

# Antiviral susceptibility testing of pandemic (H1N1) 2009 influenza viruses



## Background

There are currently two drugs recommended for the treatment of influenza in the UK, oseltamivir (Tamiflu) and zanamivir (Relenza), both neuraminidase inhibitors. The influenza A M2 inhibitor drugs amantadine and rimantadine are not recommended, since pandemic (H1N1) 2009 influenza viruses are resistant to these drugs.

Since the initial characterisation of the pandemic influenza virus in March 2009, laboratories worldwide have been monitoring the susceptibility of the virus to neuraminidase inhibitors. Testing for antiviral susceptibility of influenza viruses, including the pandemic (H1N1) 2009 strain is currently undertaken by the Respiratory Virus Unit (RVU) at the Health Protection Agency's Centre for Infections (CfI).

## Sources of influenza viruses

Specimens confirmed positive for pandemic (H1N1) 2009 influenza virus by laboratories in the Health Protection Agency's Regional Microbiology Network (RMN) are forwarded to CfI for virological investigations. These include specimens from patients hospitalised with influenza and community samples taken by GPs.

Community samples are also obtained via either the existing Royal College of General Practitioners (RCGP) influenza surveillance scheme for seasonal influenza, or through enhanced surveillance studies (National Pandemic Flu Service, Fluwatch, MOSA schools). Diagnosis of pandemic (H1N1) 2009 influenza in these samples will be made by RVU at CfI.

## Surveillance of influenza viruses

Confirmed pandemic (H1N1) 2009 influenza clinical specimens are processed for virus isolation at the RVU. An antigenic typing profile is developed for each virus isolate grown to sufficient titre.

The antigenic similarity to candidate pandemic (H1N1) 2009 influenza vaccine viruses including A/California/7/2009 and A/England/195/2009, and to other pandemic (H1N1) 2009 influenza viruses circulating in the UK is determined. This allows analysis of the antigenic drift of pandemic (H1N1) 2009 influenza as it circulates through the UK population over time.

Susceptibility to anti-influenza drugs and whole genome sequencing is performed on pandemic (H1N1) 2009 influenza clinical material and virus isolates. These investigations enable comprehensive virological analysis of pandemic (H1N1) 2009 influenza circulating in the UK following emergence in April 2009 and throughout the winter influenza season 2009/10.

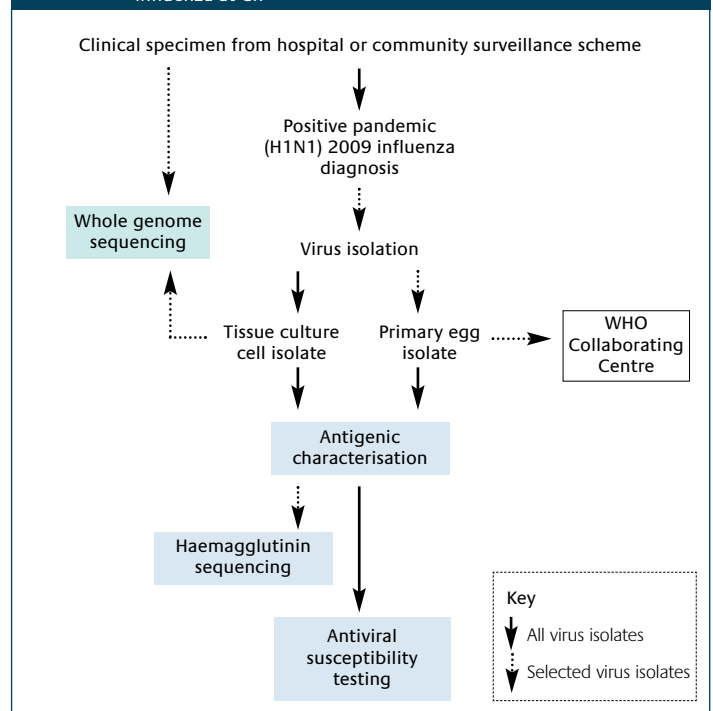
Genetic characterisation of clinical isolates of pandemic (H1N1) 2009 influenza is performed by targeted haemagglutinin (HA) sequence analysis and whole genome sequencing.

The emergence of pandemic (H1N1) 2009 influenza antigenic drift variants will be monitored by the

identification of mutations in antigenic regions and receptor binding site residues of the HA gene. Phylogenetic analysis of the sequence of each of the eight viral gene segments following whole genome nucleotide sequencing will allow in-depth analysis of virus evolution over time in the human population.

Analysis of viruses from known patient clusters may detect viral mutations required for human adaptation and efficient transmission. Analysis of virus from atypical cases may produce data on the molecular determinants of pathogenicity of pandemic (H1N1) 2009 influenza viruses.

**Figure 1** Overview of virological surveillance of pandemic (H1N1) 2009 influenza at CfI



## Virological surveillance

### Specimen selection

Specimens received from hospital laboratories are assessed for suitability for virus isolation based on sample volume and integrity. A range of specimens from hospital and community sources are selected for culture, including samples from representative and atypical patient presentations, and with a broad temporal and geographical distribution.

Virus isolates and viral sequence information is obtained from patients with severe or unusual disease presentation including hospitalised and fatal cases.

Sampling criteria have been revised as the pandemic has progressed, but current criteria relate to sentinel surveillance, and non-sentinel cases according to criteria applied locally for enhanced sampling and virus diagnosis in hospitalised cases.

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In patients undergoing treatment with antiviral drugs, pyrosequencing of virus directly from clinical samples can identify drug resistance due to known mutations, and virus isolates can be screened for resistance due to novel mutations by phenotypic methods.

Antigenic typing and HA sequencing can identify evidence of antigenic drift and antigen escape mutations in virus isolates obtained from patients who have received influenza vaccine. All laboratory testing procedures on pandemic (H1N1) 2009 influenza viruses are performed in microbiological safety cabinets.

## Growth of virus isolates

Viruses are grown in appropriate media including tissue culture cells and fertilised hens' eggs following standard procedures. Sequencing of the viral HA is performed to determine whether any adaptive changes occur during virus growth in the laboratory and will inform selection of the appropriate conditions for faithful replication of the virus.

Specimens are processed daily for virus isolation in tissue culture, by inoculation of each specimen into two different cell lines, with pandemic (H1N1) 2009 influenza viruses cultured in a separate laboratory from seasonal influenza virus culture. Currently, virus isolation in tissue culture can be performed on up to 460 samples per week at RVU.

**Table 1** Example of specimens cultured over a three-week period in 2009

Week number	Number of samples inoculated for virus isolation
42	209
43	421
44	382

Egg isolates made directly from clinical specimens could be provided to the Health Protection Agency's National Institute for Biological Standards and Control for developing candidate vaccine viruses in the event that an antigenic variant of pandemic (H1N1) 2009 influenza virus is detected through virological surveillance.

A selection of representative and atypical virus isolates will be provided to the WHO Collaborating Centre at the National Institute for Medical Research, London for further characterisation and comparison with pandemic (H1N1) 2009 influenza viruses circulating worldwide.

## Antiviral susceptibility testing

Virus isolates are tested for susceptibility to the neuraminidase inhibitor drugs oseltamivir and zanamivir, and to the M2 blocker amantadine. Antiviral susceptibility is assessed using genotypic and phenotypic methods.

## Genotypic antiviral susceptibility testing

Pyrosequencing is a nucleotide sequence-based technology that is used for analysing short stretches of DNA. This technique is used to characterise regions of

sequence known to be associated with antiviral resistance in influenza viruses and can be used on both cultured virus and directly on clinical material.

The ability to perform genotypic antiviral susceptibility testing directly on clinical samples means that it can be used to inform individual patient management.

Pyrosequencing from clinical material can be used to detect the H275Y mutation in N1 neuraminidase that is known to confer resistance to oseltamivir, and other mutations in the matrix gene of influenza A viruses that confer amantadine resistance.

At RVU all confirmed pandemic (H1N1) 2009 influenza virus samples are first screened by pyrosequencing for the H275Y mutation in N1 neuraminidase, before inoculation into cell culture, and both phenotypic antiviral characterisation and antigenic analysis can subsequently be performed.

Pandemic (H1N1) 2009 influenza viruses found by pyrosequencing to have the 275Y mutation conferring resistance to oseltamivir, cannot be cultured in the same laboratory as oseltamivir-sensitive pandemic (H1N1) 2009 influenza strains, or seasonal influenza viruses.

Hence, genotypic antiviral susceptibility testing is performed daily to not only inform patient management, but to also enable timely virus isolation of pandemic (H1N1) 2009 influenza strains in the appropriate laboratory.

## Phenotypic antiviral susceptibility testing

Phenotypic characterisation by enzyme inhibition tests are performed with cultured virus to confirm genotypic data, monitor natural variation in drug susceptibility and identify antiviral resistance from unknown mutations in the viral genome. Phenotypic characterisation by these assays determines the concentration of neuraminidase inhibitor (NI) drug that results in a 50% inhibition of neuraminidase enzyme activity (IC50 value). The assays are used to measure susceptibility to both NI drugs oseltamivir and zanamivir.

As for virus isolation in culture, phenotypic analysis of oseltamivir sensitive and resistant pandemic (H1N1) 2009 viruses and seasonal influenza viruses is performed in separate laboratories.

Cases of pandemic (H1N1) 2009 influenza infection with NI resistant virus will be followed up virologically, by analysis of pre and post-treatment specimens and sequential sampling while symptoms persist, and also clinically and epidemiologically.

The results of antiviral susceptibility testing of pandemic (H1N1) 2009 influenza viruses at RVU are reported nationally in the HPA Weekly National Influenza Report, and internationally to the European Centre for Disease Prevention and Control (ECDC), and the WHO.