

# **National Laboratory Guidelines for Pandemic Influenza**

Collection and Handling of Human  
Specimens for Laboratory Diagnosis of  
Influenza with Pandemic Potential

## Acknowledgements

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# 1 Introduction

These guidelines are intended for use by health professionals and laboratory staff to ensure safe collection, handling and transport of human specimens for diagnosis of influenza with pandemic potential. The guidelines were compiled with assistance from members of the New Zealand Virology Laboratory Network and they will be updated, as necessary, as more information becomes available.

## 2 Background

An influenza pandemic is the most likely event to cause a large-scale health emergency. The three human pandemics in the 20th century – the 1918 ‘Spanish’, 1957 ‘Asian’ and 1968 ‘Hong Kong’ pandemics – all reached New Zealand.

The current risk to human health posed by the avian influenza H5N1 panzootic has highlighted the need for pandemic planning.

During a World Health Organization (WHO) Global Influenza Pandemic Alert, and in concert with New Zealand’s pandemic response strategy, all health care providers should be alert for patients with respiratory illness that could be pandemic influenza. For all suspected patients, respiratory samples for virus detection and acute and convalescent serum samples should be collected.

The overall approach to diagnosis and management will be affected by the WHO/Ministry of Health pandemic alert status. During the early phases of an influenza pandemic in New Zealand (border management and cluster control phases, WHO phases 4–5), influenza A diagnostic tests with the maximum sensitivity and specificity and a turnaround time of less than 24 hours will be required to ensure accurate identification and to assist a rapid public health response. Once pandemic influenza has entered New Zealand, the need for highly accurate testing will diminish.

The WHO recommended strategy for initial testing of each specimen is to diagnose influenza A virus infection rapidly and exclude other common viral respiratory infections. Investigations for other potential causes of the illness as deemed appropriate by the attending physicians will require other general clinical tests such as biochemical, serological and haematological assays. These guidelines therefore cover both testing for influenza and general diagnostic laboratory testing.

Influenza can cause serious human disease. The influenza virus is predominantly spread by large droplets and may be spread by direct and indirect contact. Comprehensive biosafety procedures are required in each laboratory, especially where aerosol-producing procedures are being carried out on respiratory samples.<sup>1</sup>

The principle of biosafety protection against influenza A is to establish multiple layers of protection. This principle applies as follows:

- **Personal protection:** Laboratory staff should wear personal protective equipment (PPE, see Appendix 1 for details).
- **Aerosol and droplet precaution:** A Biological Safety Cabinet should be used for all technical procedures that may generate aerosols or droplets (eg, vortexing, opening and pipetting specimen tubes).

<sup>1</sup> WHO. 2005. *WHO laboratory biosafety guidelines for handling specimens suspected of containing avian influenza A virus*. Geneva: World Health Organization. URL: [http://www.who.int/csr/disease/avian\\_influenza/guidelines/handlingspecimens/en/index.html](http://www.who.int/csr/disease/avian_influenza/guidelines/handlingspecimens/en/index.html).

WHO. 2004. *Laboratory Biosafety Manual*. Geneva: World Health Organization. URL: <http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf>.

- **Contact precaution:** Decontamination should be performed for all technical procedures that may result in contact contamination.

These guidelines will be updated as necessary, as more information becomes available, and are to be used in conjunction with usual laboratory standards AS/NZS 2243.3:2002 and NZS/ISO 15189:2003.

### 3 Clinical Diagnosis

A critical first step in the pandemic response strategy is the clinical assessment of the probability of a true pandemic influenza (PI) case. This protocol refers only to suspected or probable cases. Specimens should not be processed unless there has been discussion with the on-call microbiologist or infectious disease physician.

The clinical case definition may be modified in the face of pandemic influenza, but the current working case definition for avian influenza (AI) is provided below.

#### **Clinical case definition for avian influenza**

Acute onset fever  $\geq 38^{\circ}\text{C}$ , sore throat and cough, **plus:**

- contact (within seven days prior to onset of symptoms) with a confirmed case of avian influenza while this case is infectious
- **or** contact (within seven days prior to onset of symptoms) with domestic poultry or contact with birds in an area known to have avian influenza
- **or** has worked in a laboratory (within seven days prior to onset of symptoms) that is processing samples from persons or animals that are suspected of having avian influenza infection.

## 4 Specimen Types for Influenza Testing

### 4.1 Specimens

#### 4.1.1 Preferred specimens

Collect upper respiratory tract samples. The specimens of choice are:

1. nasopharyngeal swab (NPS)
2. throat swab (TS).

During the early phases of a pandemic, when accurate diagnosis is crucial, take **TWO separate samples**.

NPSs pose a lower risk to staff during collection than do nasopharyngeal aspirates or nasal washes, both of which may generate aerosols and must be performed in a controlled environment – such as a negative pressure room. Nasal swabs are not recommended as they provide lower yields of virus.

- A NPS can be combined with a TS in a single Virus Transport Medium (VTM) tube if necessary.
- If VTM is unavailable, use 1 ml sterile saline, and immerse the swab in the fluid.
- Use other VTM-swab tubes only as approved by the receiving laboratory.

#### 4.1.2 Other specimens

Depending on the nature of the pandemic virus, it may be appropriate to collect other specimens. Anterior nasal swabs are less sensitive. Invasive procedures (such as bronchoalveolar lavage or lung biopsy) may also be performed for the diagnosis of virus infection. The optimal sampling strategy will only be available once the illness caused by PI virus has been defined.

Postmortem samples are not covered in these guidelines.

#### 4.1.3 When samples should be collected

It is preferable to take respiratory samples for virus isolation, or for the detection of viral nucleic acids or antigens, during the first three days after the onset of clinical symptoms. However, they may be taken up to a week after the illness onset, or even later in severely ill or immune-compromised patients.

#### 4.1.4 Blood for serology

Take an acute phase serum sample (7–10 ml whole blood) as soon as possible after the onset of clinical symptoms, and no later than seven days after onset. Collect a convalescent-phase serum sample 14 days later.

## 4.2 Safety

Persons collecting respiratory samples must be properly trained. The key points are:

- Keep yourself safe.
  - Wear personal protective equipment (PPE) and know how to correctly and safely put it on and remove it (see Appendix 1 for details).
- Keep others safe.
  - Label specimen containers before entering the specimen collection room. Labelling should include patient name, NHI number (if known), date of birth and date of collection.
  - Do not take paperwork, including request forms, into the specimen collection room. On the request form, highlight the words **suspected pandemic influenza infection**.
  - Record contact details for patient and requester. Laboratory staff need to know who to notify for critical results.
  - Alert laboratory staff that samples are being taken, to allow time for preparation.
  - Keep sample containers separate from all other samples.
  - Sample containers should be double-bagged before storage and transportation (see section 5).
- Integrate testing with patient care.
  - Don't let fear of PI interfere with diagnosis and management.

## 4.3 Sample collection

### 4.3.1 Nasopharyngeal swab

Use a pernasal swab with non-wooden shaft and synthetic fibre tip:

1. Label sample tube with patient name, NHI number (if known), date of birth and date of collection.
2. Insert swab into one nostril, parallel to the palate, rotate gently and advance until resistance is felt (one eye often waters when swab is in correct position).
3. Press swab tip on the mucosal surface of the mid-inferior portion of the inferior turbinate (see diagram), leave in place for a few seconds, then slowly withdraw with a rotating motion.
4. Place tip of swab back into swab collection tube containing VTM and carefully break or cut the shaft of the swab.
5. Close the lid tightly.
6. Place the sample tube in a plastic specimen bag, then double-bag for protection against spillage.
7. The sample in VTM should be delivered to the laboratory promptly or stored at 4°C.



### **4.3.2 Throat swab**

Use a plastic shafted dacron swab:

1. Label sample tube with patient name, NHI number (if known), date of birth and date of collection.
2. Get patient to say 'ahhh' and vigorously swab both tonsillar areas and posterior nasopharynx. Use tongue depressor to depress tongue in order to prevent contamination of swab with saliva.
3. Place swab back into swab collection tube in VTM and snap the shaft of the swab.
4. Close the lid tightly.
5. Place the sample tube in a plastic specimen bag, then double-bag it for protection against any spillage.
6. The sample in VTM should be delivered to the laboratory promptly or stored at 4°C.

## 5 Specimen Transport and Processing

### 5.1 Pandemic influenza referral laboratories

During the early phases (border management and cluster control), ensure timely and rapid forwarding of samples from suspected and probable PI cases, via regular laboratory transport network systems, to one of the referral laboratories as listed below.<sup>2</sup>

1. Auckland region: Department of Virology  
LabPLUS  
Building 31  
Auckland City Hospital  
Private Bag 92 024  
Grafton, Auckland  
Ph: (09) 307 8995  
After hours: (09) 379 7440
2. Central region: Specialist Services Laboratory  
Waioira Building Level 3  
Waikato Hospital  
Pembroke Street  
Hamilton  
Ph: (07) 839 8726 ext 8530 or 8460  
After hours: (07) 839 8899
3. Wellington region: ESR  
34 Kenepuru Drive  
PO Box 50 348  
Porirua  
Ph: (04) 914 0690  
(04) 914 0728  
(04) 914 0764  
After hours: 027 576 6367
- Specialist Services Laboratory  
Clinical Services Block, Level F  
Wellington Hospital  
Riddiford Street  
Private Bag 7902  
Wellington  
Ph (including after hours):  
(04) 385 5999 ext 6060
4. Southern region: Virology/Molecular Microbiology Section  
Microbiology Unit  
Canterbury Health Laboratories  
PO Box 151  
Christchurch  
Ph: (03) 364 0356 (Virology lab)  
(03) 364 0354 (Section Head)  
After hours: (03) 364 0640

**Contact after hours:** To contact the on-call microbiologist or infectious disease physician, phone the appropriate after hours hospital number above.

<sup>2</sup> For a list of tests available at each regional virology laboratory, see Appendix 4.

## 5.2 Specimen packaging and transport

It is essential that the laboratory receiving the sample is aware that it comes from a suspected or probable pandemic influenza case. In addition, the facilities required to safely handle the sample must be available.

The ultimate aim is to have high quality samples sent to the appropriate laboratory as fast as possible.

Package and transport specimens as per standard recommendations for infectious substances.

Transport samples between laboratories following standard procedures.

Where an isolate or suspected organism is being referred to a reference laboratory for further testing, transport the specimen as an infectious substance (packaged according to instruction 602 of the International Air Transport Association's Dangerous Goods Regulations). Make telephone contact with the receiving laboratory to facilitate safe and rapid processing of specimens.

See Appendix 2 for information on transport of specimens to the Institute of Environmental Science and Research (ESR), which has access to PC3 facilities for viral culture.

Positive isolates from early cases will be sent by the referral laboratories (following appropriate transport regulations) to the Commonwealth Serum Laboratories (CSL), the WHO Collaborating Centre in Melbourne, for confirmation.

## 5.3 Arrival of specimens

### 5.3.1 Arrival of samples in the laboratory

1. Notify the laboratory before samples arrive for testing.
2. Do **not** use a pneumatic tube system to transport specimens.
3. Reception staff should assist with assigning laboratory numbers to the outside of biohazard bags and process request forms for data entry as usual. However, they should **not** open a suspected PI package.
4. Tubes should be opened and separated in a Biological Safety Cabinet by designated personnel before being transferred to other areas for routine analyses. A sealed bucket/rotor centrifuge should be used as standard.

### 5.3.2 Arrival of samples with no prior warning

Contact the on-call microbiologist or infectious disease physician immediately if a sample labelled 'PI' arrives without prior warning or discussion. The sample should not be handled further except under their advice. The purpose of these guidelines is to ensure consistent, appropriate procedures are followed in all laboratories for all samples.

### **5.3.3 Arrival of contaminated samples**

If contaminated tubes (eg, due to leakage) arrive, do not process them. Instead, contact the clinical microbiologist.

Follow standard operating procedures and discard.

## **5.4 Specimen processing<sup>3</sup>**

### **5.4.1 Processes that can be conducted using standard precautions in PC2 facilities**

Standard precautions in PC2 facilities can be used for:

- blood and urine specimen processing (note that initial opening of blood tubes should be performed in a Biological Safety Cabinet)
- pathological examination and processing of formalin-fixed or otherwise inactivated tissues
- molecular analysis of extracted nucleic acid preparations
- electron microscopic studies with glutaraldehyde-fixed grids
- routine examination of bacterial and fungal cultures following the initial inoculation
- routine staining and microscopic analysis of fixed smears
- final packaging of specimens for transportation to diagnostic or reference laboratories for additional testing. Specimens should already be in a sealed, decontaminated primary container.

### **5.4.2 Processes that can be conducted with more stringent work practices in PC2 facilities**

More stringent work practices (see below) in PC2 facilities can be used for:

- cutting up, blocking and macroscopic description of respiratory tissue
- initial opening of blood tubes
- aliquoting and/or diluting specimens
- inoculation of bacterial, fungal and virological culture media
- performing diagnostic tests that do not involve propagation of viral agents
- nucleic acid extraction procedures involving untreated specimens
- preparation and chemical- or heat-fixing of smears for microscopic analysis.

<sup>3</sup> As per AS/NZS 2243.3:2002; see Department of Health and Ageing, Australia. 2005. Australian Management Plan for Pandemic Influenza (Section 5, Annex 5). URL: <http://www.health.gov.au/internet/wcms/publishing.nsf/Content/phd-pandemic-plan-5d.htm>.

The **stringent measures** to be employed for these activities in PC2 facilities are as follows:

- Medical laboratory staff should wear protective equipment, including:
  - disposable gloves
  - disposable solid front gowns that are either impermeable or covered with a plastic apron, and that have cuffed sleeves
  - full eye protection and respiratory protection, preferably a N-95 particulate filter mask. A surgical mask may be substituted if necessary provided that the work is carried out in a Biological Safety Cabinet. Personnel who cannot wear these masks because of facial hair or other fit-limitations should wear loose-fitting hooded or helmeted Powered Air Purifying Respirators.
- All specimen manipulations, including aerosol-producing procedures such as centrifugation, should be carried out in a Biological Safety Cabinet.

#### **5.4.3 Processing respiratory samples**

- Treat all respiratory samples as highly infectious and process them using a Biological Safety Cabinet.
- All workers must wear PPE (see Appendix 1) when handling and working with respiratory specimens.
- Air dry smears in the Biological Safety Cabinet and fix them in alcohol for five minutes before processing them in the normal way for a Gram stain.
- Inoculate bacterial culture plates in the Biological Safety Cabinet before incubation.
- Place fluids for cell counts into the counting chamber in the Biological Safety Cabinet, and perform microscopy while wearing a mask.
- The sample is infectious until:
  - a) nucleic acid extraction has been completed **or**
  - b) the specimen has been fixed in alcohol or acetone for five minutes.

#### **5.4.4 Processing blood samples**

Perform centrifugation in a sealed bucket/rotor centrifuge. The use of screw-top tubes for the storage of plasma and serum is recommended.

The registration of the sample, label printing and labelling of tubes (except for the primary one) can occur in the normal manner.

- Staff performing the centrifugation steps must wear PPE as described in Appendix 1.
- Open the bag containing patient samples in the Biological Safety Cabinet.
- Place sample tubes in a sealed centrifuge container.
- The sealed container can now be taken from the Biological Safety Cabinet and placed in the centrifuge.
- Balance the sample, then spin as per normal protocol.

- After centrifugation, remove the sealed centrifuge container and return it to the Biological Safety Cabinet.
- Open primary tubes after centrifugation in the Biological Safety Cabinet, as centrifugation may generate aerosols.
- Any separation of cells, plasma and serum will occur in the Biological Safety Cabinet.
- Transfer the samples to a screw-top tube. Decontaminate the new tube before it is taken to the core laboratory.
- Decontaminate the centrifuge bucket in the appropriate manner.

#### **5.4.5 Faecal samples**

Process faecal samples using standard laboratory precautions. Extra precautions may be necessary once the epidemiology and pathogenicity of the pandemic virus are known. These guidelines will be updated as more information becomes available.

#### **5.4.6 Urine samples**

Pipette or manipulate urine samples in a Biological Safety Cabinet, using standard laboratory precautions.

### **5.5 Decontamination**

Decontaminate work surfaces and equipment after specimen processing. Standard laboratory decontamination protocols using 0.5% hypochlorite are sufficient. Other virucidal disinfectants are also commercially available.

### **5.6 Storage of samples**

Store all diagnostic samples, including respiratory and serum samples, as per the standard national influenza surveillance protocols (and in preparation for possible forwarding to WHO reference laboratory):

- Respiratory samples should be stored at  $-70^{\circ}\text{C}$ .
- Serum samples should be stored at  $-20^{\circ}$  or  $-70^{\circ}\text{C}$ .

### **5.7 Disposal of samples**

Once the samples have been held for the required duration, dispose of them in the normal manner.

## 6 Laboratory and Staffing Issues

### 6.1 Requirements for laboratory staff

Staff who are normally involved in the collection and processing of samples, but who are in one of the recognised high-risk groups for complications following influenza,<sup>4</sup> should be excluded from these activities and instead should work in other areas. High standards of personal hygiene are important in minimising the risk to staff.

### 6.2 Laboratory staff prophylaxis

Laboratory staff should be vaccinated with the most current influenza vaccine.

As the pandemic progresses, it is anticipated that there will be laboratory staff who acquire infection in the community and recover. Where possible, these individuals should be utilised for specimen collection and processing.

### 6.3 Accidental exposure

Each laboratory should have a protocol in place for management of accidental exposure of laboratory workers to a PI strain. Staff working with a known PI virus should log the content and duration of the related work.

Any case of accidental exposure should immediately be reported to the microbiologist and the health and safety officer. Depending on the level of exposure, the staff member should be evaluated and monitored for influenza-related symptoms. If appropriate, post-exposure prophylaxis (Tamiflu) may be administered following consultation with a physician.

### 6.4 Laboratory surge capacity planning

Laboratories should assess projected nationwide needs for scaled-up diagnostic activity during the early stages of a pandemic, in terms of:

- laboratory staffing, namely:
  - training of all personnel in the use of diagnostic procedures
  - general education on PI
- laboratory testing – identifying tests that will be essential during the peak of a pandemic when staff shortages are likely
- laboratory supplies – assessing anticipated supply needs and considering adjusting stock levels.

Refer to local District Health Board (DHB) pandemic plans and also refer to the Ministry of Economic Development Business Continuity Plan.<sup>5</sup>

<sup>4</sup> See Ministry of Health. 2006. *Immunisation Handbook*. URL: <http://www.moh.govt.nz/immunisation>

<sup>5</sup> See Ministry of Economic Development. 2005. *Business Continuity Planning Guide*. URL: <http://www.med.govt.nz/upload/27552/planning-guide.pdf>

## 7 Personal Protective Equipment

How PPE will be used is dependent on each individual laboratory and each situation. Standard infection control guidelines should always be followed.

Wear full PPE for processing respiratory samples until they have been rendered non-infectious (see section 5.4.3). See Appendix 1 for guidelines on putting on and removing PPE.

PPE includes:

- disposable gloves
- surgical mask with fluid shield that is tight fitting
- eye protection – ordinary spectacles do not provide sufficient protection
- disposable single-use gown, with cuffed sleeves, that is either impermeable or covered with a plastic apron.

## 8 Procedures for Influenza Diagnosis

These diagnostic procedures relate to the early phases of a pandemic (border management and cluster control). During these phases, timely and accurate diagnosis is vital. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR, referred to as PCR in this document) is the optimal test for the detection of a novel PI virus, and it should be the first test that is conducted. Any samples that are PCR positive for a novel strain of influenza should be sent on by the PCR referral laboratory to the WHO Collaborating Centre in Melbourne for confirmation. During later phases, when infection is widespread, the testing strategy may alter.

### 8.1 PCR

The optimal test for the detection of a novel strain of influenza is PCR using broadly reactive primers capable of detecting all 16 haemagglutinin (HA) subtypes of influenza A. The matrix (M) protein gene is the target commonly used.

Concurrent testing using an HA PCR and a matrix PCR is recommended.

Conduct nucleic acid extraction at the lysis step in a Biological Safety Cabinet. Once the sample is lysed and placed into a new tube, the specimen is not regarded as infectious. It can then be processed in accordance with methods approved by International Accreditation New Zealand.

**Recommended pandemic use:** During the early stages of a pandemic, prior to the identification of the HA type involved, a broadly reactive PCR capable of detecting all 16 HA types of influenza A will be required. Once influenza cases are widespread in the community, HA type-specific diagnosis may become superfluous, unless multiple strains are circulating.

### 8.2 Viral cell culture and rapid cell culture

Viral culture procedures must be performed in a PC3 facility using PC3 work practices. The characterisation of viruses recovered in cell culture by haemagglutination inhibition (HAI) assay should be undertaken in a PC3 facility.

Viral culture using Madin Darby Canine Kidney (MDCK) cell lines is likely to detect potential new pandemic strains. Methods available include tube culture, which may take 4–7 days, and multiwell plate culture, 1–3 days. Use HAI for the characterisation (typing and subtyping) of any cell culture isolates that can provide important information on antigenic drift. PCR and Immunofluorescence assays (IFA) can be used as initial indicative typing/subtyping for any cell culture isolates.

Standard diagnostic virology laboratories should not attempt cell culture for any virus from specimens obtained from suspected or probable PI cases until influenza A PCR results are known. If viral culture from respiratory samples is to be attempted, it should be in the PC3 containment laboratory (see Appendix 2 for details on transport of samples to the National Influenza Centre in ESR – part of the WHO Global Influenza Programme).

**Recommended pandemic use:** During the border management and cluster control stages of a pandemic, forward samples known to be positive for the PI virus to ESR for viral culture. Note that all samples from early cases should be sent to CSL for confirmation (either directly from PCR laboratory or via ESR). Appendix 5 shows the minimum number of samples to be sent from each regional laboratory to CSL for viral culture during all stages of a pandemic.

### 8.3 Rapid tests for influenza

Rapid tests should not be used during the early stages of a pandemic when sensitive, accurate testing is crucial.

Commercially available rapid diagnostic tests are screening tests for influenza A and B virus infections, which provide results within 30 minutes. They may be referred to as 'near patient tests' or 'point of care tests'. The sensitivity of rapid tests is variable (median 70–75%), while specificity is high (median 90–95%). Because of their low sensitivity, false negative results are a major concern.<sup>6</sup>

All rapid testing steps should be performed in a Biological Safety Cabinet by a staff member wearing PPE (see Appendix 1).

**Recommended pandemic use:** These tests should not be used during the early stages of a pandemic. They may, however, have a use for the triaging of patients in health care settings once a pandemic virus is spreading.

### 8.4 Immunofluorescence assay

Immunofluorescence assays (IFA) are widely used for the rapid non-type-specific laboratory diagnosis of influenza A and B virus infections. These tests can provide a result within 2–4 hours. The sensitivity of these assays in comparison with cell culture ranges from 70–100%; specificity range is 80–100%. These assays use commercial reagents, which are widely available in New Zealand, and most are capable of detecting influenza A and B viruses.

In addition, the WHO collaborating centre in Centers for Disease Control and Prevention (CDC), Atlanta produces and distributes WHO influenza reagents kit including influenza type A/H5-specific monoclonal antibody pool. Positive H5 control slides (containing irradiated cellular material) are available from CSL, Melbourne.

Sample preparation should be performed in a Biological Safety Cabinet by a staff member wearing PPE (see Appendix 1).

Once alcohol fixation has been completed, the sample is no longer infectious.

<sup>6</sup> WHO. 2005. *WHO recommendations on the use of rapid testing for influenza diagnosis*. Geneva: World Health Organization. URL: [http://www.who.int/csr/disease/avian\\_influenza/guidelines/rapid\\_testing/en/index.html](http://www.who.int/csr/disease/avian_influenza/guidelines/rapid_testing/en/index.html).

**Recommended pandemic use:** During the early stages of a pandemic this test should not be used as the sole test. However, this test can be run in parallel with PCR to provide additional confirmation.

## 8.5 Serology

The serological diagnosis of PI infection is likely to be of limited use during the initial stages of a pandemic. However, serological testing may be helpful for exclusion of infection or for epidemiological purposes. Serological tests available for the measurement of influenza-specific antibody include haemagglutination inhibition (HAI) tests, enzyme immunoassays, and virus neutralisation tests. The microneutralisation assay is the WHO recommended test for the measurement of antibody to avian influenza viruses. Microneutralisation assays detect antibodies 10–14 days after the onset of illness due to avian influenza A/H5N1 virus. Because this test requires the use of live virus, its use is restricted to those laboratories with PC3 facilities. Once a novel virus adapts to humans, conventional HAI assays may be applicable.

Perform any procedure that may produce aerosols, especially pipetting, in a Biological Safety Cabinet. Once serum samples have been centrifuged, separated and placed into a new screw-top tube, automated serology may be performed in the normal way.

**Recommended pandemic use:** Acute and convalescent serum samples should be collected and stored at  $-20^{\circ}\text{C}$ . Serological assays may not be available during the early stages of a pandemic.

## 9 Criteria for a Laboratory-Confirmed Case

- The preferred assay during the early phases (border management and cluster control) of a pandemic response is PCR. If the test is run without a positive control, products should be confirmed by sequencing and comparing with sequences in deposited databases.
- Each positive PCR result must be confirmed by another laboratory during the early phases.
- A negative PCR result does not rule out the presence of influenza virus.
- Results should be interpreted together with the available clinical and epidemiological information.

WHO recommends that all laboratory results for influenza A/H5, H7 and H9 during the interpandemic and pandemic alert periods should be confirmed by a WHO reference laboratory. All PCR-positive samples in these periods will be sent to CSL in Melbourne.

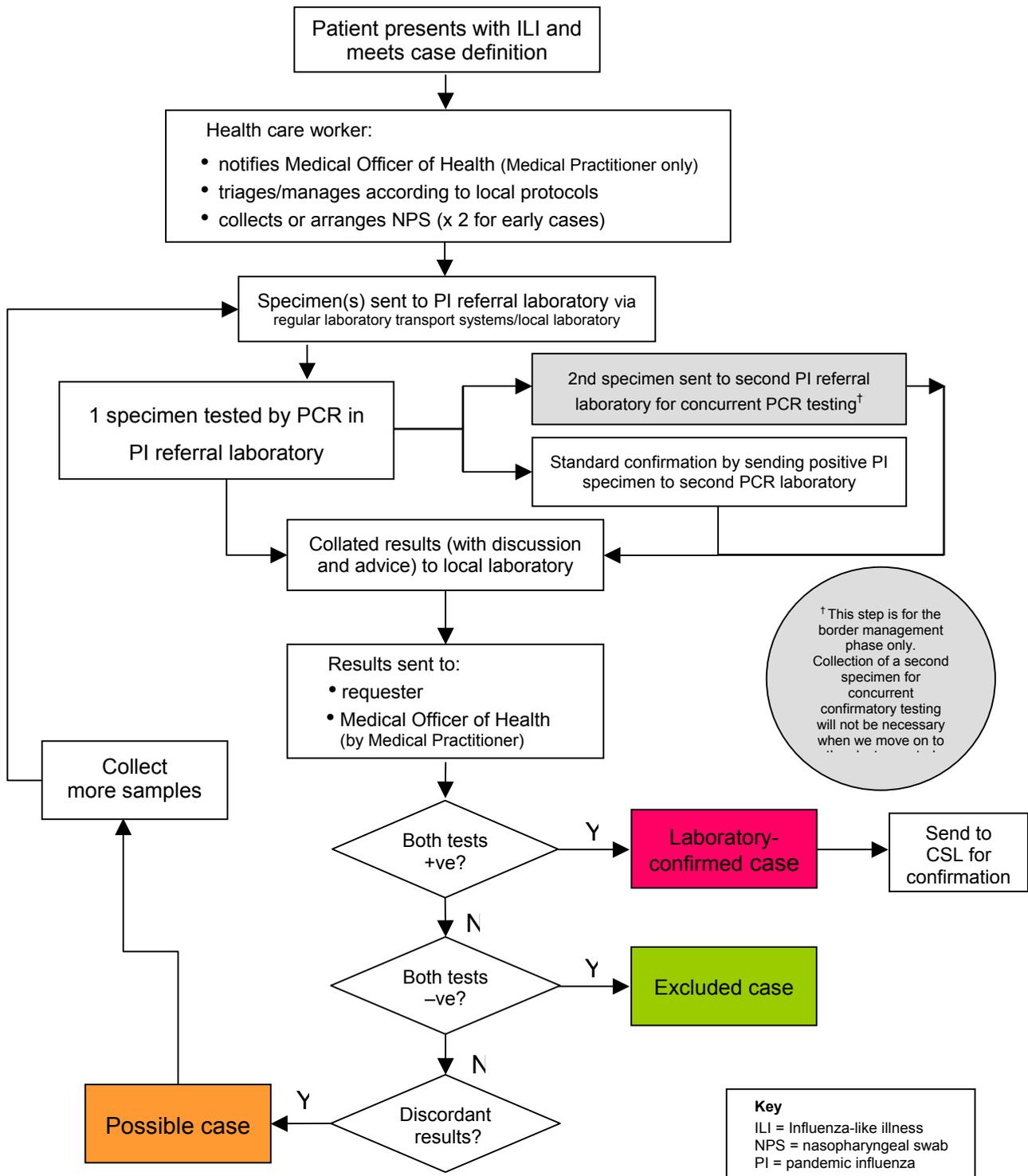
## **10 Notification of Results**

The referral laboratory must send the results to the local/requesting laboratory. At the same time, the results may also be sent to the requesting clinician.

The local laboratory must ensure that the requesting clinician receives the results.

# 11 Flow Diagram for Laboratory Diagnosis

Diagnostic strategy for patients with suspected pandemic influenza infection in the border management and cluster control phases.



## 12 Phased Approach to Diagnosis

The diagnostic testing strategy for suspected or probable cases is to use the most sensitive and specific assay to detect the presence of a novel influenza type and its specific subtype. Link any relaxation of this strategy to the staged New Zealand pandemic response strategy.

In addition, the WHO National Influenza Centre in ESR has developed a phased approach for its role during pandemic periods (see Appendix 6).

WHO phase	New Zealand strategy	Ministry of Health/DHB alert code	Objective and action
1 2	Plan for it (planning)	<b>WHITE</b> (information/advisory)	Review laboratory diagnostic capability.
3		<b>YELLOW</b> (standby)	<p>Activate enhanced surveillance and test returned travellers meeting case definition for novel subtype of influenza.</p> <p>Test by RT-PCR for influenza A matrix and H5 (most sensitive and specific method).</p> <p>Establish communication lines with Medical Officers of Health and other virology laboratories.</p>
4	Keep it out (border management)	<b>RED</b> (activation)	<p>Test returned travellers who meet case definition for novel subtype of influenza.</p> <p>Introduce dual testing strategy (ie, two samples tested in two different laboratories).</p> <p>Test by PCR for influenza A matrix and H5.</p> <p>Turnaround time should be short and 24/7 to assist public health response.</p>
5			Stamp it out (cluster control)
6	Manage it (pandemic management)		<p>Consider moving to rapid testing and IFA once pandemic influenza established in area/community.</p> <p>Test selected individuals (unusual cases, new clusters, the very ill) with PCR as required for case management.</p> <p>Use rapid testing and IFA for case management purposes. Test and turnaround times will be determined by patient management requirements and laboratory capacity.</p>
Post pandemic period	Recover from it (recovery)	<b>GREEN</b> (stand down)	<p>Resume normal interpandemic function.</p> <p>Test selected individuals to monitor new activity.</p> <p>Identify immune individuals.</p>

# Appendix 1: Personal Protective Equipment (PPE)

Follow standard laboratory protocols for what PPE to wear and when to wear it. Below is a summary of recommended PPE and how to put it on and remove it.

## Recommended PPE

- Disposable gloves
- Surgical mask with fluid shield that is tight fitting
- Eye protection – ordinary spectacles do not provide sufficient protection
- Disposable single-use gown with cuffed sleeves that is either impermeable or covered with a plastic apron

## Putting on PPE

1. Always tie back loose hair and avoid touching any part of the face when working in PPE gear.
2. Put on solid-front isolation gown and do it up; both ties must be firmly tied.
3. Put on mask. Use mask with eyeshield if available.
4. Put on protective eyewear. Always clean reusable safety goggles (see section below).
5. Put on correctly sized gloves and pull wrists of gloves over wrists of gown sleeves. Double gloving is recommended.

## Removal and disposal of PPE

It is crucial that PPE gear is removed without accidental contamination and whilst remaining in the respiratory laboratory:

1. Before removing your hands from Biological Safety Cabinet, remove contaminated outer layer of gloves and discard in waste bag in cabinet.
2. Untie both ties on gown without touching any unprotected areas. It may be necessary to seek the assistance of a colleague who is wearing clean gloves.
3. Remove gown so that it turns inside out, and immediately put it in foot-operated yellow biohazard waste container.
4. Remove final pair of gloves so that the 'dirty' outside of the glove only ever touches the 'dirty' outer surface of the other glove, and 'clean' skin inside the glove only ever touches 'clean' skin of the other hand. Immediately place inside-out gloves into a yellow biohazard waste bag.
5. Cleanse hands using standard hand hygiene.
6. Remove goggles (if worn separately), handling them by the arm of the frames, and dump immediately into container for washing (see below).

7. Remove mask, handling it **only** by its strings and taking great care **not** to touch the body of the mask (the part that covers the nose and mouth). Immediately dump into biohazard waste bin.
8. Immediately cleanse hands again using standard hand hygiene.

### **Reusable eye protection**

- Put used goggles immediately into a container (eg, ice cream container).
- Wash goggles in the respiratory laboratory in detergent and warm water, preferably in a sink (but a bucket or equivalent will suffice), following the method described below.
  
- **Wear gloves and take care not to splash**
- Fill the sink with warm water and detergent.
- Immerse goggles and clean them under the water surface.
- Wipe all surfaces with a disposable cleaning cloth.
- Discard cloth in yellow biohazard waste bag after washing.
- Drain out washing water.
- Rinse goggles in clean warm water and drain the sink.
- Remove gloves and wash your hands.
- Dry the newly clean goggles with disposable paper towel.

## Appendix 2: Transporting Human Samples Suspected of Containing Pandemic Influenza to the WHO National Influenza Centre in ESR

ESR complies with land and air transport regulations for transporting human samples suspected of containing pandemic influenza to ESR with a free courier service.

1. **The shipping materials consist of:** VTM; swabs; plastic specimen bags; plastic bubble bag; adsorbent pads; specimen request forms; chill pad; courier tickets; bio-bottle and cardboard outer box. The cardboard box outer has interchangeable address cards in separate pockets; a clear plastic adhesive backed envelope for the 'dangerous goods' forms; and a reversible 'infectious substances' label.

In addition, the following forms are included:

- two 'Shipper's Declaration for Dangerous Goods' forms
- a sample completed 'Shipper's Declaration for Dangerous Goods' form
- a pre-printed New Zealand Couriers Charge Label (ESR will be charged for delivery)
- specimen request forms.

### 2. How to send your samples

- a. Place respiratory swabs from patients with suspected pandemic influenza infection in a vial containing VTM, with the lid closed tight and wrapped with adhesive tape or parafilm. Spray the outside of the vial with 1% Virkon (or 70% ethanol) and change into a new pair of outer gloves. Place the vial in the plastic specimen bag, then place in the bubblewrap bag with chill pads and absorbent pads. The two sachets of liquid absorbing material are there to soak up any spillage. Then place it in the biobottle and close the lid tightly. Spray the outside of the biobottle with 1% Virkon (or 70% ethanol) and change into a new pair of outer gloves. **Please keep samples refrigerated until container can be couriered to ESR. Please only pack specimens suspected of containing pandemic influenza in this package.**
- b. Place the biobottle into its outer cardboard box. Place specimen request form inside the cardboard box but outside the biobottle. Close the top of the cardboard box.
- c. Swap the address cards around on the outer box so that ESR is in the 'To' pocket and your address card is in the 'From' pocket. The ESR address is: HPAI, Institute of Environmental Science and Research, 34 Kenepuru Drive, PO Box 50-348, Porirua.
- d. Complete in duplicate the 'Shipper's Declaration for Dangerous Goods' forms (see sample completed form) by: (i) filling in your name and address; (ii) writing in the weight or volume of the infectious substances; (iii) signing the declaration. Fold the two forms and place them in the clear plastic envelope on the box.

- e. Fill in the 'Sender' box of the New Zealand Couriers Charge Label and attach in the marked space.
- f. Phone New Zealand Couriers contact number 0800-800-841 to arrange pick-up of your shipment.
- g. Phone ESR virology lab (daytime phone: (04) 914 0690 (Lisa), (04) 914 0728 (Judy), or (04) 914 0764 (Sue); after hours phone: 027 576 6367) to inform the shipment of pandemic influenza samples to ESR. If you require more shipping containers or materials, contact ESR.

## Appendix 3: WHO Pandemic Phases

<b>Interpandemic period</b>	<b>Phase 1.</b> No new influenza virus subtypes have been detected in humans. An influenza virus subtype that has caused human infection may be present in animals. If present in animals, the risk <sup>a</sup> of human infection or disease is considered to be low.
	<b>Phase 2.</b> No new influenza virus subtypes have been detected in humans. However, a circulating animal influenza virus subtype poses a substantial risk <sup>a</sup> of human disease.
<b>Pandemic alert period</b>	<b>Phase 3.</b> Human infection(s) with a new subtype, but no human-to-human spread, or at most rare instances of spread to a close contact. <sup>b</sup>
	<b>Phase 4.</b> Small cluster(s) with limited human-to-human transmission but spread is highly localised, suggesting that the virus is not well adapted to humans. <sup>b</sup>
	<b>Phase 5.</b> Larger cluster(s) but human-to-human spread is still localised, suggesting that the virus is becoming increasingly better adapted to humans, but may not yet be fully transmissible (substantial pandemic risk). <sup>b</sup>
<b>Pandemic period</b>	<b>Phase 6.</b> Pandemic phase: increased and sustained transmission in general population.
<b>Postpandemic period</b>	Return to interpandemic period.

- a The distinction between *phase 1* and *phase 2* is based on the risk of human infection or disease resulting from circulating strains in animals. The distinction would be based on various factors and their relative importance according to current scientific knowledge. Factors may include: pathogenicity in animals and humans; occurrence in domesticated animals and livestock or only in wildlife; whether the virus is enzootic or epizootic, geographically localised or widespread; other information from the viral genome; and/or other scientific information.
- b The distinction between *phase 3*, *phase 4* and *phase 5* is based on an assessment of the risk of a pandemic. Various factors and their relative importance according to current scientific knowledge may be considered. Factors may include: rate of transmission; geographical location and spread; severity of illness; presence of genes from human strains (if derived from an animal strain); other information from the viral genome; and/or other scientific information.

## Appendix 4: Referral Laboratory Capability for Pandemic Influenza Detection and H5 Subtyping

	IFA A&B (commercial)	IFA A/H5 (WHO)	PCR	Viral culture	HAI (H5)
Auckland	✓		✓		
Waikato	✓		Setting up		
ESR Porirua	✓	✓	✓	✓	✓
Wellington	✓		✓		
Christchurch	✓		✓		
Dunedin	✓				

## Appendix 5: Minimum Number of PCR-Positive Pandemic Influenza Specimens to be sent to CSL

	Early pandemic*	Middle pandemic	Late pandemic	Total
Auckland	5	5	5	15
Waikato	5	5	5	15
ESR Porirua**	5	5	5	15
Wellington	5	5	5	15
Christchurch	5	5	5	15
Dunedin	5	5	5	15
<b>Total</b>	<b>30</b>	<b>30</b>	<b>30</b>	<b>90</b>

### Notes

- \* All samples from early cases must be sent to CSL, Melbourne, for confirmation.
- \*\* ESR can serve as a depository for all PCR positive specimens. A minimum number of specimens, as listed in this table, will be subjected to viral culture in the PC3 laboratory at the WHO National Influenza Centre in ESR.

## Appendix 6: Role of the WHO National Influenza Centre in ESR during Interpandemic, Pandemic Alert and Pandemic Periods

New Zealand strategy	Ministry of Health/DHB alert code	WHO National Influenza Reference Lab	National Influenza Surveillance Unit	Public Health Unit, GPs	Ministry of Agriculture and Forestry's Animal Reference Lab
<b>Plan for it</b> (planning) WHO phases 1–3	<b>White</b> (information/ advisory)	Actively communicate with New Zealand Virology Laboratory Network, Ministry of Health, CSL, WHO.  Perform tests: PCR for influenza A matrix, H5 IFA, H5 viral culture, HAI typing, microneutralisation, sequencing.  Conduct Standard Operating Procedures for biosafety and all H5 tests in PC2/3.  Maintain a quality assurance panel (H5N1 PCR) for hospital labs.  Integrate lab component into national plan.	Develop communication protocols with Ministry of Health and WHO.  Maintain capacity for weekly, monthly and annual reports.  Develop capacity for enhanced surveillance (database, IT, etc).  Develop enhanced surveillance protocol.  Frequently train staff.  Develop surge capacity and business continuity plans.	Develop communication protocols with Medical Officers of Health.  Conduct training, where appropriate, on specimen handling (biosafety, collection, transportation).  Gather information (including through media watch).	Develop Memorandum of Understanding for access to PC3.  Use SOP for sharing information rapidly.  Conduct research on influenza at human–animal interface.
	<b>Yellow</b> (standby)	Develop protocols for urgent referral to CSL.  Develop surge capacity and business continuity plans.			
<b>Keep it out</b> (border management) WHO phase 4	<b>Red</b> (activation)	Offer diagnostic tests for some DHBs.  Offer confirmatory diagnostic service for other hospital labs.  Offer viral culture and HAI typing nationally.  Make urgent referral of isolates/ specimens to CSL.	Rapidly report to Ministry of Health, MOsH, WHO.  Consider study on seroprevalence in risk groups, after discussion with Ministry of Health.  Enhance surveillance.	Assist case finding and investigation.	Assist field investigation of farmers and animal workers.
<b>Stamp it out</b> (cluster control) WHO phase 5		Activate surge capacity.  Monitor disease spread and antigenic drift.  Assist study on virus pathogenicity and transmissibility in humans.	Enhance surveillance.		
<b>Manage it</b> (pandemic management) WHO phase 6		Monitor disease spread and antigenic drift.	Adjust surveillance.		
<b>Recover from it</b> (recovery)	<b>Green</b> (stand down)	Resume normal interpandemic function.	Seroprevalence in general population.		