



South Australian Laboratory

Pandemic Influenza sub plan

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SA Health

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Introduction

SA Pathology provides routine influenza diagnostic nucleic acid testing (NAT) from the Frome Road site with testing for influenza A and B performed 7 days per week. The routine diagnostic assay is an in house produced high volume assay performed on automated platforms that is readily amenable responding to changes in test volumes in response to outbreaks. A limited number of specimens are currently subtyped by supplementary nucleic acid tests on site or are referred to the WHOCC reference laboratory for more extensive evaluation. SA Pathology does not perform influenza virus culture but this is routinely performed at WHOCC on referred specimens.

The objectives of laboratory testing change over the course of a pandemic. In the standby phase the overall aim of a pandemic plan is to identify new cases of the disease either entering Australia or emerging domestically to allow containment of an outbreak.

Since, at this stage, the rate of the disease in the general community is low it is necessary to have access to definitive tests that can differentiate a pandemic influenza strain from other respiratory infections. For example, during the 2009 pandemic, an additional type specific influenza NAT was run in parallel with all routine assays to immediately identify pandemic strains. Because quarantine and contact prophylaxis measures are most effective if implemented rapidly, these tests should be available on short notice and with rapid reporting times. This may involve establishing dedicated transport plans to the centralised laboratory

During the initial responses phase of a pandemic it is more difficult to predict precisely where a new case of pandemic influenza will present and so provision has to be made to either refer patients rapidly for specimen collection or provide collection at distributed sites. During the targeted response phase of a pandemic testing requirements may be less, and it may be easier to establish dedicated centres for specimen collection.

Because of the different testing requirements as a pandemic evolves this annex has being divided into two sections. The first is descriptive of the kinds of specimens which can be collected, possible testing methodologies, transport of specimens and locations for testing. This is intended to provide an overview of the range of possible strategies which could be used in a pandemic. The second part is designed to provide a testing protocol which is recommended to be applied during various phases of a pandemic based on the information in part one of the annex.

Part A

Specimen collection locations

Suspected early cases occurring in Adelaide should be referred to one of the influenza hospitals (Flinders Medical Centre, Royal Adelaide Hospital, Women's and Children's Hospital) for assessment, including diagnostic specimen collection.

Although the majority of cases of pandemic influenza are likely to occur in metropolitan areas, initial cases may occur in regional or remote areas. This is particularly the case with imported infections where the patient lives in a regional area and remains asymptomatic until they return home from interstate/international travel. Laboratory testing plans should include the means of collecting specimens in non-metropolitan areas and transporting them to the testing laboratory.

Recommended laboratory specimens

Respiratory tract specimens

Upper respiratory tract specimens (one deep nasal or one throat swab) should be collected within 1-3 days of onset of symptoms. A combined nasal and throat specimen (collected with the one swab) may provide more material for testing and therefore be a more sensitive specimen. Self-collected sputum samples, or tracheal aspirates from intubated patients are also suitable.

Nasopharyngeal aspirates or nasal washes are not recommended for detection of pandemic influenza virus as these procedures are aerosol producing with additional risk for collection staff. Specialist centres may choose to collect these with use of appropriate PPE.

Respiratory tract specimens will be required in the stand-by and initial response phases and again in the stand-down/recovery phase to allow the full range of influenza detection tests and typing, but in the targeted response phase (with expected surge); there may be a reduced requirement for laboratory testing.

Serum

Serum specimens for seroepidemiology may also be required, particularly in stand-by and initial response and stand-down/recovery phases of a pandemic. An acute-phase serum (7-10ml whole blood) should be collected within 1-7 days after onset and a convalescent-phase serum collected 14 days after onset (or if near death, a second anti-mortem specimen collected even if 14 days has not elapsed). SA Pathology no longer provides serological testing for retrospective influenza diagnosis and the paired serological samples are referred interstate for testing.

Other Specimens

Other specimens for influenza testing may also be collected from a patient in some circumstances, for example:

- > the need for definitive exclusion of pandemic influenza in early cases
- > where the pandemic influenza strain is trophic for tissues other than the upper respiratory tract

These additional specimens may include: lower respiratory tract specimens such as bronchoalveolar fluid or lavage (BAL), tracheal aspirates or lung biopsy, stool, whole blood, and *post mortem* specimens for virus detection and isolation.

Methods of specimen collection

Specimen collection safety precautions

Specimen collection requires close physical proximity to the patient and recommended precautions, particularly for respiratory protection, should be followed closely. The person collecting nasal or throat swabs should be wearing appropriate PPE (gloves, gown, mask and eyewear).

Site:

- > Need a wall that the patient can rest head against whether patient stands or sits
- > HCW needs enough space to be able to stand beside the patient (not in front)
- > Need to have hand washing facilities or hand disinfectant

Preparation of patient and HCW:

- > Explain the procedure to the patient
- > Ensure the HCW is protected: placement (beside the patient), wearing gloves, gown, mask & splash proof glasses

Swabs needed:

- > VTM swab (Viral transport medium swab) suitable for all virus detection methods including cell culture)

Swab specimen collection

Nasal swab:

- > Stand at the side of the patient
- > Ensure the head is resting against the wall
- > Place a hand on the forehead (non-dominant hand) and the thumb at the tip of the nose
- > Use a viral culture swab and insert the swab into the closest nostril horizontally, approximately 2-3 cm
- > Place lateral pressure on the swab in order to collect cells from the midline nasal plate (not from anterior nares)
- > Rotate the swab twice (2 x 360° turns) collecting the epithelial cells (not mucous)
- > Insert swab into viral transport medium, break off shaft of swab and recap tube
- > Place swab directly in the tube, label tube with patient name, collection date and source (nasal)



Throat swab

- > Stand at the side of the patient
- > Ensure the head is resting against the wall
- > Place a hand on the forehead (non-dominant hand)
- > Ask the patient to open their mouth widely and say 'ah'
- > Use tongue spatula to press the tongue downward to floor of the mouth
- > Use a viral culture swab and insert the swab into mouth avoiding any saliva
- > Place lateral pressure on the swab in order to collect cells from both of the tonsillar arches and the posterior nasopharynx, without touching the sides of the mouth.
- > Rotate the swab twice (2 x 360° turns) collecting the epithelial cells (not mucous)
- > Insert swab into the same transport tube containing nose swab, break off the shaft and recap firmly.
- > Label tube with patient name, collection date and source (nose/throat)



The swab specimens (in viral transport medium) should be stored and transported at 4°C and delivered promptly to the laboratory.

Specimen packaging and transport

Staff training and safety

Staff responsible for supervision of packaging and transporting specimens should be familiar with NPAAC guidelines: Information on the transport of pathology specimens. Staff responsible for packaging and shipping specimens by air, interstate or overseas should have current qualifications in

shipping of Dangerous Goods. Suitable PPE (gloves and gown) should be worn while packing specimens and standard laboratory hygiene practices should be followed.

Transport locations

When specimens are being moved within an institution, pneumatic tube delivery systems should not be used, as any breakage or leakage within the pneumatic system could contaminate the entire institution.

It is essential that the laboratory receiving the specimen is aware that it comes from a potential pandemic influenza case. Telephone contact should be made with the receiving laboratory to facilitate safe and rapid processing of the specimens.

Specimen Processing and Laboratory Biosafety

Requirements for laboratory staff involved in processing and testing of specimens:

Staff who are in one of the recognised NHMRC high risk groups for complicated influenza should be excluded from these activities unless absolutely necessary. High standards of personal hygiene are important in minimising the risk to staff.

Laboratory staff prophylaxis

Laboratory staff should be vaccinated against the currently circulating influenza strain, and if available the new pandemic strain. A protocol for management of accidental exposure of staff to a pandemic influenza strain, including post-exposure prophylaxis with a neuraminidase inhibitor antiviral drug should be in place in laboratories processing respiratory specimens. Doses of an appropriate drug should be readily available to laboratory staff for this purpose.

As the pandemic progresses, it is anticipated that there will be staff who will have acquired infection in the community and recovered. Those staff should be preferentially used for specimen collection and processing.

Personal protective equipment (PPE)

All staff potentially exposed to samples known or suspected to contain pandemic influenza should wear suitable PPE and must be trained in its proper use. It is expected that the laboratory OHS Officer will oversee this training.

Specimen processing

Blood and urine specimens processed outside microbiology or histopathology laboratories should be handled using standard precautions in BSL2 laboratories.

For microbiological and anatomical pathology laboratory specimens the following procedures can be carried out under BSL2 precautions:

- > 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 transport to diagnostic or reference laboratories for additional testing. Specimens should already be in a sealed, decontaminated primary container.

Activities involving manipulation of untreated respiratory specimens may be performed in BSL2 facilities, but with more stringent work practices as described below. These activities include:

- > cut up, blocking and macroscopic description of respiratory tissue
- > 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.

Stringent measures to be employed for these activities in BSL2 facilities include:

Medical laboratory staff should wear protective equipment, including disposable gloves, disposable solid front gowns with cuffed sleeves that are either impermeable or covered with a plastic apron, full eye protection and surgical mask.

Gowns, gloves and masks should be discarded after the specimens have been processed. Remove the mask after the gown and gloves. Do not touch the mask front when removing mask from face, mask tabs only should be touched. Careful attention should be given to hand hygiene after removal of protective clothing and especially before touching the face; contact with eyes and mucosal surfaces should be minimised.

All specimen manipulations should be carried out in a certified biological safety cabinet (BSC) class 2 or 3. Aerosol producing procedures should be carried out in a biological safety cabinet (of at least class 2) and centrifugation should be carried out using sealed centrifuge cups or rotors that are unloaded in a biological safety cabinet. During a pandemic or when handling of specimens at high risk of containing a novel influenza strain, specimens will be inactivated in a BSC before nucleic acid extraction commences

Activities that might require PC3 facilities and PC3 work practices such as viral culture and manipulation of cultures are not performed by SA Pathology.

Decontamination

Work surfaces and equipment should be decontaminated after specimen processing using standard laboratory protocols.

0.5% hypochlorite is sufficient to inactivate influenza viruses and is recommended for routine decontamination. For decontamination of metal surfaces, 70% alcohol may be used.

Diagnostic tests

The appropriate use of available diagnostic tests at each phase of the pandemic will vary according to the diagnostic information required for that phase (refer to SECTION 2). In the early containment phase, the aim will be for rapid and accurate diagnosis. Virus isolation (by cell culture) and typing will also be needed for vaccine production. In the maintenance phase, the virus will be monitored for antigenic and genetic changes to ensure any vaccine remains effective, and for development of anti-viral resistance. In the late phase, accurate detection of virus will again be used to identify the end of the pandemic. Seroepidemiology will also be required. These samples will be forwarded to WHOCC for testing.

Testing may also be undertaken for other significant viral and bacterial infections that may cause a similar illness, for example during the early phases of PI to eliminate other pathogens, or in a confirmed case where these pathogens may occur as a secondary complication of influenza infection.

According to the method used, direct testing for influenza virus may identify the virus type (A, B or C), subtype (according to haemagglutinin (H) and neuraminidase (N) proteins), or strain (point mutations in H and/or N).

Nucleic acid testing (NAT)

The test of choice for detection of influenza due to a potential new pandemic strain will be PCR using primers capable of detecting all 16 potential haemagglutinin (HA) types of influenza. Rapid testing will be essential in the early containment phase for which real time PCR will be the test of choice, with a result within 6 working hours of specimen receipt.

Rapid antigen tests (RAT)

Influenza rapid antigen tests have also been termed Point of Care (POC) tests but are not widely used for this purpose by primary care providers, other health care workers (such as pharmacists) or for home-testing in Australia as these tests have limited sensitivity.. These tests detect influenza type (A or B) using nucleoprotein as target and so should detect all subtypes. However, although the tests have high specificity (98-100%), they have limited sensitivity (less than 70-80%). Lower sensitivity may be seen with new strains of influenza. Thus, false negative results occur more commonly than false positive results, and if negative, more sensitive & specific tests, such as NAT are essential. Currently influenza RATs are not performed by SA Pathology laboratories.

Virus Culture

Viral cell culture procedures should be performed in a PC3 facility. Specimens are transferred to WHOCC when culture is required e.g. routine surveillance of influenza and especially in the stand-by and early response phases of a pandemic.

Typing and subtyping

Definitive typing of the pandemic influenza strain will be undertaken by the WHOCC using reference methods, including serological typing employing WHO reference antisera, and nucleic acid sequencing.

Serology

Due to the delays in serological responses, the utility of serology tests for identifying pandemic activity will be limited. However they may be required for determining immunity in HCW, as a final exclusion of infection, or to maximise the case ascertainment rate in cases, especially where direct detection was not performed or was inadequate.

Specimens from suspected case should be submitted to the local public health laboratory for testing either at that laboratory or be referred to a NIC or the WHOCC.

PART B

Phased approach to diagnosis

Diagnostic activities may usefully be considered in 4 phases:

> preparedness, stand-by, response (initial and targeted), and stand-down and recovery.

Phase	Activity	Testing scheme	Performed by
Preparedness	Specimen	Nose/throat swab	GP, hospital ED/in-patient
	Direct Tests	(1) type specific NAT (2) Selected samples for sub-type specific NAT	(1) Central lab (2) Central or reference lab
	Strain Typing	Serotyping or sequencing on selected samples	Reference Lab
	Resistance Testing	Neuraminidase testing, sequencing on selected samples	Reference Lab
	Serology	(1) Type specific CFT/EIA on paired samples	Reference lab
	Additional Specimens	For positive case	Hospital
Standby	specimen	Nasal/throat swab	GP, hospital ED/in-patient
	direct tests	(1) type specific NAT if RAT screen, follow with NAT (2) sub-type specific NAT,	(1) central lab (2) Central lab or reference lab
	strain typing	serotyping or sequencing	reference lab
	resistance testing	neuraminidase enzyme assay or sequencing	reference lab
	serology	(1) type specific CFT/EIA	(1)reference lab
		(2) sub-type specific EIA	(2) referencel lab
	additional specimens/tests	for positive PI cases	designated PI hospital
Response Initial	Aim	Detect first cases PI for therapy, contact tracing. Small number of specimens.	

		Specimens tested by NAT (results within 6 working hours of specimen receipt). Rapid referral of specimens to WHOCC.	
	specimen	Nasal/throat swab	designated PI hospital
	direct tests	(1) type specific NAT if RAT screen, follow with NAT (2) sub-type specific NAT	(1) central lab (2) central lab (NAT within 6 working hours of receipt)
	strain typing	serotyping or sequencing	reference lab
	resistance testing	Neuraminidase enzyme assay or sequencing	reference lab
	serology	(1) CFT/EIA type specific (2) EIA sub-type specific	(1) local and/or central lab (2) central lab
	additional specimens/tests	for positive PI cases	designated PI hospital
Response Targeted	Aim	Surge in cases. Testing now only of selected individuals as required for monitoring change in PI strain, for clinical management or to identify spread into new areas. Specimens tested by NAT (results within 6 working hours of specimen receipt) Limited referral of specimens to WHOCC	

	specimen	Nose/throat swab	Fever Clinic
	direct tests	(1) NAT	(1) central lab
		(2) RAT	(2) ± designated local lab
	strain typing	serotyping or sequencing	reference lab
	resistance testing	neuraminidase enzyme assay or sequencing	reference lab
	serology	EIA sub-type specific	reference lab
	additional specimens/tests	for positive PI cases	designated PI hospital
Stand Down and Recovery	Aim	Detect residual cases.	
	specimen	Nasal/throat swab	Fever Clinic
	direct tests	NAT	central lab
	strain typing	serotyping or sequencing	reference lab
	resistance testing	neuraminidase enzyme assay or sequencing	reference lab
	serology	EIA sub-type specific	reference lab
	additional specimens/tests	for positive PI cases	designated PI hospital

KEY:

- CFT complement fixation test
- EIA enzyme immunoassay
- HI haemagglutination inhibition
- NAT nucleic acid test
- NT neutralisation test
- PI pandemic influenza
- RAT rapid antigen test

For more information

SA Pathology

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