immediate vicinity of patients with pandemic influenza is fundamental to our understanding of the routes and mechanisms of transmission.

Objectives: To collect data on patients infected with pandemic H1N1 2009 (swine flu). Primary objectives were to correlate the amount of virus detected in a patient’s nose with that recovered from his/her immediate environment, and with symptom duration and severity. Secondary objectives were to describe virus shedding and duration according to major patient characteristics: adults versus children, and those with mild illness (community patients) versus those with more severe disease (hospitalised patients).

Methods: Adults and children, both in hospital and from the community, who had symptoms of pandemic H1N1 infection, were enrolled and visited every day during follow-up for a maximum of 12 days. Symptom data was collected and samples were taken, including nose swabs and swabs from surfaces and objects around patients. Samples of air were obtained using validated sampling equipment. The samples were tested for the presence of pandemic H1N1 virus, using polymerase chain reaction (PCR) to detect virus genome and an immunofluorescence technique to detect viable virus.

Results: Forty-three subjects were followed up, and 19 of them were subsequently proven to be infected with pandemic H1N1 virus. The median duration of virus shedding from the 19 infected cases was 6 days when detection was performed by PCR, and 3 days when detection was performed by a culture technique. Over 30% of cases remained potentially infectious for at least 5 days. Only 0.5% of all community and none of the hospital swabs taken revealed virus on surfaces. Five subjects had samples of the air around them collected and virus was detected by PCR from four; some of the air particles in which virus was detected were small enough to be inhaled and deposited deep in the lungs.

Limitation: Small number of subjects recruited.

Conclusions: The finding that over 30% of infected individuals have infectious virus in their noses for 5 days or more has infection control implications. The data suggest that contact transmission of pandemic influenza via fomites may be less important than previously thought, but transmission via bioaerosols at short range may be possible, meaning that high-level
personal protective equipment may be needed by health-care workers when attending patients with pandemic influenza. Further work is being undertaken to consolidate these findings, as they have important potential implications for the protection of health-care workers and the formulation of advice to households, nationally and internationally.
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<tr>
<td>AC</td>
<td>adult in the community</td>
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<tr>
<td>AH</td>
<td>adult in hospital</td>
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<tr>
<td>ARI</td>
<td>acute respiratory infection</td>
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<td>CC</td>
<td>child in the community</td>
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<tr>
<td>CH</td>
<td>child in hospital</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
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<tr>
<td>HA</td>
<td>haemaglutinin</td>
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<td>HPA</td>
<td>Health Protection Agency</td>
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<tr>
<td>ILI</td>
<td>influenza-like illness</td>
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<tr>
<td>LRT</td>
<td>lower respiratory tract</td>
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<tr>
<td>MDCK</td>
<td>Madin-Darby Canine Kidney</td>
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<tr>
<td>NIHR</td>
<td>National Institute for Health Research</td>
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<tr>
<td>NIOSH</td>
<td>National Institute of Occupational Safety and Health</td>
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<tr>
<td>NPA</td>
<td>nasopharyngeal aspirate</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>PCT</td>
<td>Primary Care Trust</td>
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<tr>
<td>PPE</td>
<td>personal protective equipment</td>
</tr>
<tr>
<td>RSV</td>
<td>respiratory syncitial virus</td>
</tr>
<tr>
<td>SFM</td>
<td>serum-free medium</td>
</tr>
<tr>
<td>TCID</td>
<td>tissue culture infectious dose</td>
</tr>
<tr>
<td>URT</td>
<td>upper respiratory tract</td>
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<tr>
<td>VTM</td>
<td>viral transport medium</td>
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All abbreviations that have been used in this report are listed here unless the abbreviation is well known (e.g. NHS), or it has been used only once, or it is a non-standard abbreviation used only in figures/tables/appendices, in which case the abbreviation is defined in the figure legend or in the notes at the end of the table.
Executive summary

Background
The threat posed by pandemic influenza is high on the agenda of health-care organisations and governments around the world. As pandemic mitigation strategies have been developed over recent years it has become very clear that influenza transmission is an area that is poorly understood and hotly debated. The biggest controversy relates to whether influenza is mainly transmitted by touching virus deposited on surfaces, or by droplets or bioaerosols in the air. If touch is important then hand washing offers a major defence. If droplets are important, simple barriers, such as a surgical mask, will stop transmission. But if bioaerosols are important, specialised respirators are needed. Thus, infection control guidance is difficult to formulate and mainly based on weak evidence. Current evidence suggests that infectious virus is not typically released from adults after 5 days of illness (slightly longer in children). However, little is known about the extent to which virus is deposited by infected individuals into the environment and whether deposited virus has the ability to infect new hosts, i.e. whether it remains viable. The generation of information about the deposition of viable influenza virus in the immediate vicinity of patients with pandemic influenza is fundamental to our understanding of the routes and mechanisms of transmission.

Objectives
This study was conducted to collect data on patients who had pandemic H1N1 2009 infection (swine flu). The primary objectives were to correlate the amount of virus detected in a patient’s nose with that recovered from his/her immediate environment (on fomites and in the air), and with symptom duration and severity. Secondary objectives were to describe virus shedding and duration according to major patient characteristics: adults versus children, and those with mild illness (community patients) versus those with more severe disease (hospitalised patients).

Methods
Adults and children, both in hospital and from the community, who had symptoms of pandemic H1N1 infection, were enrolled and visited every day during follow-up for a maximum of 12 days. Information about symptoms was collected and samples were taken, including nose swabs and swabs from surfaces and objects (fomites) around patients (e.g. door handles, remote controls). Samples of air were obtained using validated sampling equipment. These samples were tested for the presence of pandemic H1N1 virus, using polymerase chain reaction (PCR) to detect virus genome and an immunofluorescence technique to detect viable (live) virus.

Results
Forty-three subjects were followed up, and 19 of them were subsequently proven to be infected with pandemic H1N1 virus. The median duration of virus shedding from the 19 infected cases was 6 days when detection was performed by PCR, and 3 days when detection was performed by a culture technique. Over 30% of cases remained potentially infectious for at least 5 days. However, contrary to conventional understanding, virus shedding was not always greatest when an individual was most symptomatic. Few fomites were found to be contaminated with virus – in fact only 0.5% of all community and none of the hospital swabs revealed virus. Five subjects had samples of the air around them collected and virus was detected by PCR from four. Some of the air particles in which virus was detected were small enough to be inhaled and deposited deep in the lungs.

Conclusions
Despite some limitations caused by the small number of subjects recruited, important observations have been made. The finding that over 30% of infected individuals have infectious virus in their noses for 5 days or more has infection...
control implications. The evidence for the significance of both contact and bioaerosol routes of transmission, depends upon demonstrating that viable virus is deposited from an infected patient. This has been shown for touched fomites. Virus has been demonstrated by PCR in air samples, but the results of live virus testing are inconclusive. The data generated suggest that contact transmission of pandemic influenza via fomites may be less important than hitherto emphasised, whereas transmission via bioaerosols at short range may be possible, meaning that high-level personal protective equipment (PPE) might be needed by health-care workers when attending patients with pandemic influenza. Further work is being undertaken to consolidate these findings as they have important potential implications for the protection of health-care workers and the formulation of advice to households, nationally and internationally.
Chapter I
Introduction

As pandemic mitigation strategies have been developed over recent years it has become very clear that influenza transmission is one area that is poorly understood and hotly debated. Distinguishing the relative importance of the various modes of transmission (Box 1) is critical for the development of infection control precautions in health-care settings and in the home.

If contact transmission is dominant then hand hygiene becomes the most critical intervention. However, if respiratory droplet transmission is significant, surgical face masks that provide a barrier against droplets may be important, and the safe distance away from an infected person without a mask might be as close as 4 feet (ft), because droplets fall out of the air quickly and do not travel far. At present, opinions are sharply divided on the importance of bioaerosol transmission.1,2 Tellier3 in particular, argues that the potential of short-range bioaerosol transmission has largely been ignored. At present, the UK recommends droplet precautions as opposed to bioaerosol precautions (surgical masks rather than respirators) for most forms of contact with patients with pandemic influenza,3 based on the current balance of limited evidence; however, this is contested by some frontline health-care workers who believe that these safeguards are inadequate, and there is little evidence with which to reassure them.

In parallel, the dynamics of viral shedding in relation to symptom onset and severity are important factors, highly relevant to estimates of the period of infectivity and to therapeutic management. 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, i.e. virus has been recovered by a deliberately performed invasive technique. These so called ‘viral shedding’ studies measure virus shed from infected cells; they do not actually measure virus that is deposited into the touched or respired environment; i.e. they do not define environmental contamination and the hazard posed to others. While such data are useful, if they could be linked to near-patient environmental sampling, estimates of the extent to which infectious virus is deposited on to surfaces and into the air in the patient’s immediate vicinity could be made.

Background data

It is well established that viral titres in nasopharyngeal samples taken from adults are proportional to symptom severity and decline steadily from symptom onset.4–7 Studies in the community of patients who are infected with influenza A show that the mean duration of viral shedding [as measured by polymerase chain reaction (PCR)] for seasonal influenza A viruses is 5–6 days from symptom onset6,7 compared with culture methods that are normally negative by day 6.4,5 It is also well documented that children, patients with chronic illnesses and the immunocompromised can shed live virus for longer periods.8–11 Published data are now available that describe viral shedding from patients with pandemic H1N1 virus infection. Shedding (as determined by nasal sampling) detectable by PCR lasts for approximately 6 days,12–15 but culture-positive specimens, i.e. detecting viable virus, appear rare after 5 days of illness.15,16

While PCR is almost certainly more sensitive because it detects both viable and non-viable virus, its interpretation is far more problematic because it is not possible to determine the presence of viable (transmissible) virus from this technique; it can be used only to illustrate the potential for viable virus to be present. However, there have also been difficulties in deciphering studies looking at live virus because of the range of techniques used for detection (cell lines, animal models and human subjects) and variation in sensitivities between, and even within, such methods, for example a human infectious dose is likely to differ from a tissue culture infectious dose (TCID).

Fomites

A role for fomites, including surfaces, in the transmission of influenza A appears widely accepted but limited data are available to directly support the possibility of contact transmission.
of influenza. In contrast, studies of rhinovirus\textsuperscript{17} and respiratory syncytial virus (RSV)\textsuperscript{18} have shown contact transmission to be significant. Furthermore, there is a paucity of scientific data on virus survival on fomites. An experimental study of influenza virus survival on a range of porous and non-porous surfaces is often cited, but was conducted over 25 years ago.\textsuperscript{19} In this study both influenza A (H1N1) and B viruses could be cultured from experimentally contaminated, non-porous surfaces, such as steel and plastic, for between 24 and 48 hours. However, they survived for < 12 hours on porous materials such as cloth, paper and tissues. Viable virus could be transferred from non-porous surfaces to hands for 24 hours, and from tissues to hands for 15 minutes, but live viruses could be recovered from hands only within 5 minutes of their transfer. Banknotes have been experimentally contaminated with influenza A viruses, and live virus has been shown to be present for up to 3 days, although this period of time was dependent on the concentration of inocula. Interestingly, the presence of respiratory mucus significantly increased survival times.\textsuperscript{20} Other studies have looked at fomite contamination in the environment of individuals with acute respiratory infections (ARIs), but they have either not looked for or not found viable influenza virus.\textsuperscript{21,22}

**Air**

If influenza virus can transmit via bioaerosols then we would expect to be able to detect virus in such aerosols, and we might expect to find evidence of long-range transmission of infection. Studies performed over 40 years ago showed that artificially aerosolised influenza could be recovered (by using infection in animals as a detection method) for up to 24 hours after release,\textsuperscript{23,24} and that aerosolised virus is able to infect humans.\textsuperscript{25} More recently, influenza virus was detected by PCR in aerosol samples taken from medical facilities.\textsuperscript{26,27} Despite the above, the detection of live virus in aerosols, generated by humans has not been demonstrated before. In addition, there is a striking absence of robust epidemiological proof for the long-range transmission of influenza. Studies that have reported such an occurrence\textsuperscript{28,29} are confounded by the fact that droplet and contact transmission cannot be excluded. However, it must also be said that literature claiming that bioaerosols are unlikely to play a significant role have often ignored the potential for short-range bioaerosol transmission.\textsuperscript{2,30}

Assimilating the available evidence leads us to conclude that infectious virus is not typically

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**BOX 1 Definitions**

Airborne transmission has generally been used to refer to infections that spread over long distances through particles in the air, for example tuberculosis. Only bioaerosols (aerosols that contain living organisms) suspended in the air can travel over long distances but some confusion can arise because:

- droplets could also be considered to be airborne, although only for a short period of time and over short distances
- bioaerosols can transmit infection over short distances as well as long; in fact, because bioaerosols are more concentrated nearer their source, they are more likely to transmit over short distances than long

Because of this confusion, we prefer the terms respiratory and contact transmission to ‘airborne transmission’ when discussing influenza.

Respiratory transmission can include:

- **Bioaerosol transmission** Bioaerosols are particles typically < 5 µm in diameter, which carry microorganisms and are capable of both remaining suspended for long periods and travelling distances greater than 6 ft. They can be generated by coughing, talking and even breathing and may transmit infection on being inhaled into the respiratory tract (reviewed by Tellier\textsuperscript{2})
- **Droplet transmission** Respiratory droplets are larger particles (≥ 20 µm) that fall out of circulation typically within 3–4 ft. They are generated by coughing and sneezing, and transmit infection on coming into contact with the respiratory tract, often the mucous membranes of the nose and mouth (reviewed by Nicas et al.\textsuperscript{31})

It should be recognised that there is no absolute cut-off between aerosols and droplets; particles lie on a continuum, with larger particles tending towards droplet behaviour

Contact transmission concerns physical contact with respiratory secretions, for example hands coming into contact with contaminated fomites or person-to-person contact, such as a handshake. We recognise that traditionally this type of contact has been referred to as ‘indirect contact’ and that droplets have been regarded as a form of direct contact transmission, but we find the term ‘contact transmission’ more intuitive.
released from adults after 5 days of illness (slightly longer in children), and that little is known about deposition patterns and persistence of virus released into the environment or its ability to infect new hosts. The generation of information about the presence of viable influenza virus in the environment is fundamental to our understanding of the routes and mechanisms of transmission. This study was therefore conducted to collect data on conventional virus shedding and environmental contamination (fomites and air), and to investigate the relationships between them.
Chapter 2
Methods

This multicentre, prospective, observational descriptive cohort study recruited subjects between 14 September 2009 and 25 January 2010, in accordance with the principles of the Declaration of Helsinki and UK regulatory requirements. It was approved by Leicestershire, Northamptonshire & Rutland Research Ethics Committee 1 (09/H0406/94).

Research objectives

The primary objectives were to correlate the amount of virus detected in a patient’s nose with:

1. that recovered from the environment around them
2. symptom duration, and
3. symptom severity.

Secondary objectives were to describe virus shedding and duration according to important patient subgroups: adults versus children, and those with mild illness (community patients) versus those with more severe disease (hospitalised patients). An additional secondary objective concerned the environmental deposition of virus in association with aerosol-generating procedures.

A number of ‘policy’ objectives were also stated, which included: (1) ‘safety distances’ around patients with pandemic and seasonal influenza; (2) appropriate use of respiratory personal protective equipment (PPE) and infection control practices for pandemic and seasonal influenza, according to patient type, illness severity and time since symptom onset; and (3) antiviral treatment duration for patients with pandemic influenza. Due to a lack of data, these points cannot be adequately addressed and are therefore not discussed further in this report.

Participants

Subjects were recruited from the following groups:

- adults in hospital (AH)
- children in hospital (CH): age range 1 month–16 years
- adults in the community (AC)
- children in the community (CC): age range 1 month–16 years.

Recruiting centres were Nottingham University Hospitals NHS Trust (AH + CH), Nottingham City Primary Care Trust (PCT) (AC + CC), Nottingham County PCT (AC + CC), Leicester University Hospitals NHS Trust (AH) and Sheffield Teaching Hospitals NHS Trust (AH). [Note: the designation AH and CH denote that the patient (adult or child, respectively) was enrolled during hospital admission. However, subjects discharged from hospital before the end of follow-up were then seen in the community; so, while initial environmental specimens will have been taken in hospital, later ones will be from the subject’s home. No subjects initially enrolled in the community were subsequently admitted to hospital.]

Sampling frames

- Hospital All cases of suspected pandemic H1N1 influenza identified to researchers by clinical care teams who had agreed to be approached by a researcher. Hospitals involved in recruitment were: Queens Medical Centre and City Hospital, Nottingham; Leicester Royal Infirmary; and Royal Hallamshire Hospital, Sheffield.
- Community Individuals living in the Nottingham area, who had symptoms of pandemic H1N1 virus infection, received an invitation to take part in the research and had use of a telephone. Invitations were given by the following methods: local newspapers, posters sited in community areas, 3000 leaflets posted in the NG2 area, 15,000 letters given to parents via schools, and 3000 invitations given out at antiviral collection points in areas covered by Nottingham City and Nottinghamshire County PCTs.

A formal sampling fraction was not used to identify cases.
Eligibility criteria

Subjects were eligible to take part if they fulfilled our definition of influenza-like illness (ILI):

- fever (or recent history of fever) plus any one of cough, sore throat, runny nose, fatigue or headache

or

- any two of cough, sore throat, runny nose, fatigue or headache.

Exclusions

Subjects were excluded if they: had experienced illness for > 48 hours (community cases) or > 96 hours (hospital cases); were PCR-negative for pandemic H1N1 (as part of NHS care); had taken part in influenza research involving an investigational medicinal product within the last 3 months (including vaccination). See Appendix 4, Eligibility checklist.

Enrolment

Informed consent was obtained and an influenza rapid antigen test (Quidel QuickVue® Influenza A+B test) was performed on a nasal swab. A positive rapid antigen test was initially an inclusion criterion, but it was abandoned as an entry requirement after 2 weeks because of perceived low sensitivity (see Discussion, below).

A subject was defined as a case if:

- he/she met our criteria for ILI, and
- tested PCR-positive on a nasal swab for pandemic H1N1.

It had not been our intention to recruit and follow up patients who were pandemic H1N1-negative, but this did occur. Data on these subjects are presented below (see Results).

Study procedures

Adult subjects were followed for up to 10 days from the start of symptoms and children < 13 years of age were followed for up to 12 days. In addition to collecting initial symptom data to confirm a subject’s eligibility, daily records of were taken of symptoms, temperature readings, medications, bioaerosol-generating procedures (if hospitalised), room temperature and humidity. A symptom diary was completed by each subject on a daily basis; symptoms were given a severity score on a scale of 0–3 (see Appendix 1, Symptom diary card). The following samples were collected:

- **Daily nasal swabs** A dry cotton swab with a polystyrene shaft (FB57835, Fisherbrand) was passed around one nostril in a circular motion three times and then immersed in viral transport medium (VTM).

- **Surface swabs** Samples were taken approximately every other day during the period of follow-up. Three surfaces were swabbed in hospital rooms: patient table; Patientline® console or nurse call button and window sill. In the home, samples were taken from the dining table, kettle handle, TV remote control, bedside table, bathroom tap and bathroom door handle. Cotton swabs with polystyrene shafts (FB57835) were moistened with VTM and then rubbed across a maximum area of 4 × 5 cm² in three different directions, applying even pressure. The same part of any fomite was swabbed each day. This sampling method was validated during a previous study (B Killingley, University of Nottingham, May 2010, personal communication). In addition to using swabs, the use of sponges was trialled to sample the patient or bedside tables. The sponges (TS/15-B:PBS, Technical Service Consultants) were 50 cm² in size, sterile, and dosed with 10 ml of a neutralising buffer. They were wiped over a 4 × 5 cm² area (a different area to that sampled by swab) and then sealed in a sterile medical grade plastic bag. No specific cleaning instructions were given to households, and hospital cleaning continued as normal during follow-up of any subjects. If other household members became ill during the period of follow-up, sampling of the original participant continued, and the age and symptoms of any potential secondary cases were recorded.

Swabs (in VTM) and sponges were kept on ‘wet’ ice for no longer than 3 hours before being frozen at −80°C.

Air particles were collected using a National Institute for Occupational Safety and Health (NIOSH) two-stage cyclone bioaerosol sampler, which has been validated for use with influenza. The first stage of the sampler has a 3-mm inlet, a 6-mm outlet and a disposable 15-ml collection tube (35–2096, Falcon). The second stage has a 1.3-mm inlet, a 2.5-mm outlet and a disposable 1.5-ml tube
The samples then pass through a 37-mm polytetrafluoroethylene (PTFE) filter with 2-mm pores (225–27–07, SKC). At 3.5 l/min, the first stage will collect particles with a diameter > 4 µm, the second stage collects particles with a diameter of 1–4 µm, and the filter collects particles with a diameter of < 1 µm. The sampler conforms to the American Conference of Governmental Industrial Hygienists/International Organization for Standardization criteria for respirable particle sampling. The flow rate through each sampler was set with a flow calibrator (Model 4143, TSI) before use. Samplers were mounted on tripods at a height of 150 cm, were placed at distances of either 3 ft or 7 ft from the subject, and ran for either 1, 2 or 3 hours. Not all subjects were stationary during the sampling period (though they were asked to remain in the same position if they could), so the distance from the subject to the sampler may have varied a little over time. Sampling was performed on just one follow-up day. After sampling, intact samplers were taken straight to a laboratory, where 750 µl of VTM was added to both stage-one and stage-two tubes, and the filter paper was immersed in a 15-ml tube, also containing 750 µl of VTM. These procedures were carried out in sterile conditions, under a microbiological safety hood. Samples were then stored at –80°C.

**Laboratory methods**

The following sample-processing ‘rules’ were instituted:

- Nasal swabs from day 4 onwards were not tested if days 1–3 were all PCR-negative.
- Culture was only performed on PCR-positive samples.
- Environmental swabs were not processed if nasal swabs, taken on the three previous days from a case, were PCR-negative.
- Sponges were tested on day 1 only.

Laboratory work was carried out at Health Protection Agency (HPA) and University of Cambridge virology laboratories at Addenbrooke’s Hospital, Cambridge, UK. Each sample was defrosted and split into six aliquots – three for PCR and three for culture – and then refrozen at –70°C. On the day of testing, the sponges were defrosted and the liquid removed by squeezing the sponge within its bag. The liquid was separated into aliquots for testing. PCR was performed once the RNA was extracted and samples for potential culture were refrozen at –80°C.

**Polymerase chain reaction**

Nucleic acid was extracted from the samples using the Qiagen Symphony SP extractor mini kits, including onboard lysis and a bacteriophage (MS2) as internal control. A novel influenza A H1N1 pentaplex assay was devised to detect virus genome in the samples. The assay was designed to detect novel H1N1 influenza A, seasonal H1 influenza A, seasonal H3 influenza A, influenza B, and the internal control, MS2. Details of the primers, probes and protocol used can be found in Appendix 6 (see PCR protocol). Reactions were carried out on a Rotorgene™ 6000 (Corbett Research) real-time DNA detection system. Viral load data were generated using the PCR assay and plasmids containing the gene target to create a standard curve, such that the concentration of genome present in each sample could be calculated.

**Culture**

Cultures were performed from the last day of nasal swab PCR positivity, for example if a swab was PCR positive on day 5, cultures were performed in the following order: day 5, 4, 3, 2 and 1. If a culture was positive on any given day then an assumption was made that previous days would also have been culture-positive and no further testing was done. Pandemic H1N1 did not form plaques readily and gave only a weak cytopathic effect, the latter meant that the TCID of 50 was difficult to calculate. Consequently, immunofluorescence to detect the influenza A nucleoprotein was used to demonstrate the presence of live replicating virus in the nuclei of cultured nasopharyngeal cells. See Appendix 6 (Culture protocol) for further details.

Genomic sequencing was performed by Geneservice™.

**Outcome measures**

1. *Virus shedding (nose swab) and environmental deposition (fomites and air) as measured by PCR and virus culture techniques*. Laboratory confirmation was defined as a positive result of any specimen tested for pandemic H1N1 virus. The duration of viral shedding was defined as the time between symptom onset and the last day that a
positive specimen was taken. Because patients were seldom recruited on the day symptoms began, an assumption has been made that they were shedding virus from the first day of symptoms to the last positive specimen.

2. **Daily symptom scores** Each symptom score within a category is summed to give an overall category score, for example cough – 2, shortness of breath – 1 = lower respiratory tract (LRT) score of 3.
   - upper respiratory tract (URT) score – stuffy nose, runny nose, sneezing, sore throat, sinus tenderness, earache
   - LRT score – cough, shortness of breath
   - systemic score – fatigue, myalgia, headache
   - total symptom score is the sum of URT, LRT and systemic symptom scores, plus a score for diarrhoea and a score for vomiting.

3. **Medication logs** If the day symptoms began is assigned as day 1, then we have assumed that patients received oseltamivir within 48 hours if they received it on or before day 3.

### Statistical methods

The recruitment target was 100 subjects in total, comprising approximately 25 patients in each of the four groups. Statistical analysis was planned to examine correlations between virus shedding and virus deposition in the environment. Subgroup sizes of 25 [which 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 \( n = 100 \). Viral shedding data is primarily descriptive, but it was important to be able to make formal statistical comparisons of the duration of shedding between adults and children. By pooling data into adults versus children (n = 50 per group), a difference of one day (two tailed-test) could be detected with power > 80%, provided that the coefficient of variation in shedding was ≤0.3. For larger differences, for example 2 or 3 days, the study was well powered to coefficients of variation up to 0.6.

A detailed descriptive analysis of the data is presented. The Student’s t-test was used to compare mean values. The Pearson’s product–moment correlation test was used to test associations between variables. Fisher’s exact test was used to test the significance of risk ratios.

### Changes to protocol

**Minor amendments to protocol 1.0:**
- application of corrected document version numbers to adult and parent/guardian consent forms
- creation of a new study document: ‘letter to ward managers’
- abandonment of a positive influenza rapid antigen test as an inclusion criterion.

**Substantial amendment resulting in protocol version 1.1:**
- addition of stool sample collection for a substudy involving The Centre for Ecology & Hydrology. (Note, this substudy did not ultimately take place.)
- clarification of the role of clinical teams in recruiting patients.

**Minor amendment to protocol 1.1:**
- creation of new study documents: ‘letter to parent/guardians’, ‘study poster’ and ‘study leaflet’
- extended study duration to 31 August 2010
- extended virology testing on samples already collected.
Chapter 3

Results

One hundred and fifty subjects were screened between 14 September 2009 and 25 January 2010; 107 were ineligible, and 43 were enrolled and followed up. Reasons for exclusion at screening included: symptoms being present for too long (48%), influenza PCR test (as part of medical care)-negative (15%), declined to take part (9%). Pandemic H1N1 virus was detected in 19 subjects. The group of 24 pandemic-negative cases consisted of: RSV = 5 (all children); rhinovirus = 5; coronavirus = 2; rhinovirus + coronavirus = 1; NHS-pandemic H1N1 test-positive, study laboratory pandemic H1N1 test-negative = 2; unknown = 9.

In the final analyses, one subject was excluded on the basis of having received pandemic H1N1 vaccine prior to enrolment, and three subjects were removed (all of whom tested negative for pandemic H1N1 according to the study laboratory); two because clinical (as part of medical care) and study pandemic H1N1 2009 PCR tests did not agree; and one because study documents were lost. Recruitment by group of the 39 remaining subjects was as follows: 9 AC, 12 AH, 15 CC and 3 CH (Figure 1).

Of the remaining 39 subjects, 19 (49%) tested positive for pandemic H1N1 virus and 20 (51%) were negative. Follow-up of at least 8 days occurred in 16/19 positives and 12/20 negatives. The numbers enrolled, along with a demographic description of pandemic H1N1 cases, is shown in Table 1.

Pandemic H1N1 cases

Of the 19 cases recruited, 10 (53%) were female, 11 (58%) were children and 11 (58%) were community cases. Seven subjects reported comorbidities and in six cases these were respiratory conditions. Table 2

---

FIGURE 1 Participant flow diagram.
Results

Lists the 19 cases of pandemic H1N1 recruited into the study and shows some of the key outcome measures for each. No recruited cases needed high-dependency care or died during follow-up.

Symptoms

The most frequently reported symptoms in our subjects with pandemic H1N1 were: stuffy nose (100%), runny nose (100%), cough (100%), fatigue (95%) and sneezing (89%) (Table 3). Fever was reported on the day illness began in 13/19 (68%) cases, and was measured as high (≥38°C) during follow-up in 7/19 (37%) of cases.

In general, symptom scores declined over time. URT and systemic symptoms peaked on day 2 of illness and LRT symptoms peaked on day 3. However, it should be noted that most subjects were recruited >36 hours after illness onset, which may give misleading information on maximal symptom scores; there was only one patient with information available on day 1, and only five for day 2 (Figure 2a). Figure 2b shows mean symptom scores of subjects with pandemic H1N1 influenza as a function of the number of days since illness onset.

In a comparison of subjects who were positive for pandemic H1N1 infection with others recruited, no significant difference was seen in the average time from symptom onset to treatment initiation: positive cases (1.7 days), others (1.7 days) (p = 0.90). Visual inspection of plots showing mean symptom scores (broken down into categories) over time suggests that subjects who were negative for pandemic H1N1 infection had higher URT symptom scores and LRT symptoms that peaked 3 days after pandemic H1N1-positive subjects (Figure 3). However, no significant differences between these two groups were detected when comparing symptoms scores on the day of recruitment (URT p = 0.11, LRT p = 0.18 or systemic symptoms p = 0.20) or in the total mean symptom score over time (46.5 for subjects with pandemic H1N1 vs 52.3 for others, p = 0.54).

Antiviral drugs

Overall, 21/39 (54%) of enrolled subjects took an antiviral drug [either oseltamivir (20/21) or zanamivir (1/21)] and this occurred within 2 days of illness onset in 12/17 cases (71%) for which data are available. Of the pandemic H1N1-positive cases, 11/19 (58%) received an antiviral drug (all oseltamivir); hospital cases 7/8 (88%) and community cases 4/11 (36%). A total of 44% of pandemic H1N1 cases took oseltamivir within 48 hours, and the average time from symptom onset to treatment initiation in these subjects was 1.7 days (data on when treatment was begun for one patient is not available). The mean total symptom score on the first day of enrolment in

<table>
<thead>
<tr>
<th>TABLE 1 Numbers enrolled and overall demographic description of subjects with pandemic H1N1 influenza</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Enrolled</td>
</tr>
<tr>
<td>Excluded/removed from analyses</td>
</tr>
<tr>
<td>Pandemic H1N1-positive subjects</td>
</tr>
<tr>
<td>Pandemic H1N1 subjects only</td>
</tr>
<tr>
<td>Male sex (%)</td>
</tr>
<tr>
<td>Median age (years), range</td>
</tr>
<tr>
<td>Ethnic group</td>
</tr>
<tr>
<td>White</td>
</tr>
<tr>
<td>Black</td>
</tr>
<tr>
<td>Asian</td>
</tr>
<tr>
<td>Mixed</td>
</tr>
<tr>
<td>Mean time from symptom start to enrolment (days)</td>
</tr>
</tbody>
</table>
| Subject | Age (years) | Sex (M/F) | Ethnicity | Comorbidity         | Time from symptom onset to enrolment (days) | Peak total symptom score (day of follow-up) | Peak viral load (day of follow-up) | Duration of viral shedding by PCR (days) | Last day culture-positive by IF | Day oseltamivir begun
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
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<td>21</td>
<td>F</td>
<td>Asian</td>
<td>Nil</td>
<td>2</td>
<td>6 (1)</td>
<td>4.2 x 10^5 (1)</td>
<td>6</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>AC04</td>
<td>28</td>
<td>F</td>
<td>Black</td>
<td>Asthma</td>
<td>1</td>
<td>13 (1)</td>
<td>1.3 x 10^6 (1)</td>
<td>3</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>AH01</td>
<td>19</td>
<td>F</td>
<td>White</td>
<td>Cystic fibrosis</td>
<td>2</td>
<td>13 (1)</td>
<td>1.8 x 10^6 (6)</td>
<td>9</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>AH03</td>
<td>27</td>
<td>F</td>
<td>Asian</td>
<td>Nil</td>
<td>2</td>
<td>28 (1)</td>
<td>1.0 x 10^6 (1)</td>
<td>9</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>AH04</td>
<td>30</td>
<td>F</td>
<td>Asian</td>
<td>Nil</td>
<td>2</td>
<td>17 (1)</td>
<td>1.6 x 10^7 (6)</td>
<td>10</td>
<td>8</td>
<td>3</td>
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<tr>
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<td>Asian</td>
<td>Nil</td>
<td>3</td>
<td>12 (1)</td>
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<td>5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>AC01</td>
<td>21</td>
<td>F</td>
<td>Asian</td>
<td>Nil</td>
<td>2</td>
<td>20 (6)</td>
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<td>5</td>
<td>4</td>
<td>N/A</td>
</tr>
<tr>
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<td>Black</td>
<td>Asthma</td>
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<td>25 (1)</td>
<td>3.9 x 10^4 (1)</td>
<td>3</td>
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<td>2</td>
</tr>
<tr>
<td>CC01</td>
<td>12</td>
<td>M</td>
<td>Mixed</td>
<td>Asthma</td>
<td>2</td>
<td>18 (1)</td>
<td>1.8 x 10^6 (1)</td>
<td>5</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>CC02</td>
<td>11</td>
<td>M</td>
<td>Asian</td>
<td>Nil</td>
<td>2</td>
<td>18 (1)</td>
<td>3.4 x 10^6 (1)</td>
<td>8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CC03</td>
<td>6</td>
<td>M</td>
<td>Asian</td>
<td>Nil</td>
<td>2</td>
<td>5 (2)</td>
<td>4.7 x 10^6 (1)</td>
<td>6</td>
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<td>4</td>
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<tr>
<td>CC04</td>
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<td>M</td>
<td>White</td>
<td>Nil</td>
<td>1</td>
<td>10 (1)</td>
<td>3.5 x 10^7 (3)</td>
<td>4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CC05</td>
<td>9</td>
<td>M</td>
<td>White</td>
<td>Asthma</td>
<td>1</td>
<td>23 (1)</td>
<td>2.1 x 10^6 (1)</td>
<td>7</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>CC06</td>
<td>4</td>
<td>M</td>
<td>White</td>
<td>Eczema</td>
<td>0</td>
<td>8 (2)</td>
<td>1.0 x 10^6 (1)</td>
<td>6</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>CC07</td>
<td>3</td>
<td>F</td>
<td>White</td>
<td>Nil</td>
<td>1</td>
<td>8 (1)</td>
<td>1.2 x 10^3 (2)</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>CC14</td>
<td>6</td>
<td>M</td>
<td>White</td>
<td>Nil</td>
<td>1</td>
<td>12 (1)</td>
<td>5.3 x 10^7 (1)</td>
<td>7</td>
<td>6</td>
<td>–</td>
</tr>
<tr>
<td>CC15</td>
<td>2</td>
<td>F</td>
<td>White</td>
<td>Nil</td>
<td>2</td>
<td>10 (4)</td>
<td>2.3 x 10^11 (3)</td>
<td>8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CH01</td>
<td>15</td>
<td>F</td>
<td>White</td>
<td>Nil</td>
<td>2</td>
<td>10 (1)</td>
<td>3.3 x 10^6 (1)</td>
<td>6</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>CH03</td>
<td>0</td>
<td>F</td>
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<td>3</td>
<td>4 (1)</td>
<td>3.1 x 10^7 (1)</td>
<td>7</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

IF, immunofluorescence; N/A; not available.

a Time from symptom onset to last day swab positive.
b First day of symptoms = day 1, therefore treatment at day 3 is within 48 hours.
c Only data on one viral load available.
TABLE 3 Symptom reported over the course of study follow-up in both patients with pandemic H1N1 influenza and others

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Pandemic H1N1 subjects (n=19)</th>
<th>Others (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever (on day of onset)*</td>
<td>13 (68)</td>
<td>12 (57)</td>
</tr>
<tr>
<td>Runny nose</td>
<td>19 (100)</td>
<td>20 (95)</td>
</tr>
<tr>
<td>Sore throat</td>
<td>12 (63)</td>
<td>17 (81)</td>
</tr>
<tr>
<td>Cough</td>
<td>19 (100)</td>
<td>21 (100)</td>
</tr>
<tr>
<td>Shortness of breath</td>
<td>14 (74)</td>
<td>20 (95)</td>
</tr>
<tr>
<td>Stuffy nose</td>
<td>19 (100)</td>
<td>18 (86)</td>
</tr>
<tr>
<td>Sneezing</td>
<td>17 (89)</td>
<td>17 (81)</td>
</tr>
<tr>
<td>Earache</td>
<td>3 (16)</td>
<td>8 (38)</td>
</tr>
<tr>
<td>Sinus tenderness</td>
<td>12 (63)</td>
<td>15 (71)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>6 (32)</td>
<td>8 (38)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>10 (53)</td>
<td>10 (48)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>18 (95)</td>
<td>20 (95)</td>
</tr>
<tr>
<td>Headache</td>
<td>15 (79)</td>
<td>12 (57)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>14 (74)</td>
<td>15 (71)</td>
</tr>
</tbody>
</table>

* The symptom of fever was not recorded on a daily basis, although an oral measurement of body temperature was.

FIGURE 2 Mean symptom scores of pandemic H1N1 cases over time. (a) Number of observations (subject data) available for each day. Day 1 is the day of symptom onset. (b) Mean symptom scores of subjects with pandemic H1N1 as a function of the number of days since symptoms started.
FIGURE 3 Upper respiratory tract, lower respiratory tract and systemic symptom scores over time. First row: mean symptom scores for positive pandemic H1N1 cases (solid line) and negative cases (dashed line) as a function of the number of days since symptoms started. Second row: number of observations available for each day; pandemic H1N1 cases (solid line) and negative cases (dashed line). Day 1 is the day of symptom onset.
the study was significantly higher for subjects with pandemic H1N1 who received antiviral drugs within 48 hours of symptom onset (mean score 16.6) than subjects with pandemic H1N1 who either did not take oseltamivir or did so after 48 hours of illness onset (8.6) \((p = 0.018)\) (Figure 4a).

**Viral load**

Subject viral loads were examined over time and in relation to symptom scores. Nasal swab viral loads, measured by PCR, varied widely across our pandemic H1N1-positive subjects, ranging from \(0.9 \times 10^5\) to \(1.7 \times 10^{11}\) copies/ml. Viral loads plotted over time are shown for four subjects from whom the most complete data were obtained (Figure 5a). All subject viral loads over time are shown in Figure 5b, which illustrates the heterogeneity of the data; for each individual trajectory, viral loads tend to decrease with time, but there is an apparent increase in the mean value, because individuals with high viral loads tend to shed for longer.

The mean peak viral loads of the four recruitment groups were \(5.9 \times 10^5\) for AH, \(2.4 \times 10^5\) for AC, \(1.0 \times 10^7\) for CH and \(1.6 \times 10^6\) for CC. No significant differences were detected between any of the groups, although there was a trend towards higher peak loads in children (Figure 6). The mean peak viral load of adults was \(4.4 \times 10^5\), and that of children was \(2.2 \times 10^6\), with no significant difference detected between them \((p = 0.28)\).

Neither total, URT or systemic symptom scores correlated with viral loads at different points in time. However, the LRT symptoms score on day 5 was significantly correlated \((p = 0.049)\) (Figure 7).

**Rapid antigen tests**

Overall, 10/19 (53%) of subjects with pandemic H1N1 influenza were antigen test-positive: 2/8 (25%) adults and 8/11 (73%) children. No pandemic H1N1-negative patients were antigen test-positive. There were no significant differences in symptom scores on the first day of the study between subjects who had a positive rapid antigen test and those who had a negative one. For URT, LRT and systemic symptoms, the mean symptom score on the first days of study were 5.32 and 3.7, respectively, for those with a positive test, and 6.30 and 3.7 for those with a negative test \((p\)-values 0.53,
FIGURE 5 Viral loads plotted over time. (a) Viral loads from selected subjects are shown. (b) All viral load data are shown. Subject trajectories are shown as dashed lines and the mean of these is shown as a solid line. (c) Number of observations available for each day. Day 1 is the day of symptom onset.

FIGURE 6 Peak viral loads of subjects categorised into their recruitment groups.
Results

0.72 and 0.67, respectively). Among the 13 subjects who had a viral load measurement performed on the first day of the study, eight (62%) had a positive rapid test. The mean viral load on the first day of study was larger for the eight patients with a positive rapid test (198 × 10^4 copies/ml) than for the five patients with a negative rapid test (4 × 10^4 copies/ml), although the difference was not statistically significant (p = 0.15).

Virus shedding

The duration of virus shedding measured by PCR had mean of 6.2 days and a range of 3–10 days. There was no difference between children (mean 6.1 days) and adults (mean 6.3 days) (p = 0.89). Based on the numbers involved, the power to detect a difference was 19% if adult shedding was 6 days and child shedding was 7 days. The duration of shedding of hospital cases (mean 6.8 days) was slightly longer than that of community cases (mean 5.7 days), although the difference was not significant (p = 0.33) (Figure 8).

Figure 8 shows the distribution of live virus shedding for the 12 positive cases, and highlights the recruitment group to which each subject belongs. There was no significant correlation between the duration of the live virus shedding and total symptom score of these 12 cases on the day of recruitment was detected, with coefficients of correlation with URT symptoms of 6% (p = 0.8), with LRT symptoms 19% (p = 0.43) and with systemic symptoms 8% (p = 0.75).

A total of 12/19 cases (63%) were culture positive for pandemic H1N1. The mean duration of live virus shedding from these 12 cases was 4.7 days (range 3–8 days). However, because cases with no positive culture were excluded (durations too short to be observed or false-negative testing), this represents an upper bound for the duration of shedding. To obtain a lower bound for the duration, the calculation was repeated with the assumption that ‘negative’ patients do not shed live virus (duration of shedding = 0). This gives a mean duration of 2.9 days (range 0–8). The median value when all 19 subjects were included was 3 days, and 6/19 (31%) subjects shed live virus for at least 5 days from the onset of illness.

Figure 7 shows the distribution of live virus shedding for the 12 positive cases, and highlights the recruitment group to which each subject belongs. There was no significant correlation between the duration of the live virus shedding and total symptom score of these 12 cases on the day of recruitment.
of recruitment [correlation coefficient −0.09, 95% confidence interval (CI) −0.63 to 0.51, \( p = 0.78 \)] or the sum of total symptom scores during the whole follow-up (correlation coefficient −0.22, 95% CI −0.71 to 0.40, \( p = 0.48 \)).

The mean duration of shedding determined by both PCR and culture was not significantly different for subjects who received antivirals within 48 hours and those who received them after 48 hours or not at all [PCR: 6.4 days vs 5.9 days, \( p = 0.61 \); culture-positives: 4.6 days vs 4.8 days, \( p = 0.88 \)]. All culture results (assuming six have 0 days): 3.4 days vs 2.4 days, \( p = 0.43 \).

Box 2 summarises symptom and virus shedding findings.

### Environmental deposition

**Surfaces**

In total, 414 community swabs (+52 sponges) and 45 hospital swabs (+seven sponges) were taken, of which 397 swabs and 12 sponges were tested (not all swabs were tested because of sample processing rules, see Chapter 2, Laboratory methods). Pandemic H1N1 virus was detected by PCR on two occasions on surfaces from around one patient in the community (following discharge from hospital), giving a swab positivity rate of 0.5%. Quantitative PCR could only be performed on one sample because the amount of sample available in the other was insufficient. Live virus was recovered from one of these surfaces. The subject from around whom the swabs were taken was found to

---

**FIGURE 8** Distribution of the duration of virus shedding by PCR. The duration of viral shedding is defined as the time between symptom onset and the last day that a positive specimen was taken. Because patients were seldom recruited on the day symptoms began, an assumption has been made that they were shedding virus from the first day of symptoms to the last positive specimen.

**FIGURE 9** Distribution of the duration of virus shedding by culture positivity (\( n = 12 \)). The duration of viral shedding is defined as the time between symptom onset and the last day that a positive culture was obtained. Cultures were performed from the last day of nasal swab PCR positivity. If a culture was positive on any given day then it was assumed that previous days’ would also have been culture-positive.
be shedding virus from the nose on the same day, although other household members were also unwell on these days; a 5-year-old was unwell with cough and fever on day 4, and a 2-year-old was unwell with cough and fever on day 10 (Table 4).

**TABLE 4** Details of surface swabs that were positive for pandemic H1N1 virus

<table>
<thead>
<tr>
<th>Specimen no.</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject ID</td>
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<td>AH04</td>
</tr>
<tr>
<td>Surface (setting)</td>
<td>Kettle handle (home)</td>
<td>Bathroom tap (home)</td>
</tr>
<tr>
<td>Surface material</td>
<td>Plastic</td>
<td>Metal</td>
</tr>
<tr>
<td>Swab method</td>
<td>Cotton swab</td>
<td>Cotton swab</td>
</tr>
<tr>
<td>Number of days after symptoms began that swab was taken</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Viral load from surface swab (copies/ml)</td>
<td>91,205</td>
<td>N/A</td>
</tr>
<tr>
<td>Viral load from nose on day swab collected (copies/ml)</td>
<td>902,703</td>
<td>N/A</td>
</tr>
<tr>
<td>Culture</td>
<td>Positive</td>
<td>Negative</td>
</tr>
</tbody>
</table>

N/A, not available.
Note, at the time swabs were taken other household members in this subject’s family were also unwell with symptoms of ILI.

**Air**

Air samples were collected from the immediate environment of five subjects (all of whom were rapid antigen test positive): three while in hospital and two in the community. Seventeen separate collections were undertaken, generating 51 samples (although one could not be processed because of insufficient sample volume). Air samples were positive from four out of five subjects. Eight out of 17 (47%) collections and 22/50 (44%) samples were positive for PCR. No samples were confirmed to contain live virus (Table 5).

Quantitative PCR demonstrated a range of values between 238 and 24,231 copies/ml; higher values were recorded in instances when more than one infected person was present in the sampling room. Samples collected over a 1-hour period generated 8/24 PCR-positives (33%), those over a 2-hour period zero out of three positives, and those over a 3-hour period 14/23 positives (61%). The risk ratio for a sample to be positive over a 3-hour period relative to a 1-hour period was 1.83 (95% CI 0.95 to 3.51, \( p = 0.082 \)). Samples collected at a distance close to the subject (approximately 3 ft) generated 13/23 PCR-positives (57%), whereas those collected further away (at least 7 ft) generated 9/27 PCR-positives (33%). The risk ratio for a sample to be PCR positive at a distance of 3 ft versus ≥ 7 ft was 1.70 (95% CI 0.89 to 3.22, \( p = 0.15 \)). Virus was detected in all particle sizes collected: particles < 1 \( \mu \)m gave 7/16 positives (44%); particles 1–4 \( \mu \)m gave 8/17 positives (47%) and particles > 4 \( \mu \)m gave 7/17 positives (41%). Among particles of size 1–4 \( \mu \)m and > 4 \( \mu \)m, the relative risk of obtaining a positive sample relative to particles of size < 1 \( \mu \)m was 1.08 (95% CI 0.51 to 2.28, \( p > 0.99 \)) and 0.94 (95% CI 0.43 to 2.08, \( p > 0.99 \)), respectively (Table 5).

Initially it appeared that 3 samples were culture positive for virus. To verify that the cultured virus in the air samples was the same as that from subject’s nose, PCR was carried out on
TABLE 5 Description of air particle samples collected

<table>
<thead>
<tr>
<th>Subject</th>
<th>AH03</th>
<th>AH04</th>
<th>CC05</th>
<th>CC15</th>
<th>CH03</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject setting (+ infected others)</td>
<td>Hospital bed in side room</td>
<td>Hospital bed in side room</td>
<td>Playing in bedroom</td>
<td>Playing in living room (6-year-old infected child also present)</td>
<td>Cot on neonatal unit (two infected neonates also present)</td>
</tr>
<tr>
<td>Room temperature (°C)</td>
<td>21.6</td>
<td>23.3</td>
<td>20.0</td>
<td>18.0</td>
<td>24.0</td>
</tr>
<tr>
<td>Room humidity (relative %)</td>
<td>50</td>
<td>50</td>
<td>64</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>No. of days after symptoms began that sample was taken</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Viral load from nose on day sample collected (copies/ml)</td>
<td>238,091</td>
<td>10,625,714</td>
<td>699,723</td>
<td>178,923,317,453</td>
<td>24,208</td>
</tr>
<tr>
<td>Virus cultured in the nose on sampling day?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Duration of sampling (hours)</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Approximate distance from subject (ft)</td>
<td>3</td>
<td>7</td>
<td>3</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Pandemic H1N1 virus detected by PCR</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Particle size virus detected in µm</td>
<td>&lt; 1</td>
<td>–</td>
<td>&lt; 1</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td>1–4</td>
<td>1–4</td>
<td>1–4</td>
<td>1–4</td>
<td>1–4</td>
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<td>&gt; 4</td>
<td>&gt; 4</td>
<td>&gt; 4</td>
<td>&gt; 4</td>
</tr>
</tbody>
</table>

N/A, not available.
the harvested virus to confirm the presence of pandemic H1N1. However, as well as the clear presence of pandemic H1N1 there was a signal that indicated the presence of another virus. Work was then undertaken to try and identify this virus though it is important to note the following: (1) there were no original samples left to reanalyse; (2) the signal was detected only in harvested, amplified virus; and (3) this signal was not seen in the air sample on which the initial PCR was done.

- PCR assays were performed (see Appendix 6, PCR protocol), which confirmed the contaminating virus to be influenza A, H1. Plaque assay on the harvested air sample virus was strongly positive (titre $30 \times 10^7 \times 2.5$/ml = $7.5 \times 10^8$ plaque-forming units (pfu)/ml). (Note: pandemic H1N1 does not plaque in these cells.)

- Contamination with another influenza virus did not preclude there being live pandemic H1N1 virus in the cells as well. Therefore, an experiment was performed whereby diluted virus was cultured and an attempt made to quantify the amount of virus by PCR. If live pandemic H1N1 was present in the original sample, we postulated that extracted nucleic acid should be at higher concentration in the re-amplified aliquots. Harvested virus was diluted in 10-fold steps from neat to $10^{-7}$. Each dilution was split into two aliquots: one frozen and the other inoculated into fresh MDCK (Madin–Darby Canine Kidney) cells. The MDCK cells were incubated for 48 hours before the virus was again harvested. Results indicate that there was no live pandemic H1N1 virus in these samples (at least by these methods). Three out of 11 dilutions were positive for pandemic H1N1 influenza prior to reamplification, but none of the dilutions was positive post re-amplification.

- Finally, in an attempt to determine conclusively the identity of the contaminating virus, samples of the matrix gene amplicons were sequenced. Results show that influenza A PR8 was the contaminating isolate (undoubtedly from the laboratory).

Findings from the environmental sampling are summarised in Box 3.

**Composite charts**

In order to best demonstrate the information we have generated for each subject, charts integrating data from nasal swabs and environmental samples are shown below for selected patients (Figure 10). All patient charts are shown in Appendix 7.

**BOX 3 Environmental sampling data summary**

- Almost no fomite contamination was found (0.5% of all specimens taken)
- Five subjects had samples of the air around them taken and virus was detected by PCR from four of them; PCR positive specimens were equally well represented across all of the particle size ranges measured
- Although viable virus was recovered from three samples, we were unable to prove that this virus was pandemic H1N1, as opposed to a contaminant
FIGURE 10 Composite charts for subjects. (a) AH04; (b) AH03; (c) CC15; (d) CH01. The ‘symptom’ bar shows the number of days for which symptoms were present. The ‘nasal swab’ bar shows the last day that a swab was either PCR-positive or culture-positive. The ‘surface’ and ‘air’ bars show days up to the time that a positive sample was obtained. Arrows show the days when oseltamivir was started and when follow-up ended. ‘Day 1’ is the day of illness onset.
Chapter 4
Discussion

This is the first study that has attempted to assess actual viral shedding from patients with influenza, by examining the near-patient environment for virus as opposed to simply taking respiratory specimens. Sampling virus, particularly live virus, in the environment is challenging; getting to the subject in time, executing optimal sampling while preserving virus viability and performing sensitive detection tests in the laboratory are all key factors that necessitate very extensive and complex logistic arrangements. An attempt to overcome this first problem was carried out by targeting recruitment in the community, as well as in hospital (when presentation is often delayed), enabling an approach to subjects early in their illness when virus shedding is usually at its highest. In addition, the use of a bioaerosol sampler, designed and validated by collaborators at NIOSH enabled us to sample air around infected subjects.

Subjects’ with pandemic H1N1 experienced a range of symptoms, but a mild illness was evident in the majority of cases, as has been reported elsewhere. There were no significant differences with respect to symptom type or duration between those positive for pandemic H1N1 virus and those who were non-confirmed (negative). Although the non-confirmed cases included some individuals who were infected with other respiratory viruses, undoubtedly some were falsely negative on pandemic H1N1 virus testing.

Viral loads, in general, declined over time, although a lack of data hinders further interpretation. Only 5/19 subjects had data available at four or more time points. The wide range of results seen may in part be reflected by differences in sample quality. The peak viral load was found to be higher in children than adults, in line with other studies, although this was not significant. There was a significant association, however, found between viral load and LRT symptoms on day 5 of a subject’s illness, suggesting that persistent LRT symptoms might be a clinical marker for prolonged shedding. However, cautious interpretation of this result is necessary, given the lack of data.

Our findings on virus shedding, as conventionally described, are broadly in agreement with other published findings relating to pandemic H1N1 virus (Table 5). The median duration of virus shedding from the 19 infected cases was 6 days when detection was performed by PCR, and 3 days when detection was performed by a culture technique. Forty-four per cent of these subjects received oseltamivir within 2 days of illness onset. Fifty-eight per cent of subjects were recruited directly from the community, and these cases shed virus for a shorter period of time than the hospital cases (5.7 vs 6.8 days). Although this finding was not significant it accords, nevertheless, with data suggesting that hospitalised influenza cases shed virus for longer, with potential infection control implications for health-care institutions.

When comparing studies (Table 6), it should be borne in mind that differences in study populations may exist (children vs adults, hospital vs community cases), a variety of sampling methods are used and that the proportions of cases receiving antiviral drugs (particularly whether they received them within 48 hours) may differ. In a Vietnamese hospitalised cohort of 292 pandemic H1N1 cases, PCR detected virus in combined nose and throat swabs in the following proportion of patients: after 1 day of treatment 86% (165/192); day 2 59% (45/76); day 3 38% (27/72); day 4 25% (34/138); and day 5 14% (11/76). After 5 days of treatment, 7% (12/179) were still positive, although no positive cultures were obtained after day 5.17 Laboratory findings from a study of 70 cases in Singapore gave a mean duration of viral shedding of 6 days, with shedding > 7 days in 37% of patients. The mean duration of positive culture results on six patients was 4 days. Finally, in a Canadian study, 43 community patients with pandemic H1N1 had a nasopharyngeal specimen collected on day 8 of their illness: 74% were PCR-positive and 19% were culture-positive.

One subject from our study who demonstrated the shedding of live virus up to day 8 will be considered further. She was a 34-year-old woman, of South-Asian origin, who had no comorbidities, and did not take regular medicine. She spent one
night in hospital on the first day of her illness and began taking oseltamivir on day 2 (the subject reported taking oseltamivir each day and, while there is no reason to suspect non-compliance, this cannot be excluded). Prominent symptoms early in her illness were fever, cough, sore throat and fatigue. The virus was sequenced across the HA gene during the period of time that it was shed, and no changes were detected. In addition, no common oseltamivir resistance mutations were detected. All of the other household family members subsequently developed symptoms of cough and fever; a 5-year-old daughter became unwell on day 4 of the mother’s illness, followed by a 2-year-old son on day 5 and her 30-year-old husband on day 6. Thus, a high secondary attack rate in this family was associated with high levels and prolonged shedding of virus, despite the index case being treated with oseltamivir.

It is interesting to note that no difference was found in the duration of viral shedding (PCR or culture) between those who took oseltamivir within 48 hours and those who did not, although our numbers are small (10 vs 8), and it is impossible to draw conclusions because a sample size of at least several hundred subjects would have been needed. Other studies have demonstrated a shortened duration or suppressed levels of shedding in association with oseltamivir when it is given early.\textsuperscript{13,34,35} Subjects with pandemic H1N1 who did receive antiviral drugs had significantly higher initial symptom scores than those who did not, perhaps indicating that patients with more severe symptoms were more likely to access to early treatment. This difference might mask any effect of antiviral drugs on duration of shedding. In addition, it may explain why symptom scores were consistently lower among those who received no or late treatment than among those who received early treatment.

Our findings relating to the duration of live virus shedding have infection control implications. They suggest that over 30\% of cases remain potentially infectious for at least 5 days and, given that live virus may persist in the environment for up to 48 hours,\textsuperscript{19} viable virus may be present for 7 days after an index case first develops symptoms. These data are consistent with other recent studies that suggest that pandemic H1N1 may be contagious for a longer period of time than seasonal flu.\textsuperscript{15,35} This has clear implications for pandemic infection control and self-isolation guidelines.

| TABLE 6 Published studies describing shedding patterns from cases of pandemic H1N1 |
|---------------------------------|-----------|-----------|-----------|-----------|
| Setting                         | UK (this study) | China\textsuperscript{13} | Hong Kong\textsuperscript{36} | Singapore\textsuperscript{14} | Germany\textsuperscript{15} |
| No. of cases                    | 19        | 421       | 22        | 70        | 15        |
| Adults and children             | Yes       | Yes       | Yes       | Yes       | Yes       |
| Percentage who received oseltamivir within 48 hours | 44        | 72.4      | 95        | 51        | 40 (three were given prophylactically) |
| Duration of viral shedding by PCR | 6.2 (mean) | 6 (median) | 4 (median) | 6 (mean) | 6.6 (mean) |
| Duration of viral shedding by culture | 3 (median) | Range 0–8 | –         | –         | 4 (mean, n = 6) |
| Risk factors for prolonged shedding | –         | Age < 14 years, male sex, delayed oseltamivir | Younger age | –         | –         |
However, despite finding that live virus shedding continued for over 4 days in most subjects, fomites contaminated with virus were found in only two instances, involving only one subject. Therefore, only 0.5% of all community fomites, and none of the hospital fomites, swabbed revealed virus, although on one occasion live virus was found. This instance occurred in a household where, at the time of taking the surface swab, a 5-year-old child was also experiencing her first day of symptoms, but the surface contamination was from a kettle handle and so is unlikely to have been directly handled by the secondary case. These findings are in contrast with those of Boone and Gerba, who detected influenza virus (by PCR) on over 50% of all swabs taken from a number of fomites in the home and in child-care centres. They also differ from the findings of a study that involved subjects who were experimentally infected with influenza virus. Swabs taken from fomites in subjects’ rooms (two subjects shared a room) revealed influenza (detected by PCR) in 9/48 swabs (19%), although no live virus was found (B Killingley, University of Nottingham, May 2010, personal communication). It is also likely that more than one individual contributed to virus deposition in Boone and Gerba’s study. This contrasts with the circumstances of the current study, where only one individual was ill when the vast majority of swabs were taken. In addition, the homes used in Boone and Gerba’s study contained a symptomatic child 100% of the time compared with 79% of homes in the current study. It is also worth noting that no specific cleaning instructions were given during the follow-up of our subjects, so, for example, daily cleaning of hospital rooms would have continued, which may have contributed to the low positive swab rate. A more speculative suggestion would be that pandemic H1N1 is less stable in the environment than other influenza strains, and indeed there is some evidence to suggest that some influenza viruses may be more robust than others. In experimental conditions an avian virus survived for up to 6 days on some surfaces and unpublished observations (J Greatorex, HPA, May 2010, personal communication) suggest a laboratory-adapted PR8 (H1N1) virus is more hardy than seasonal wild-type strains. The finding of influenza RNA on fomites on its own does not prove that disease can be spread via the contact route – demonstration of live virus transmitted in an infectious dose would be required for this. Despite an isolated discovery of live virus, our findings overall suggest that the contact route of transmission for pandemic H1N1 may well play a more minor role in the transmission of influenza than hitherto suggested by experts, and by the current emphasis placed on hand hygiene as a means of interrupting transmission.

A noteworthy finding of this study is the demonstration of virus in particles collected from the air around subjects who have influenza; this has not previously been attempted in a community setting. Five subjects had samples of the air around them taken, and virus was detected by PCR from four of them. In two instances there were additional patients with pandemic H1N1 (children) present in the room as well as the study subject during air sampling, and it was these samples that revealed the most virus. All particle sizes collected contained virus detectable by PCR, including the < 1-µm and 1–4-µm fraction sizes, which are bioaerosols of a respirable size, i.e. they can reach the distal airways of the respiratory tract. Sampling for a longer time period, and nearer to the subject, led to the detection of more virus as one might expect, although analyses did not reveal any statistical significance because numbers were small.

Unfortunately, we have been unable to conclusively demonstrate the presence of live pandemic H1N1 in any samples. Initial culture results indicated the presence of live virus in three samples from one subject (AH03) and PCR detected only pandemic H1N1 in the original samples. However, following amplification of the virus to permit further analysis, it appears that the sample became contaminated with a laboratory influenza strain. It was not possible to go back to the original sample (as none remained) or subsequently prove that the live virus detected was pandemic H1N1 as opposed to the contaminant.

There were no unusual room temperature or humidity readings recorded during sampling, but there are insufficient data to study the effects of these variables further.

It is unclear why it was not possible to culture live virus from specimens when most subjects had live virus detected on nasal swabs, although detecting live virus in samples is challenging and the techniques are still relatively new. Difficulties include the fragility of the virus particle (especially its susceptibility to desiccation) and the fact that sufficient virus needs to be collected to enable culture. Because the amount and concentration of virus being sampled in air is much lower than that from nasal swabs, detection is more difficult. The use of VTM during sample collection (as opposed to its addition afterwards) to help preserve
Discussion

Evidence backing up at least the potential for bioaerosol transmission of influenza infection has recently been reviewed;39 supporting evidence comes from the detection of influenza virus (by PCR) in the air around patients,26,27 the demonstration of bioaerosol transmission in animal models,40,41 and increasingly sophisticated mathematical modelling techniques, which suggest a role for bioaerosol spread.42 Detecting the presence of influenza in the air is the first step in a chain of evidence needed to confirm that influenza viruses – emitted from an infected individual and existing as bioaerosols – can initiate infection in a person exposed to them. The other steps in this sequence are (1) confirming that live, i.e. infectious, virus is present and (2) confirming that sufficient live virus exists that can be inhaled by an individual to initiate infection. Couch et al.35 conducted a series of experiments in 1966, culminating in a human-to-human transmission study attempting to follow this line of evidence for coxackie virus, and came to the conclusion that bioaerosol transmission ‘unquestionably occurred’. Similar data on influenza are lacking and it remains that the human infectious dose of influenza in natural conditions is not known for any route. Alford et al.25 showed that three times the TCID50 was needed to infect volunteers via bioaerosols; this compares to other studies showing that 127–320 TCID50 are needed to initiate infection by the intranasal route.44 Using these data, attempts have been made to estimate the risk of infection attributable to the different routes of infection,15 but the outputs of such models are only ever as good as the input assumptions. However, if Alford et al.’s25 supposition is true then even small quantities of viable virus expressed via bioaerosols might have significant infectious potential.

Detection of virus by PCR was seen from air samples collected at close range (3ft) to subjects, well within the contact distance of an attending health-care worker suggesting that the theory of short range bioaerosol transmission advanced by Tellier39 cannot be dismissed. Although clearly based on extremely limited data, these finding are of sufficient importance to justify further efforts to reproduce them including further attempts to detect of live virus.

There are several limitations to this study. First, the numbers of subjects recruited was well below target. The study began recruiting just prior to the beginning of the second wave of the pandemic in England, but the overall number of people infected during the second wave was well below what had been predicted46 and seroconversions during the first wave were far higher than expected.47 In addition a mild illness, including a high asymptomatic infection rate49 contributed to our difficulty. It is also evident that enrolling people early in the course of their illness is challenging. Over one-half of the volunteers we saw were ineligible because symptoms had been present for too long. A further problem was difficulty in identifying subjects as having influenza as opposed to other ARIs. It has been shown that the standard definition of ILI cannot be relied upon to distinguish pandemic H1N1 from other ARIs,48,49 and the low numbers of people with illness in the local population made the positive predictive value of even our modified definition of ILI low (48%). A near-patient rapid antigen test was used to help reveal influenza cases, but our original inclusion criteria that required a positive antigen test were modified because the sensitivity of the test in our hands (with a nasal swab) was low. Overall, 10/19 (53%) of our cases were antigen test-positive; the sensitivity in adults was 25% and in children 73%. These findings concur with a number of other reports about the low sensitivity of these tests to detect pandemic H1N1.50–52 This resulted in a difficulty in reliably recruiting only subjects with pandemic H1N1, such that we followed up subjects who had other ARI. For technical and logistic reasons, the capacity to generate PCR results on samples quickly enough to limit this follow-up in most cases did not exist. The modest recruitment of pandemic H1N1 cases limits the study in several ways, including the generalisability of our findings and because of a lack of data the ability to address our primary aim – to correlate virus shedding on nose swabs with environmental samples.

Second, the sampling methods used require further consideration, as care is needed during interpretation of the results:

- **Nasal swab** Although a nasopharyngeal aspirate (NPA) is considered to be the best specimen for detecting influenza A viruses,53–55 this procedure causes more discomfort and is more difficult to perform, particularly in children. Indeed, studies attempting to collect daily NPA samples from subjects have reported problems with subjects’ tolerance and compliance with
the procedure. A nasal swab, however, has been shown to be an acceptable alternative that is not statistically less sensitive than a NPA, although suboptimal sampling (caused by interoperator variation in technique) can still occur.

- **Fomite swabbing** Despite adopting a similar swabbing technique to other comparable studies and validating this in advance using experimentally deposited virus (B Killingley, University of Nottingham, May 2010, personal communication), virus was rarely isolated from fomites. Furthermore, the fomites sampled were similar, except that four of our nine chosen surfaces (bedside table, dining table, patient table and window sill) are not items that are actually picked up or grasped by the hand. Virus may well be transferred to, or settle on, such surfaces, but sampling was performed from only a small proportion of the surface area. Furthermore, many of these surfaces were made of wood, a material that does not support virus survival (J Greatorex, HPA, May 2010, personal communication). In future, consideration will be given to alternative sampling methods, for example using a sponge (wiping a surface may collect more material and can cover a larger surface area) and increasing our focus on ‘grasped’ items. We used and tested the sponge too infrequently during this study to draw any firm conclusions about its performance compared with a cotton swab.

Finally, all subjects from whom air samples were obtained tested positively on rapid antigen testing. This may have biased the group somewhat, as a positive rapid antigen test has been associated with higher viral loads in nasal samples. On the other hand, our intention was to prove whether viable virus deposition on surfaces or in the air was possible in practice; so selection of these individuals was important. Also no measurements or estimates of room air flow patterns or ventilation were made when collecting samples. Such parameters are likely to have an influence on the ability to detect virus in the air.
Chapter 5
Conclusion

Despite limitations resulting in an inability to fully address the primary aims of the study, important observations have been made. Our findings show that live pandemic H1N1 virus can be found in the noses of over 30% of infected individuals for at least 5 days after symptoms begin. The evidence for the significance of both contact and bioaerosol routes of transmission, depends upon demonstrating that viable virus is deposited from an infected patient. This has been shown for touched surfaces, although the data suggest that contact transmission via fomites may be less important than hitherto emphasised. Transmission via bioaerosols at short range is not ruled out; virus was detected by PCR in aerosols, but we were unable to conclusively demonstrate the presence of live virus.

Implications for health care/recommendations for research

As the current data are inconclusive further work is being undertaken to consolidate these findings, as they have important potential implications for PPE requirements in health-care workers, nationally and internationally. In order to address recruitment difficulties, involvement of specific groups (for example university students) and targeting contacts of index cases who present to a general practitioner or hospital will be attempted during the influenza season 2010–11.
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Professor Jonathan Nguyen-Van-Tam (Professor of Health Protection) contributed to study design, data interpretation and was chief investigator.

Dr Ben Killingley (MRC Clinical Research Fellow) contributed to study design, data collection, patient enrolment and data interpretation, as well as being responsible for project management, including co-ordination across sites, study logistics, data management and preparation of regulatory submissions. He drafted the study protocol and this report, which were both reviewed by all authors.

Dr Jane Greatorex (Senior Research Scientist) contributed to study design and data interpretation, and was responsible for laboratory analysis.

Dr Simon Cauchemez (Research Councils UK Research Fellow) contributed to data interpretation and was responsible for statistical analysis.

Ms Joanne Enstone (Research Co-ordinator) contributed to study design and data interpretation.

Dr Martin Curran (Head of Molecular Diagnostic Microbiology) contributed to study design, data interpretation and laboratory analysis.

Professor Robert Read (Professor, Infectious Diseases) contributed to study design and data interpretation and was the principal investigator at the Sheffield site.

Dr Wei Shen Lim (Consultant, Respiratory Medicine) contributed to study design and data interpretation, and was the principal investigator at the Nottingham site.

Dr Andrew Hayward (Senior Lecturer, Infection and Population Health) contributed to study design and data interpretation.

Professor Karl Nicholson (Professor, Infectious Diseases) contributed to study design and data interpretation, and was the principal investigator at the Leicester site.
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Appendix 1

Protocol, version 1.1, 8 October 2009
CONFIDENTIAL PROTOCOL

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

Sponsor: University of Nottingham
Funding Source: National Institute of Health Research
REC Reference: Leicester 1 – 09/H0406/94
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<td>Professor Jonathan Van-Tam</td>
</tr>
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<td>Objectives</td>
<td>The objectives of the proposed study are:</td>
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| 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. |
<table>
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<tr>
<th>Study Configuration</th>
<th>Multi-centre, observational + interventional</th>
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<td>Setting</td>
<td>Community and Hospital</td>
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| 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**

**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)
- Existing case of ILI in the household
- 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 = 6 months**
- Maximum for a participant; Adult = 10, Child = 12
- Planned 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°C. 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 x 10^6 to 2.0 x 10^5). 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 of 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 + Intervventional

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 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 midway through sampling we would attempt to follow them up in hospital.

**Recruitment**

**HOSPITAL CASES:**

Hospital cases will be identified through the clinical teams (including Flu-CIN nurses – see below) looking after patients in the 3 participating centres; Nottingham, Leicester and Sheffield. 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.

COMMUNITY CASES:
We plan to recruit via 2 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.

**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 fulfills 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;

<table>
<thead>
<tr>
<th>Symptom</th>
<th>UK</th>
<th>US</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>91%</td>
<td>94% (371 / 394)</td>
</tr>
<tr>
<td>Cough</td>
<td>76%</td>
<td>92% (365 / 397)</td>
</tr>
<tr>
<td>Sore Throat</td>
<td>75%</td>
<td>66% (242 / 367)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>79%</td>
<td>-</td>
</tr>
<tr>
<td>Headache</td>
<td>74%</td>
<td>-</td>
</tr>
<tr>
<td>Runny Nose</td>
<td>69%</td>
<td>-</td>
</tr>
<tr>
<td>Sneezing</td>
<td>60%</td>
<td>-</td>
</tr>
</tbody>
</table>

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)
- Existing case of ILI in the household
- 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

**Randomisation**
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

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 cm² areas on selected surfaces from within the rooms housing patients. For consistency we have chosen the following surfaces;

Hospital;
- Patient table (mid-point or nearest to midpoint)
- Patient line console (e.g. on/off button) / Nurse call button – depending on circumstances
- Window sill

Home;
- Kitchen – Dining table + kettle handle
- Lounge – TV remote control (mid point on the back of the device)
- Bedroom – Bedside table
- Bathroom – Tap + door handle

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 swabs to be dipped in tube containing 1.5 ml viral transport medium and then rubbed across 2 cm² 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, whereas 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°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:

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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 quadruplex 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 -80°C 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 events 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
MBE, BMedSci, BMBS, DM, FFPH, FRIPH
GMC No. 3241998

Date: 08 Oct 2009
19. REFERENCES


Appendix 1 – Study outline

Identification of possible cases

FLU-CIN

FluWatch

Fulfil case definition and inclusion / exclusion criteria

Hospitalised Adults (25)
Hospitalised Children (25)

Community Adults (25)
Community Children (25)

Nasal Swabs
Surface Swabs
Air Samples

Virological Analysis – PCR and Plaque Assay
<table>
<thead>
<tr>
<th>Procedures</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
<th>Day 11</th>
<th>Day 12</th>
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<tbody>
<tr>
<td>Meets case definition</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Fulfils entry criteria</td>
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<td>x</td>
<td></td>
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<tr>
<td>Consent</td>
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<tr>
<td>Symptom diary card</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Oral temperature</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Room temperature and humidity</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>Concomitant meds?</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
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<tr>
<td>Adverse event?</td>
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<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasal swab</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
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</tr>
<tr>
<td>Surface swabs</td>
<td>x</td>
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<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<td>x</td>
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<tr>
<td>Air sampling</td>
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<td>x</td>
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<tr>
<td>Stool sample (optional)</td>
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<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 3 - Symptom Diary

<table>
<thead>
<tr>
<th>Subject Number</th>
<th>Subject Initials</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F M L</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>D D M M Y Y Y Y</td>
</tr>
</tbody>
</table>

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.

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

Consent forms
CONSENT FORM (adults)

Virus Shedding Study

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

Patient Identification Number for this trial: _____________

Please Initial Boxes

1. I confirm that I have read and understood the information sheet for the above study dated 06 August 2009 (version 1.1). I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my taking part is voluntary and that I am free to pull out at any time, without giving any reason, without my medical care or legal rights being affected.

3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by members of the research team, responsible individuals from the University of Nottingham (inspectors) or regulatory authorities where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

4. I agree that should I lose the capacity to consent during the study, my full participation in it can continue.

5. I agree to my GP/hospital clinician being informed of my taking part in the study.

6. I agree to take part in the study.

_________________________      _____________  _______________________
Name of person                 Date            Signature

_________________________     _____________        _______________________
Name of person taking consent            Date           Signature
CONSENT FORM (Parent / Guardian)

Virus Shedding Study

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

Patient Identification Number for this trial: ______________

Please initial boxes

1. I confirm that I have read and understood the information sheet for the above study, dated 06 August 2009 (version 1.1). I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily

2. I understand that my child’s participation is voluntary and that they are free to withdraw at any time, without giving any reason, without their medical care or legal rights being affected

3. I understand that relevant sections of my child’s medical notes and data collected during the study may be looked at by members of the research team, responsible individuals from the University of Nottingham (inspectors) or regulatory authorities where it is relevant to his / her taking part in this research. I give permission for these individuals to have access to their records

4. I agree to my child’s GP/hospital clinician being informed of their taking part in the study.

5. I agree to my child taking part in the study.

_________________________      _____________  _______________________
Name of person                 Date            Signature

_________________________       _____________      _______________________
Name of person taking consent            Date           Signature

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Appendix 3

Information sheets
Adult

Adult Information Sheet

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

You are being invited to take part in this University of Nottingham sponsored medical research. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

Ask us if there is anything that is not clear or if you or your child would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the research project?
An influenza pandemic has recently been declared, involving the novel A(H1N1) ‘swine flu’ virus. This has spread to almost 100 countries worldwide in less than two months, causing widespread disease so far in Mexico, USA and Canada. It is highly likely that over the next 12 months, many countries including the UK will be affected by widespread illness. In the UK this wave of intense flu activity is most likely to occur in late autumn 2009.

Very little is known about the new H1N1 pandemic virus. For example we do not know how long the virus is excreted by infected humans and how much virus is spread to surfaces and carried in the air. This is very important to know as soon as possible because it affects the advice that will be given to healthcare workers about controlling the spread of infection to themselves and other patients. Similarly we need this information so we can give good quality advice to families who will have to look after each other in their own homes.

The best way to obtain this information is to ask patients who get pandemic flu soon (in August, September and October) to help us by agreeing to give a daily nose swab sample for just over one week so we can see how much virus is in the nose day by day and how quickly this disappears. At the same time we will take samples from hard surfaces in a patient’s room or home and sample the air using a special filter device. We can then work out how much virus is being excreted, how long the ‘danger period’ is, whether surfaces are more or less important than the air that we breathe (in terms of catching the virus) and if we can advise on a ‘safe distance’ from the patient, beyond which there is relatively little chance of catching the illness. We need to do these studies in children as well as adults.
The study involves a simple daily nasal swab and subjects who agree to take part will be inconvenienced to some extent. However, the technique of sampling from the nose is quick and not painful and should not present any problems. Normal medical care will not be affected in any way.

The team has been performing this kind of work for some time and is well qualified and experienced to carry out the study. Several members of the study team are leading international experts on influenza.

Why have I been chosen?
You have been chosen as you have had a diagnosis of swine flu made. This trial will include about 100 adults and children from Nottingham, Leicester and Sheffield. We are recruiting patients both from the community and in hospital.

Do I have to take part?
No. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time, without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

If you do withdraw, we will ask why, as it might be important for other people, but you don’t have to give a reason if you don’t want to.

What will happen to me if we agree to take part?
If you choose to take part, the care you receive will not be different from that should you choose not to take part. You will be asked to sign a consent form. You will be given a copy of the information sheet and signed consent form to keep for your records.

We will confirm your entry into the study following a few questions. We will ask about your symptoms and their duration and if anyone else in your household has been ill. If your answers fit our criteria we might also then do a test for influenza by taking a nose swab. The test will be done whilst we are with you. If the test is positive you are eligible, if the test is negative you won’t be able to take any further part. This test is only being done for our research purposes, the result will not change the way you are being managed by your GP or anyone else.

If eligible, you will be involved in the trial for a maximum of 10 days and a minimum of 7. The number of days will depend on how long you have had symptoms before we meet you. If we meet on the day your symptoms begin we would like to visit every day for 10 days. If we meet 2 days after symptoms begin we will visit every day for 8 days. A member of the research team will carry out the visit, the person will usually be a nurse but maybe another healthcare professional. All staff will have undergone the necessary checks and training needed to conduct such work. We will arrange appointment times with you.
We would like to visit you every day during the study and perform the following procedures (in addition to what has been mentioned above already):

- **Symptom assessment** – At the first visit you will be asked to complete a number of assessment forms that cover your medical history and current symptoms. Subsequently we will ask you to complete a diary of your symptoms. You will complete a simple chart which asks whether you are feeling certain symptoms and how severe they are. In addition to this we will take an oral temperature reading.

- **Nose swab** – A large cotton bud will be used to take a swab from the inside of the nose, it does not need to go very far back! This will be collected once every day (except on the first day when it might be done twice).

- **Surface sampling** – We have already chosen a number of common household and hospital room surfaces that we would like to swab, e.g. dining table, taps, door handles, remote control. We want to see if we can find influenza virus on these surfaces. After swabbing we will clean these surfaces. We will take swabs every other day when we visit. You will be randomly split into 2 groups for this; Group 1 will have swabs done on Days 1, 3, 5, 7 and 9. Group 2 will be done on Days 2, 4, 6, 8 and 10.

- **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. We want to see if we can find influenza virus in these particles. The machines will stand in a room and run for a maximum of 3 hours. They do make a small amount of noise. This will be done every other day during the study. A member of the research team will be present to set the machine up and collect it afterwards.

Each of the visits will last for up to one hour except when air sampling is performed (see above) which will take longer. The researcher may set up the air sampling equipment, leave it running and then return before if finishes.

If you have been recruited in hospital and are later sent home, we would wish to follow you up at home for the remainder of the study period. Similarly, if you have been recruited in the community and need to be admitted to hospital we would follow you up in hospital.

This study will not interfere with the normal medical care you may receive. This includes the use of any medicines, e.g. antivirals

If for any reason you lose the capacity to consent during the study (e.g. the remote possibility that they are admitted to hospital and need to be sedated to help with breathing) we have included a box in the consent form to tick if you are happy for us to continue with our sampling during this period.

Initially your diagnosis of swine flu is likely to have been made on clinical grounds, i.e. the symptoms that you have. Some people may have a test to confirm this diagnosis (this will be different from the test we might have done initially on the nose
swab). If swine flu is confirmed you will remain in the study but should this test come back as negative, we will not perform any further sampling on or around you and you will be excluded from the study.

**What are the possible benefits of taking part?**
There is no specific treatment benefit as we will not influence your 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.

You 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 you or your family so that you can contact your GP or other responsible medical professional.

**Contact details**
If you have any problems, concerns or other questions about this trial, you should contact the research member of staff who visits each day. If you have any complaints about the way the research staff are carrying out the study you can make a complaint to the study Chief Investigator, Professor Jonathan Van-Tam, Clinical Sciences Building, City Hospital, Hucknall Road, Nottingham, NG5 1PB. Tel 0115 823 0276.

**What will happen if I don’t want to carry on with the trial?**
You can withdraw from the study at any time but it would be best to stay in contact with us and keep to the study assessments if possible. We will ask for your reasons for withdrawing, as they might be important for other people. You don’t have to give any reasons if you don’t want to.

**What if there is a problem?**
In the event that something goes wrong and you are harmed during the trial the University of Nottingham carries insurance to make sure that if any participant incurs any unexpected adverse event that leads to their being harmed and that the event occurred as a consequence of the protocol (i.e. non-negligent harm), then the participant will be compensated. In addition, all research staff have their own professional indemnity insurance which will cover any unexpected adverse event that leads to participant harm caused by negligence.

This study will be conducted in accordance with International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) guidelines (directive CPMP/ICH/135/95), local regulatory requirements and the declaration of Helsinki, and all relevant local laws and regulations.
Will my participation in this trial be kept confidential?
When you enter the trial the researcher will record information about your illness, medical history and the subsequent course of the illness. Some of this information may be taken from your medical notes (if you are in hospital). Collection and analysis of this information is an important part of the research. Your contact details will also be recorded but will be kept separate from the study data on a secure database.

The results of the trial will be published in medical journals and sent to regulatory authorities. However, all identifying personal details will be kept strictly confidential and no information will be published or given out through which you could be identified.

What will happen to the results of the trial?
Any results will be presented to the Department of Health in the first instance. Subsequently, results may be presented at scientific medical meetings and published in a leading medical journal and possibly in national and local media too. You will not be individually identified in any report or publication.

Who is organising and funding the research?
The University of Nottingham is organising this study. The NHS Health Technology Assessment (HTA) Programme has provided the research grant and no member of the research team are being directly paid for including you in this study.

Who has reviewed the study?
The trial was peer reviewed before funding by the HTA. This study was given a favourable ethical opinion for conduct in the public-health sector by the Leicester Research Ethics Committee, and was approved by the local NHS Trust Research & Development departments.

You will be given a copy of this Adult Information Sheet and a copy of the signed Consent Form to keep.

THANK YOU FOR TAKING THE TIME TO READ THIS INFORMATION SHEET
Parent/guardian

Parent / Guardian Information Sheet

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

You and your child, or teenager, are being invited to take part in this University of Nottingham sponsored medical research. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

Ask us if there is anything that is not clear or if you or your child would like more information. Take time to decide whether or not you wish to take part.

**What is the purpose of the research project?**

An influenza pandemic has recently been declared, involving the novel A(H1N1) ‘swine flu’ virus. This has spread to almost 100 countries worldwide in less than two months, causing widespread disease so far in Mexico, USA and Canada. It is highly likely that over the next 12 months, many countries including the UK will be affected by widespread illness. In the UK this wave of intense flu activity is most likely to occur in late autumn 2009.

Very little is known about the new H1N1 pandemic virus. For example we do not know how long the virus is excreted by infected humans and how much virus is spread to surfaces and carried in the air. This is very important to know as soon as possible because it affects the advice that will be given to healthcare workers about controlling the spread of infection to themselves and other patients. Similarly we need this information so we can give good quality advice to families who will have to look after each other in their own homes.

The best way to obtain this information is to ask patients who get pandemic flu soon (in August, September and October) to help us by agreeing to give a daily nose swab sample for just over one week so we can see how much virus is in the nose day by day and how quickly this disappears. At the same time we will take samples from hard surfaces in a patient’s room or home and sample the air using a special filter device. We can then work out how much virus is being excreted, how long the ‘danger period’ is, whether surfaces are more or less important than the air that we breathe (in terms of catching the virus) and if we can advise on a ‘safe distance’ from the patient, beyond which there is relatively little chance of catching the illness. We need to do these studies in children as well as adults because we already know that
children seem to hold on to the flu virus for longer and are not very good at respiratory hygiene!

The study involves a simple daily nasal swab and subjects who agree to take part will be inconvenienced to some extent. However, the technique of sampling from the nose is quick and not painful and should not present any problems, even in children. Normal medical care will not be affected in any way.

The team has been performing this kind of work for some time and is well qualified and experienced to carry out the study. Several members of the study team are leading international experts on influenza.

Why has my child been chosen?
Your child has been chosen as they have had a diagnosis of swine flu made. This trial will include about 50 children, aged 0 to 16 years primarily from Nottingham. We are recruiting patients both from the community and in hospital.

Does my child have to take part?
No. You and your child decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time, without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care your child receives.

If you do withdraw, we will ask why, as it might be important for other children, but you don’t have to give a reason if you don’t want to.

What will happen to my child if we agree to take part?
If you and your child choose to take part, the care your child receives will not be different from that should you choose not to take part. You will be asked to sign a consent form. You will be given a copy of the information sheet and signed consent / assent forms to keep for your records.

We will confirm your child’s entry into the study following a few questions. We will ask about their symptoms and their duration and if anyone else in the household has been ill. If the answers fit our criteria we might also then do a test for influenza by taking a nose swab. The test will be done whilst we are with you. If the test is positive your child will be eligible, if the test is negative they won’t be able to take any further part. This test is only being done for our research purposes, the result will not change the way your child is being managed by your GP or anyone else.

If eligible your child will be involved in the trial for a maximum of 12 days and a minimum of 9. The number of days will depend on how long your child has had symptoms before we meet you. If we meet on the day your child’s symptoms begin we would like to visit every day for 12 days. If we meet 2 days after symptoms begin we will visit every day for 10 days. A member of the research team will carry out the visit (the person will usually be a nurse but maybe another healthcare professional).
All staff will have undergone the necessary checks and training needed to conduct such work. We will arrange appointment times with you.

We would like to visit your child every day during the study and perform the following procedures (in addition to what has been mentioned above already);

- **Symptom assessment** – At the first visit you and your child will be asked to complete a number of assessment forms that cover your child’s medical history and their current symptoms. Subsequently we will ask your child (with your help if necessary) to complete a diary of their 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.

- **Nose swab** – A large cotton bud will be used to take a swab from the inside of the nose, it does not need to go very far back! This will be collected once every day (except the first day when it might be done twice).

- **Surface sampling** – We have already chosen a number of common household and hospital room surfaces that we would like to swab, e.g. dining table, taps, door handles, remote control. We want to see if we can find influenza virus on these surfaces. After swabbing we will clean these surfaces. We will take swabs every other day when we visit. You will be randomly split into 2 groups for this; Group 1 will have swabs done on Days 1, 3, 5, 7, 9, 11. Group 2 will be done on Days 2, 4, 6, 8, 10 and 12.

- **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. We want to see if we can find influenza virus in these particles. The machines will stand in a room and run for a maximum of 3 hours. They do produce a little bit of noise. This will be done every other day during the study. A member of the research team will be present to set the machine up and collect it afterwards.

Each of the visits will last for up to one hour except when air sampling is performed (see above) which will take longer. The researcher may set up the air sampling equipment, leave it running and then return before it finishes.

If your child has been recruited in hospital and is later sent home, we would wish to follow them up at home for the remainder of their study period. Similarly, if your child has been recruited in the community and needs to be admitted to hospital we would follow them up in hospital.

This study will not interfere with the normal medical care your child may receive. This includes the use of any medicines, e.g. antivirals.

If for any reason your child loses the capacity to consent / assent during the study (e.g. the remote possibility that they are admitted to hospital and need to be sedated to help with breathing) we have included a box in the consent form to tick if you and your child are happy for us to continue with our sampling during this period.
Initially your child’s diagnosis of swine flu is likely to have been made on clinical grounds, i.e. the symptoms that they have. Some people may have a test to confirm this diagnosis (this will be different from the test we might have done initially on the nose swab). If swine flu is confirmed your child will remain in the study but should this test come back as negative we will not perform any further sampling on or around your child and they will be excluded from the study.

What are the possible benefits of taking part?
There is no specific treatment benefit as we will not influence your child’s 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.

You 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 you or your family so that you can contact your GP or other responsible medical professional.

Contact details
If you have any problems, concerns or other questions about this trial, you should contact the research member of staff who visits each day. If you have any complaints about the way the research staff are carrying out the study you can make a complaint to the study Chief Investigator, Professor Jonathan Van-Tam, Clinical Sciences Building, City Hospital, Hucknall Road, Nottingham, NG5 1PB. Tel 0115 823 0276.

What will happen if I don’t want to carry on with the trial?
You and your child can withdraw from the study at any time but it would be best to stay in contact with us and keep to the study assessments if possible. We will ask for your reasons for withdrawing, as they might be important for other families. You don’t have to give any reasons if you don’t want to.

What if there is a problem?
In the event that something goes wrong and your child is harmed during the trial The University of Nottingham carries insurance to make sure that if any participant incurs any unexpected adverse event that leads to their being harmed and that the event occurred as a consequence of the protocol (i.e. non-negligent harm), then the participant will be compensated. In addition, all research staff have their own professional indemnity insurance which will cover any unexpected adverse event that leads to participant harm caused by negligence.

This study will be conducted in accordance with International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) guidelines (directive CPMP/ICH/135/95), local regulatory requirements and the declaration of Helsinki, and all relevant local laws and regulations.
Will my child’s taking part in this trial be kept confidential?
When your child enters the trial the researcher will record information about your child’s illness, medical history and the subsequent course of the illness. Some of this information may be taken from their medical notes (if they are in hospital). Collection and analysis of this information is an important part of the research. Your contact details will also be recorded but will be kept separate from the study data on a secure database.

The results of the trial will be published in medical journals and sent to regulatory authorities. However, all identifying personal details will be kept strictly confidential and no information will be published or given out through which you or your child could be identified.

What will happen to the results of the trial?
Any results will be presented to the Department of Health in the first instance. Subsequently, results may be presented at scientific medical meetings and published in leading medical journals and possibly in national and local media too. You or your child will not be individually identified in any report or publication.

Who is organising and funding the research?
The University of Nottingham is organising this study. The NHS Health Technology Assessment (HTA) Programme has provided the research grant and no member of the research team are being directly paid for including you in this study.

Who has reviewed the study?
The trial was peer reviewed before funding by the HTA. This study was given a favourable ethical opinion for conduct in the public-health sector by the Leicester Research Ethics Committee, and was approved by the local NHS Trust Research & Development departments.

You will be given a copy of this Parent / Guardian Information Sheet and a copy of the signed Consent Form to keep.

THANK YOU FOR TAKING THE TIME TO READ THIS INFORMATION SHEET
Children 0–8 years

Child Information Sheet (0-8 year olds)

A Study To Find Out How Much Flu Is Around You

Your invitation:
Can you help us do this study?
Talk about it with your family, friends, doctor or nurse.
And ask us lots of questions!

Why have I been asked to help?
Because you are unwell with flu. 50 children aged 0 to 16 years will be helping.

Do I have to take part?
No! It’s up to you. If you do help, you can change your mind later. This won’t upset anyone.

What will happen to me?
We would like to take a sample from your nose using a cotton bud and we will take some samples from objects and even the air around you. When we take samples from your nose it won’t hurt.

We will visit you every day, for about 10 days. You may be in hospital or at home, we will follow you wherever you go!

You will be visited by a member of our team, usually a nurse. They will make appointments to see you and your parents.

Will joining in help me?
It won’t help to make you better faster but the information we get might help us prevent other people from catching flu.

What if something goes wrong?
Any trouble you or your parents have will be looked into. Details about this are in the Parent / Guardian Information Sheet.

Will my medical details be kept private? Will anyone else know?
Yes. Some people (called research inspectors) may see your medical notes to make sure the study is done properly.

What if I don’t want to do the trial any more?
You and your parents can pull out of the trial treatment at any time.

You will have a copy of this Information Sheet to keep.

THANKS FOR READING THIS – please ask us anything you want.
Contact details:
If you have any worries or questions, please tell your parents. You can also contact;

Study Doctor: Prof Jonathan Van-Tam - 0115 823 0276
Appendix 3

Children 9–15 years

Young Person Information Sheet (9-15 year olds)

A Study To Find Out How Much Flu Is Around You

What is research?
Research helps us to improve how much we know about things. This study is research to find out how much flu people carry around with them when they are ill.

Your invitation:
Would you like to be in this trial?

Before you decide, read this leaflet carefully. Talk about it with your family, friends, doctor or nurse.

Ask us if there is anything that’s not clear or if you want to know more.

Why have I been asked to help?
Because you are unwell with flu. 50 children aged 0 to 16 years will be helping.

Do I have to take part?
No! It’s up to you. If you do help, you can still pull out at any time. If you do decide to stop this won’t upset anyone.

If you do pull out, we will ask you why, as it might be important for other young people. You don’t have to give a reason if you don’t want to.

What will happen to me?
We would like to take a sample from your nose using a cotton bud and we will take some samples from objects and even the air around you. When we take samples from your nose it won’t hurt.

We will also ask you to answer some questions about how you are feeling each day and we will take your temperature.

We will visit you every day, for about 10 days. You may be in hospital or at home, we will follow you wherever you go!

You will be visited by a member of our team, usually a nurse. They will make appointments to see you and your parents.

Might anything else about the research upset me?
We don’t think so!

Will joining in help me?
It won’t help to make you better faster but the information we get might help us prevent other people from catching flu.
What happens when the trial stops?
Nothing! You should be feeling better and we have the samples we need.

What if something goes wrong?
Any trouble you or your parents have will be looked into. Details about this are in the Parent / Guardian Information Sheet.

Will my medical details be kept private? Will anyone else know?
Yes. Some people (called research inspectors) may see your medical notes to make sure the study is done properly.

What if I don’t want to do the trial any more?
You and your parents can pull out of the trial treatment at any time.

You will have a copy of this Information Sheet to keep.

THANKS FOR READING THIS – please ask us anything you want.

Contact details:
If you have any worries or questions, please tell your parents.

You can also contact;
Study Doctor:
Prof Jonathan Van-Tam
0115 823 0276
Appendix 4

Eligibility checklist
### Eligibility checklist

**VIRUS SHEDDING STUDY**

**ELIGIBILITY CRITERIA**

| Date: _____/_____/2009 | Participant Code = □□□□□ |

<table>
<thead>
<tr>
<th>Consent</th>
<th>Yes / Positive</th>
<th>No / Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td></td>
<td></td>
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<tr>
<td>Cough</td>
<td></td>
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<tr>
<td>Sore throat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Runny nose</td>
<td></td>
<td></td>
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<tr>
<td>• Fever + 1 other or • 2 of the above</td>
<td>Yes / Positive</td>
<td>No / Negative</td>
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</table>

<table>
<thead>
<tr>
<th>Symptoms for &lt;48 hrs (Community)</th>
<th>Yes / Positive</th>
<th>No / Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms for &lt;96 hrs (Hospital)</td>
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<table>
<thead>
<tr>
<th>Near Patient Test for influenza done?</th>
<th>Yes / Positive</th>
<th>No / Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>• If Yes, positive or negative?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific test for swine flu</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• If Yes, positive or negative?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Any other household member with symptoms?</th>
<th>Yes / Positive</th>
<th>No / Negative</th>
</tr>
</thead>
</table>

| Taken part in other influenza research testing medicinal products in last 3 months? | Yes / Positive | No / Negative |

---

**If only Green Boxes ticked = Eligible**

**Any Red boxes ticked = Not Eligible**
Appendix 5

Recruitment leaflet
Swine Flu Research:

If you or any of your family have flu we need your help!

Should you or other members of your family / household become unwell with symptoms such as cough, fever, sore throat, tiredness and runny nose over the next few weeks, we would like to invite you to take part in some medical research being run by the University of Nottingham.

The Department of Health has provided funding for this vital research. The study involves a nurse or doctor visiting daily to collect a nose swab and swabs from some surfaces in your home. Your help is really important to us. We hope to improve our understanding of how swine flu is spread which may lead to fewer people becoming infected.

So, if you or any family or household member develops flu-like symptoms and you/they feel able to take part in our study, please ring us and speak to one of our team. We are looking for people who have had symptoms for no more than 2 days so please call as soon as you think you are unwell. It does not matter whether medication is being taken or not.

Keep this card and call 0115 823 1813 anytime
Appendix 6

PCR protocol and culture protocol

**PCR protocol**

*PCR*

Nucleic acid was extracted from the samples using the Qiagen Symphony SP extractor mini kits, including onboard lysis and a bacteriophage (MS2) as internal control. A novel influenza A H1N1 pentaplex assay was devised to detect virus genome in the samples. The assay was designed to detect novel H1N1 influenza A, seasonal H1 influenza A, seasonal H3 influenza A, influenza B and the internal control, MS2. Reactions were carried out on a Rotorgene™ 6000 (Corbett Research) real-time DNA detection system. Viral load data were generated using the PCR assay and plasmids containing the gene target to create a standard curve, such that the concentration of genome present in each sample could be calculated.

The primers and probes used were as shown below.

**Primers**

Novel H1N1 influenza A (Metabion):

- H1FORSW: 5′–TCA ACA GAC ACT GTA GAC ACA GTA CT–3′
- H1REVSW: 5′–GTT TCC CGT TAT GCT TGT CTT CTA G–3′

Seasonal H1 influenza A (MWG Biotech):

- AH1 Forward: 5′–GGA ATA GCC CCC CTA CAA TTG–3′
- AH1 Reverse: 5′–AAT TCG CAT TCT GGG TTT CCT A–3′

Seasonal H3 influenza A (MWG Biotech):

- AH3 Forward: 5′–CCT TTT TGT TGA ACG CAG CAA–3′
- AH3 Reverse: 5′–CGG ATG AGG CAA CTA GTG ACC TA–3′

Influenza B (Metabion):

- BNP-F: 5′–GCA GCT CTG ATG TCC ATC AAG CT–3′
- BNP-R: 5′–CAG CTT GCT TGC TTA RAG CAA TAG GTC T–3′

**MS2 control (MWG Biotech):**

- MS2 Forward: 5′–TGG CAC TAC CCC TCT CCG TAT TCA CG–3′
- MS2 Reverse: 5′–GTA CGG GCG ACC CCA CGA TGT=A C–3′

**Probes**

Novel H1N1 influenza A (Metabion):  
- H1SWp3: 5′–Cy5-AAT GTA ACA GTA ACA CAC T CGT TTA ACC BHQ-3

Seasonal H1 influenza A (ABI):  
- AH1 Probe: 5′–6FAM CGT TGC CGG ATG GA-MGBNFQ–3′

Seasonal H3 influenza A (ABI):  
- AH3 Probe: 5′–VIC-CCT ACA GCA ACT GTT ACC-MGBNFQ–3′

Influenza B (Biosearch Technologies):  
- Flu-B Probe: 5′–Quasar 705-CCA GAT CTG GTC ATT GGR GCC CAR AAC TG-BHQ-2–3′

**Culture protocol**

Cultures were performed from the last day of nasal swab PCR positivity. If a culture was positive on any given day then an assumption was made that previous days would also have been culture positive.

**Technique**

Pandemic H1N1 did not form plaques readily and gave only a weak cytopathic effect, the latter meaning that the tissue culture infectious dose (TCID) 50 was difficult to calculate. Consequently, immunofluorescence to detect the influenza A nucleoprotein was used to demonstrate the
Appendix 6

presence of live replicating virus in the nuclei of infected cells.

Madin–Darby Canine Kidney (MDCK) cells were used to propagate the virus. Initially, cells were plated on to six-well tissue culture dishes (Corning), at a concentration of $7.5 \times 10^5$ cells/well. Following 24 hours’ incubation, the samples were defrosted. The cells were washed $\times 2$ in serum-free medium [SFM – Dulbecco’s Modified Eagle’s Medium (DMEM)] and 400 µl of each sample was applied to the respective well. After 30 minutes the cells were overlaid with 2 ml of SFM containing 0.14% fetal calf serum (FCS) and 0.1% Worthington’s trypsin. Dilutions (1 : 10) of influenza A (H1N1 human influenza virus A/PuertoRico/8/34) and a novel H1N1 influenza A isolate (A H1N1 Cambridge AHO4/2009) were also inoculated on to cells as positive controls. The cells were then incubated for 48 hours at 37°C. The following day, 24-well tissue culture dishes were seeded with $1 \times 10^5$ MDCK cells per well. Then, 48 hours after infection the virus was harvested. Two dilutions were made in SFM: 1 : 2 and 1 : 10. After washing the cells in the 24-well dishes $\times 2$ in SFM, 250 µl of each dilution was added to the appropriate well. Following 30 minutes’ incubation at 37°C, 1 ml of overlay (as before) was added to each well and the cells were incubated overnight. After overnight incubation, the virus dilutions were aspirated off the cells. The cells were washed $\times 2$ with phosphate-buffered saline (PBS) and then fixed with 250 µl of 4% formaldehyde at room temperature for 20 minutes. The fix was aspirated off and the cells were washed $\times 3$ with blocking solution (1% FCS in PBS). The cells were permeabilised in detergent (0.2% Triton 100 in PBS) and then washed $\times 3$ with blocking solution (1% FCS in PBS). The cells were permeabilised in detergent (0.2% Triton 100 in PBS) and then washed $\times 2$ in block. Then 250 µl of a mouse monoclonal antibody (anti-NP, Abcam, ab43821) was added to each well and the plates were incubated for 60 minutes before washing $\times 3$ with block. The secondary antibody (goat anti-mouse 488 IgG2a, Molecular Probes) was diluted 1 : 1000 in block, and 4’,6 diamino-2-phenylindole (DAPI) diluted 1 : 2000. Then 250 µl of this mix was added to the cells. Incubation was in the dark for 30–45 minutes. Cells were washed thoroughly with block, left in PBS and examined on the fluorescence microscope.

---

<table>
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<tr>
<th>Stock concentration (pmol/µl)</th>
<th>Volume of stock/reaction (µl)</th>
<th>For 80 reactions (µl)</th>
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<tbody>
<tr>
<td>H1FORSW (20)</td>
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<td>H1REVSW (20)</td>
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<tr>
<td>AH3 Reverse (50)</td>
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</tr>
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<td>0.2</td>
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<tr>
<td>MS2 Probe (10)</td>
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<td>16</td>
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<tr>
<td>2 RT platinum buffer (Invitrogen)</td>
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<td>1000</td>
</tr>
<tr>
<td>Superscript III platinum enzyme</td>
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<td>40</td>
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<tr>
<td>Water</td>
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<td>Total volume</td>
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Appendix 7

Composite subject charts
**AC01 – 21 years, female, no comorbidity, no oseltamivir**

Day 1 3 5 7 9 11 13

- **Symptoms**
- **Nose swab**
- **Surfaces**
- **Air**

**Follow-up ends**

**AC04 – 28 years, female, asthma, no oseltamivir**

Day 1 3 5 7 9 11 13

- **Symptoms**
- **Nose swab**
- **Surfaces**
- **Air**

**Follow-up ends**

**AH01 – 19 years, female, cystic fibrosis, received oseltamivir**

Day 1 3 5 7 9 11 13

- **Symptoms**
- **Nose swab**
- **Surfaces**
- **Air**

**Oseltamivir started**
AH03 – 27 years, female, no comorbidity, received oseltamivir

Symptoms

Nose swab

Surfaces

Air

PCR

Culture

Oseltamivir started

Follow-up ends

Day

1

3

5

7

9

11

13

PCR

Culture

Also positive on day 8 but other household members unwell

AH04 – 30 years, female, no comorbidity, received oseltamivir

Symptoms

Nose swab

Surfaces

Air

PCR

Culture

Follow-up ends

Day

1

3

5

7

9

11

13

Oseltamivir started

AH05 – 24 years, female, no comorbidity, no oseltamivir

Symptoms

Nose swab

Surfaces

Air

PCR

Culture

Follow-up ends

Day

1

3

5

7

9

11

13
AH07 – 34 years, male, no comorbidity, no oseltamivir

Symptoms

Nose swab

Surfaces

Air

PCR
Culture

Follow-up ends

AH08 – 33 years, male, asthma, received oseltamivir

Symptoms

Nose swab

Surfaces

Air

PCR
Culture

Oseltamivir started

CC01 – 12 years, male, asthma, received oseltamivir

Symptoms

Nose swab

Surfaces

Air

PCR
Culture

Oseltamivir started
CC02 – 11 years, male, no comorbidity, no oseltamivir

CC03 – 6 years, male, no comorbidity, received oseltamivir

CC04 – 2 years, male, no comorbidity, no oseltamivir
CC05 – 9 years, male, asthma, received oseltamivir

Symptoms

Nose swab
PCR
Culture

Surfaces
PCR
Culture

Air
PCR
Culture

CC06 – 4 years, male, no comorbidity, no oseltamivir

Symptoms

Nose swab
PCR
Culture

Surfaces
PCR
Culture

Air
PCR
Culture

CC07 – 3 years, female, no comorbidity, received oseltamivir

Symptoms

Nose swab
PCR
Culture

Surfaces
PCR
Culture

Air
PCR
Culture

Oseltamivir started

Follow-up ends
CC14 – 6 years, male, no comorbidity, no oseltamivir

Day 1 3 5 7 9 11 13

Symptoms

Nose swab PCR Culture

Surfaces PCR Culture

Air PCR Culture

Follow-up ends

CC15 – 2 years, female, no comorbidity, no oseltamivir

Day 1 3 5 7 9 11 13

Symptoms

Nose swab PCR Culture

Surfaces PCR Culture

Air PCR Culture

Follow-up ends

CC14 present

CH01 – 15 years, female, no comorbidity, received oseltamivir

Day 1 3 5 7 9 11 13

Symptoms

Nose swab PCR Culture

Surfaces PCR Culture

Air PCR Culture

Oseltamivir started

Follow-up ends

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CH03 – 0 years, female, cystic fibrosis, received oseltamivir

Day 1 3 5 7 9 11 13
Symptoms
Nose swab
PCR
Culture
Surfaces
PCR
Culture
Air
PCR
Culture

Follow-up ends

Oseltamivir started