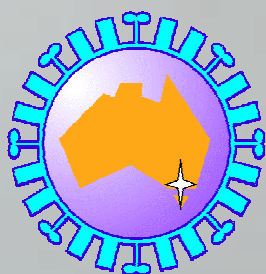


# ANNUAL REPORT 2006

## WHO COLLABORATING CENTRE FOR REFERENCE & RESEARCH ON INFLUENZA

MELBOURNE, AUSTRALIA





# WHO COLLABORATING CENTRE FOR REFERENCE & RESEARCH ON INFLUENZA



## WHO TERMS OF REFERENCE

- ◆ to obtain and preserve representative strains from outbreaks and sporadic cases of influenza in most countries of the Southern Hemisphere, fully characterise their antigenic properties and distribute them to research and production laboratories;
- ◆ to exchange information and new antigenic variants of influenza viruses with the WHO Collaborating Centres for Reference and Research on Influenza in Atlanta and London\*;
- ◆ to advise on the strains which should be included in influenza vaccines;
- ◆ to arrange for the training of research workers in specialised techniques for isolation, diagnosis and studies of influenza viruses;
- ◆ to collect epidemiological information on the prevalence of influenza in most countries of the Southern Hemisphere; and
- ◆ to assist WHO and national health authorities in developing plans for responding to pandemic influenza and to undertake work programs which will improve the pandemic response.

\* the current Terms of Reference were drafted prior to the designation of the WHO Collaborating Centre in Tokyo



Location of WHO Collaborating Centres for Reference & Research on Influenza

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# CONTENTS

1. Director's Report.....	2
2. Structure and Management.....	3
3. 2006 Surveillance Report	
Epidemiological Data .....	4
Virus Isolation from Clinical Samples.....	5
Analysis of Influenza Isolates .....	5
Influenza A(H1N1) Antigenic & Genetic Analysis.....	7
Influenza A(H3N2) Antigenic & Genetic Analysis.....	9
Influenza B Antigenic & Genetic Analysis.....	10
Serological Studies .....	12
Influenza A(H1N1).....	13
Influenza A(H3N2).....	13
Influenza B .....	14
Surveillance of Influenza Strains for Resistance to Antiviral Drugs.....	14
Preparation and Distribution of Diagnostic Reagents.....	15
Candidate Vaccine Strains .....	16
4. Research Studies and Other Activities	
Rapid Diagnostic Tests .....	17
Genetic Studies.....	17
Honours Students.....	17
Sequence Analysis for Research Purposes.....	18
Exchange of Materials with other WHO Collaborating Centres .....	18
Regional Collaboration.....	18
Web Site.....	18
The WILD System.....	18
Influenza Awareness Programs .....	18
Pandemic Preparedness.....	19
Migratory Bird Study.....	19
Influenza Vaccine Study .....	19
5. Training, Visitors and Other Activities.....	22
6. Publications and Meetings .....	23
7. Appendices .....	26

# 1. DIRECTORS REPORT

This is my last report as Director of the Centre as I will be retiring on November 30. It has been a privilege to be involved with the Centre since 1990 and to see its role recognized locally and internationally.

The emergence of highly pathogenic avian influenza has reinforced the importance of influenza as a disease and the ongoing threat of pandemics. This has led to renewed investment in control measures, expanded production of vaccines and a recognition of the vital role of government investment to address public health issues which are not commercially attractive to industry.

The role that the Centre plays in surveillance of influenza in the region, through characterization of novel strains, production and distribution of reference agents, training of staff and providing expert advice and consultancy services, has been recognized through additional utilization of the Centre's facilities and additional support for its service and research programs.

This support has enabled the Centre to obtain several items of urgently needed equipment, increase staff to meet an increased workload and to play a greater role in National and Regional activities.

As Director, I have been fortunate to be assisted by an excellent and hard working team and an involved and effective board of management. Through their combined efforts, funds have been provided to enable the Centre to relocate to purpose built laboratories at the Victorian Infectious Diseases Reference Laboratory, part of the Melbourne Health Network, within the next 12 months. I would like to express my appreciation to the Director of VIDRL, Mike Catton for his support and collegiately, through the negotiation and transaction period.

Although physical relocation of the laboratories will probably not take place until the end of 2007, Melbourne Health will assume administrative responsibility for the Centre from December 1 2006.

Relocation of the Centre will end almost 60 years of continuous involvement with WHO by the laboratory on its existing site as it was one of the first influenza laboratories designated by WHO, when the Global Influenza Program commenced

in 1948. The Centre is extremely grateful to its successive hosts, the Government owned Commonwealth Serum Laboratories which hosted it from 1948 to 1994 and to its successor CSL Ltd, which continued to house it, with government support, after the organisation had been privatized.

The next decade promises to be an extremely exciting period for the Centre as the move into new facilities will provide not only improved working conditions and access to new techniques, but the opportunity for a range of clinical, epidemiological and research collaborations and access to additional sources of funding.

It merely remains to welcome my successor, Anne Kelso, who will be taking up her position in February 2007, to thank all the colleagues who have made the position so fruitful and to wish the Centre all the best in the future.

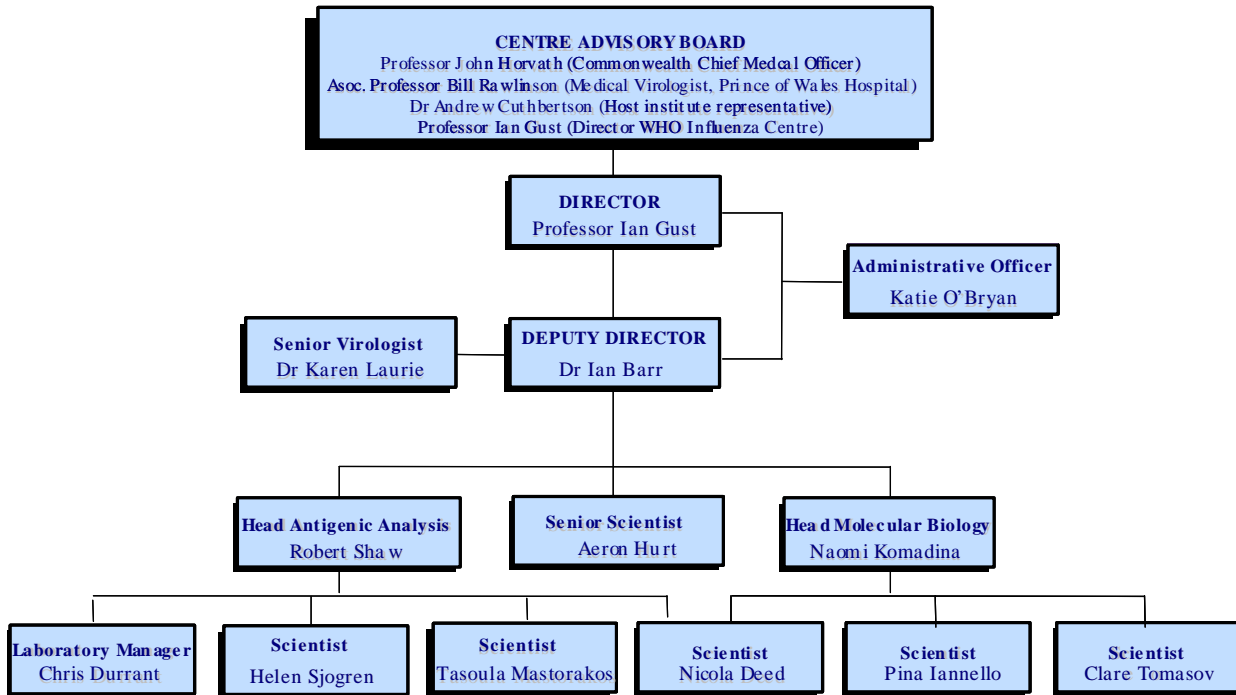


Ian D. Gust  
Director



*WHO Flu Centre retreat in Portsea, August 2006*

## 2. STRUCTURE AND MANAGEMENT



Ian Gust



Ian Barr



Katie O'Bryan



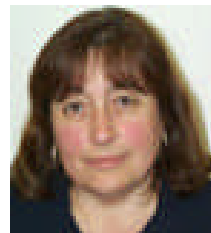
Karen Laurie



Rob Shaw



Aeron Hurt



Naomi Komadina



Chris Durrant



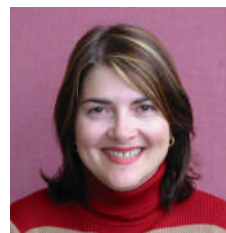
Helen Sjogren



Tasoula Mastorakos



Nicola Deed



Pina Iannello



Clare Tomasov

## 3. 2006 SURVEILLANCE REPORT

### INTRODUCTION

Two types of influenza virus are responsible for epidemic disease in humans, Type A and Type B. These can be readily distinguished by tests using antibodies produced against internal proteins of the virus. Both influenza A and B viruses have two proteins on their surface, haemagglutinin (H) and neuraminidase (N). For influenza A viruses, these proteins exist in a number of antigenically distinct forms – 16 different haemagglutinins (H1 – H16) and nine different neuraminidases (N1 – N9). Haemagglutinin is the more abundant of the surface proteins and antibody against it is the most important in immunity to influenza.

The WHO program is directed to monitoring changes in the surface antigens of influenza A and B viruses (antigenic drift) and detecting the appearance of new antigenic forms of influenza A viruses in the human population (antigenic shift).

Since 1977, three families of influenza viruses (influenza A(H1N1), influenza A(H3N2) and influenza B) have co-circulated in the human population and a representative strain of each is included in current vaccines. The monitoring of H5N1 viruses has also become an important part of WHO's influenza surveillance program.

### EPIDEMIOLOGICAL DATA

Many countries undertake surveillance for the occurrence of influenza viruses or influenza-like disease. In conjunction with strain analysis this information can be useful in determining the need to update influenza vaccines. To assist these decisions, where possible, the Centre sources epidemiological data in addition to virus isolates.

During 2006 epidemiological data was obtained from the following sources:

#### Australia

The Communicable Diseases Network Australia (Australian Department of Health and Ageing <http://www.cda.gov.au/surveil/ozflu/flucurr.htm>).

Australian Sentinel Practice Research Network (Royal Australian College of General Practitioners) Laboratory Surveillance (labWISE [http://www.health.gov.au/internet/wcms/publishing.nsf/content/laboratory%20surveillance%20\(labwise\)-2.](http://www.health.gov.au/internet/wcms/publishing.nsf/content/laboratory%20surveillance%20(labwise)-2.))

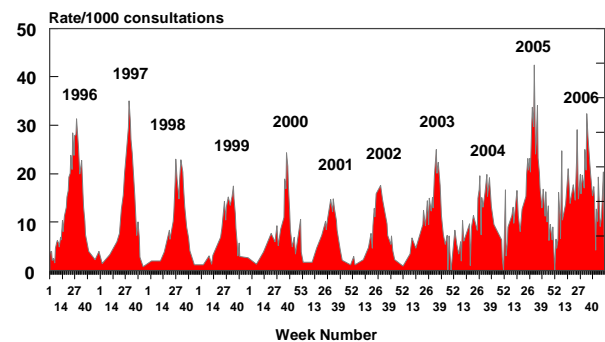
WHO Collaborating Centre for Reference and Research on Influenza (<http://www.influenzacentre.org/>)

#### New Zealand

The Health Communicable Diseases Centre, NZ Ministry of Health  
[http://www.surv.esr.cri.nz/virology/influenza\\_weekly\\_update.php](http://www.surv.esr.cri.nz/virology/influenza_weekly_update.php)

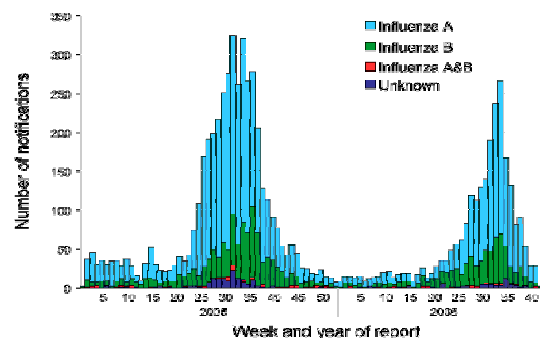
### Australia

The overall level of influenza-like illness (ILI) was moderate in 2006. The figure below shows the incidence of ILI reported through the Australian Sentinel Practice Research Network in 2006 and the previous 11 years.

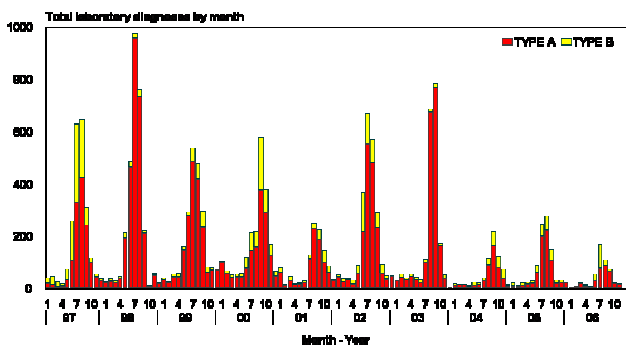


*Influenza-like illness in Australia 1996-2006*  
Source: Australian Sentinel Practice

The influenza that occurred in Australia in 2006 was mainly caused by influenza A(H3N2) and influenza B viruses. Of the 650 isolates received for analysis by the Centre, A(H3N2) was the major subtype (58.7%), followed by influenza B (37.8%) and influenza A(H1) (3.5%). The number of isolates was the second lowest in the past 10 years reflecting the mild influenza season in 2006 in Australia.



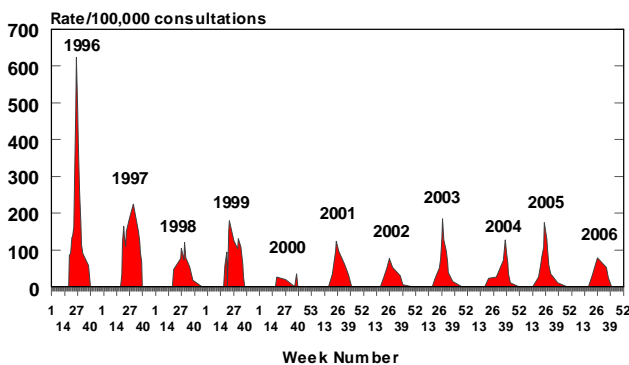
Source: National Notifiable Diseases Surveillance System



Laboratory-confirmed influenza - Australia 1997-2006  
Source: Australian CDI

### New Zealand

Based on sentinel consultation data, influenza activity in New Zealand in 2006 was low. Of the 439 New Zealand influenza samples analysed at the Centre 5 (1.1%) were type B, 360 (82%) were A(H3N2) and 74 (16.9%) were A(H1N1).



Influenza-like illness in New Zealand 1996 – 2006

### Thailand

Thailand had significant levels of influenza, with A(H3N2) strains predominating. Of 143 isolates from Thailand analysed at the Centre, 42.5% were A(H3N2) 22.2% were A(H1) and 35.3% were B strains.

### VIRUS ISOLATION FROM CLINICAL SAMPLES

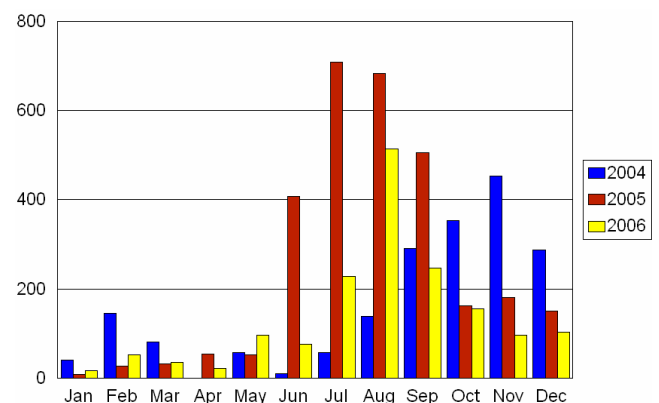
The Centre undertakes limited primary isolation of influenza viruses in cell culture in support of surveillance programs. During 2006 a total of 253 clinical samples were processed in MDCK cell culture, yielding 117 influenza isolates as shown below. In addition, direct virus isolation in embryonated hens eggs was undertaken to generate reference strains suitable for provision to vaccine manufacturers.

Source	Number of Isolates	Influenza type	
		Type A	Type B
Darwin (Aust.)	3	1	2
Perth (Aust.)	60	11	49
Melbourne (Aust.)	31	25	6
Sydney (Aust.)	3	3	0
Cambodia	2	2	0
New Caledonia	7	7	0
New Zealand	2	2	0
Solomon Islands	3	3	0
Sri Lanka	6	5	1
<b>TOTAL</b>	<b>117</b>	<b>59</b>	<b>58</b>

Virus isolation from clinical samples

### ANALYSIS OF INFLUENZA ISOLATES

Local and regional laboratories submitted 1874 isolates to the Centre for analysis during 2006.



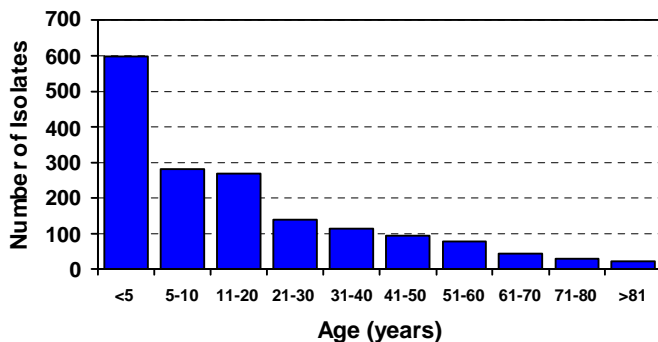
Isolates received by Centre 2004 – 2006

A total of 1721 isolates (91.8%) were recovered from these samples and these were subjected to detailed antigenic analysis as shown below.

Country	Influenza A isolates	Influenza B isolates	A + B isolates	Isolates not recovered	Total isolates submitted
Australia	419	231	650	81	731
Cambodia	5	2	7	4	11
Malaysia	61	64	125	12	137
Macau	35	15	50	0	50
New Caledonia	9	7	16	1	17
New Zealand	434	5	439	33	472
Philippines	117	13	130	9	139
Singapore	27	4	31	1	32
South Africa	19	6	25	5	30
Sri Lanka	2	0	2	3	5
Taiwan	14	9	23	0	23
Thailand	143	78	221	4	225
Others	1	0	2	0	2
<b>TOTAL</b>	<b>1286</b>	<b>434</b>	<b>1721</b>	<b>153</b>	<b>1874</b>

*Analysis of influenza isolates received in 2006*

The age distribution of the subjects from whom isolates were obtained is shown below. The majority of samples were from children less than 5 years old.



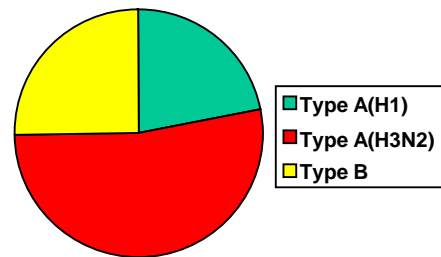
*Age distribution of subjects with isolates analysed at the Centre in 2006*

## ANTIGENIC ANALYSIS OF INFLUENZA ISOLATES

Antigenic characterisation of influenza viruses is based on the ability of the virus to agglutinate erythrocytes from various animals and the capacity of antibodies to interfere with this phenomenon. The reaction of virus isolates is compared in a haemagglutinin-inhibition (HI) test with that of a panel of reference virus strains using specific antisera raised against these viruses by infection of ferrets. Highly specific murine

monoclonal antibodies, which may be useful in further differentiating virus isolates, are often included in the tests. We have also found it useful to include pools of pre-and post-vaccination human sera from people receiving the current influenza vaccine formulation to provide an early indication of antigenic changes in the circulating viruses.

The majority of isolates received from all centres in 2006 were influenza A(H3N2) (52.7%), with 25.2% typed as B and 22% as A(H1) (see below).



*Influenza subtype of isolates analysed in 2006*

## GENETIC ANALYSIS OF INFLUENZA ISOLATES

It is now routine to determine the genetic composition of the circulating influenza viruses by sequencing a number of viral RNA genes using the reverse transcriptase polymerase chain reaction (RT-PCR). Determining the amino acid sequence of a portion of the haemagglutinin protein containing the antigenic regions (the HA1 domain) provides a sensitive method to examine the degree of change in the haemagglutinins of virus isolates and the direction that these changes may be taking. While these sequence changes can not yet be fully correlated with antigenic changes, nor can the direction of future changes be predicted with certainty, these analyses provide supporting data for the selection of vaccine strains and allow the rapid comparison of viruses occurring in different parts of the world.

The second minor viral surface antigen, the neuraminidase also undergoes antigenic and genetic changes. Antigenic changes of the neuraminidase are difficult to detect and current tests generally lack precision. Changes in the neuraminidase of influenza isolates are generally monitored by sequence analysis alone.

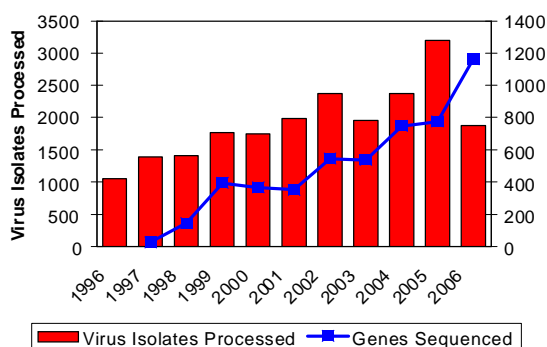
A breakdown of the routine genetic analyses undertaken by the Centre in 2006 is shown in the table below.

The sequence of the viral haemagglutinin (HA1) domain was determined for 218 isolates and the neuraminidase gene for 102 isolates. The genes of a number of reference strains were also characterised. Several virus isolates which failed to give clear-cut type or subtype reactions in serological tests were positively identified by polymerase chain reaction (RT-PCR) and this was also used to identify a number of mixed viral cultures (see table below).

Type of analysis	No. tests
HA gene sequences	
A/H1N1 isolates	53
A/H1N2 isolates	1
A/H3N2 isolates	85
B isolates	80
Mixed	0
NA gene sequences	
A/H1N1 isolates	28
A/H1N2 isolates	1
A/H3N2 isolates	47
B isolates	27
Mixed	0
Vaccine strain sequences	37
Other gene sequences	283
<b>Total sequences analysed</b>	<b>642</b>
Strains identified by PCR	35
Vaccine NA ID by PCR	2
<b>Total PCR ID analysed</b>	<b>37</b>

#### Genetic analysis of influenza isolates obtained in 2006

A summary of the number of isolates received and the total number of genes sequenced over the past 11 years at the Centre is shown below.



#### Isolates received and genes sequenced for surveillance activities at the Centre

## INFLUENZA A (H1N1)

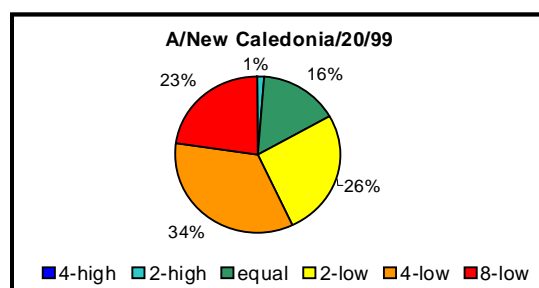
### H1 Haemagglutinin

379 A(H1) viruses were available for study and most were found to be antigenically A/New Caledonia/20/99-like. A summary of the A(H1) strains analysed, their origins and neuraminidase type are shown in the following table.

Country	A/New Caledonia/20/99-like viruses	
	H1N1	H1N2
Australia	24	0
Cambodia	3	0
Indonesia	0	0
Macau	34	0
Malaysia	38	0
New Caledonia	2	0
New Zealand	74	0
Philippines	116	0
Singapore	23	0
South Africa	2	1
Sri Lanka	2	0
Taiwan	10	0
Thailand	49	0
Other	1	0
<b>TOTAL</b>	<b>378</b>	<b>1</b>

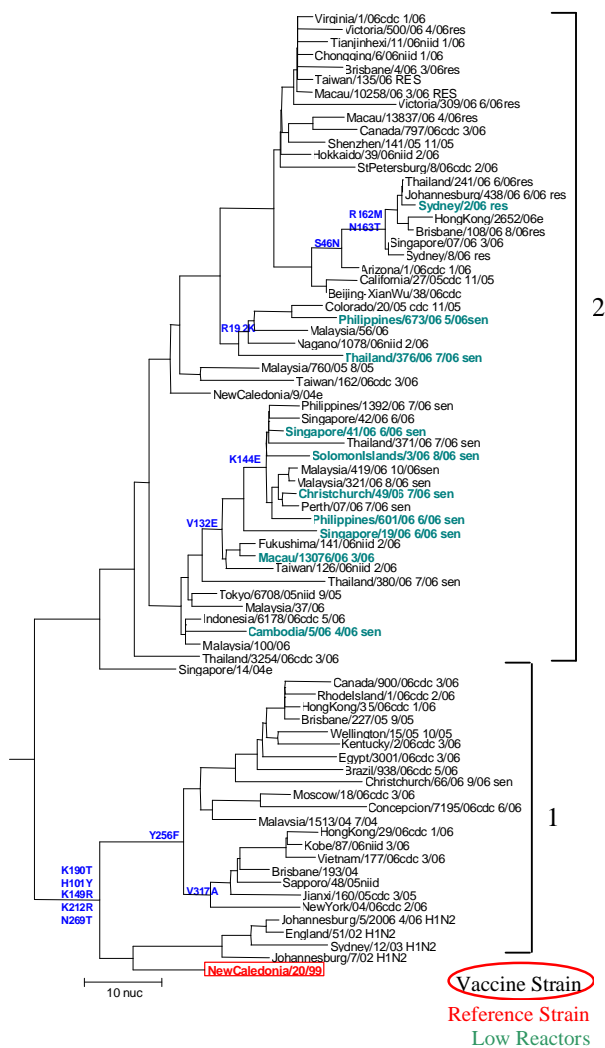
#### Summary of influenza A(H1N1) strains analysed in 2006

Antigenic analysis of the A(H1) viruses received in 2006 indicated that the majority were similar to isolates obtained during recent years and showed little antigenic drift from the reference strain (A/New Caledonia/20/99). However a proportion (23%) reacted 8-fold or lower compared to the A/New Caledonia/20/99 homologous titre. All A(H1) isolates except one from South Africa (H1N2) carried an N1 subtype neuraminidase. Representative examples of the HI results obtained for a number of 2006 H1N1 viruses are shown in Appendix 2.



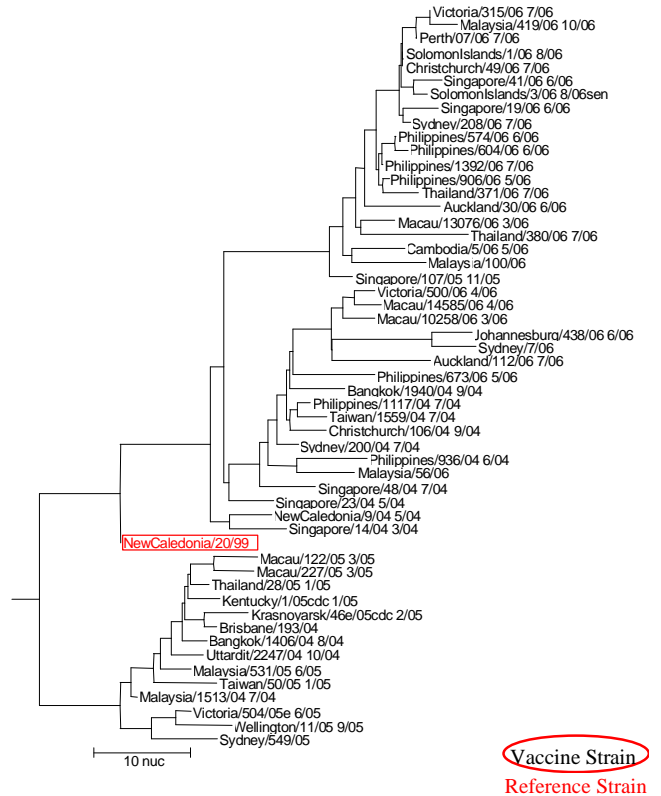
#### Summary of HI fold differences in HI titre compared to the A/New Caledonia/20/99 homologous titre

Sequencing was performed on the HA1 region of the haemagglutinin gene from 53 isolates. These isolates were selected based on their reduced reactivity with reference antisera in the HI test, their origin, or date of isolation. Phylogenetic analysis showed that while some recent isolates were genetically similar to A/New Caledonia/20/99 (Clade 1), the majority showed further genetic drift and formed a second clade (Clade 2) with 2 further sublineages. One of these had the K144E change and was represented by A/Solomon Islands/3/2006.



Phylogenetic tree for influenza A(H1N1) haemagglutinin (HA1)

neuraminidase of the reference virus A/New Caledonia/20/99.



Phylogenetic tree for influenza A(H1N1) neuraminidase



New robot for performing HI assays

## N1 Neuraminidase

N1 neuraminidase sequence analysis was performed on 28 A(H1) isolates. The 2006 A(H1) isolates bearing N1 neuraminidase clustered in to 2 distinct groups with minor changes to the

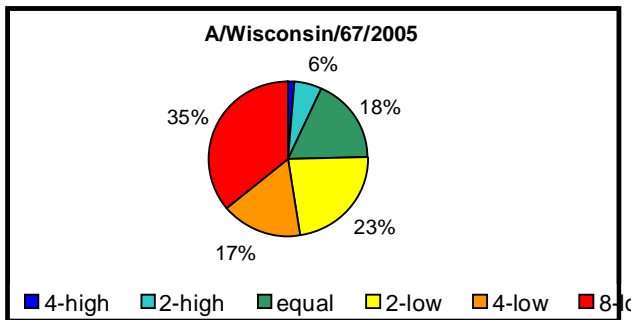
## INFLUENZA A(H3N2)

### H3 Haemagglutinin

A total of 906 subtype A(H3N2) viruses were available for further analysis. An increasing proportion of isolates in 2006 gave low reactivity against ferret sera used in the reference antisera panel. This was especially evident for sera raised against egg derived viruses. Some 35% of viruses had 8-fold or greater reductions in titre compared to the homologous titre obtained with the reference virus A/Wisconsin/67/2005 (see table below).

Country	A/Wisconsin/67/2005-like strains	A/Wisconsin/67/2005-like low reacting strains	TOTAL
Australia	246	148	394
Cambodia	1	1	2
Macau	1	0	1
Malaysia	8	15	23
New Caledonia	6	1	7
New Zealand	225	135	360
Philippines	1	0	1
Singapore	2	2	4
South Africa	9	7	16
Taiwan	2	2	4
Thailand	59	35	94
<b>TOTAL</b>	<b>560</b>	<b>346</b>	<b>906</b>

Representative examples of HI results for a number of 2006 H3N2 viruses are shown in Appendix 2.

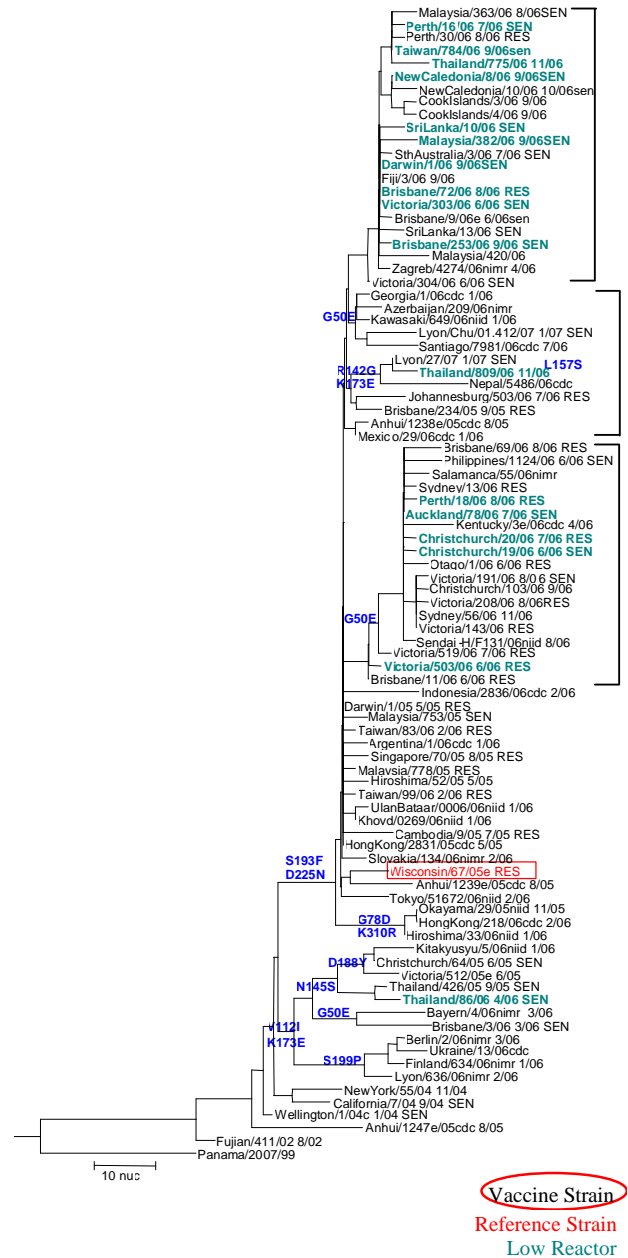


Summary of H3 HI-fold differences of isolates compared to A/California/7/2004 homologous titre

Sequencing was performed on the HA1 region of the haemagglutinin gene from 85 isolates.

These isolates were selected based on their reduced reactivity with reference antisera in the HI test, their origin, or date of isolation.

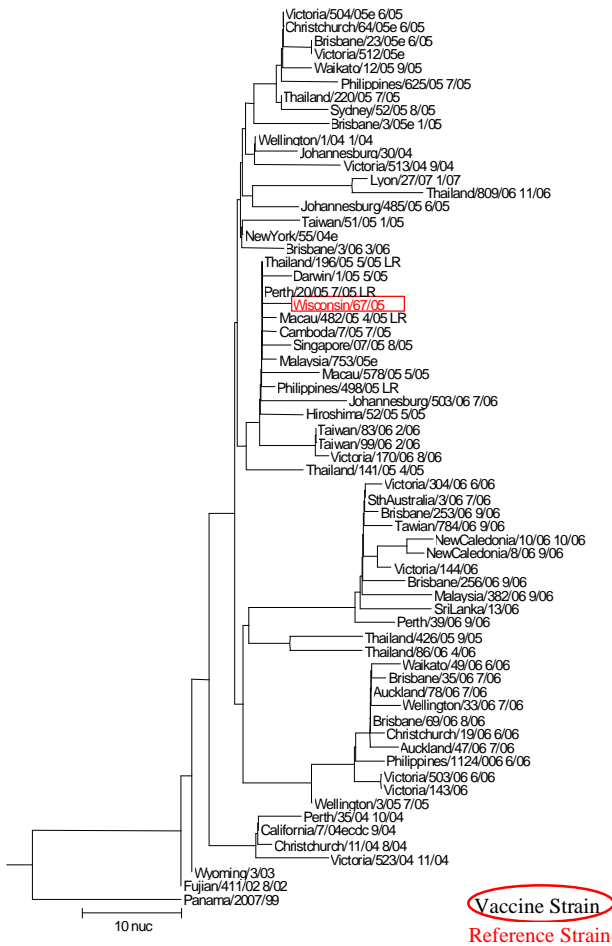
Phylogenetic analysis (see below) showed that the A(H3) 2006 virus sequences fell mainly into 2 main groups with either a V112I/K173E or S193F/D225N with the latter group having a subclade with the G50E change.



Phylogenetic tree of influenza A(H3N2) haemagglutinin (HA1)

## N2 Neuraminidase

Sequence analysis of the N2 gene for 47 A(H3N2) isolates was performed. The 2006 viruses clustered into 3 groups represented by A/Brisbane/3/2006, A/Wisconsin/67/2005 or A/Victoria/503/2006.



Phylogenetic tree of A(H3N2) neuraminidase

## INFLUENZA B

There are currently two antigenically and genetically distinct lineages of influenza B virus circulating, the B/Yamagata/16/88 lineage (which has recently been represented by B/Shanghai/361/2002-like viruses) and the B/Victoria/2/87 lineage (recently represented by B/Malaysia/2506/2004-like viruses). Until 2001 B/Victoria lineage viruses had been restricted to Asia where they tended to alternate with the B/Yamagata lineage. In 2002 the B/Victoria lineage became the predominant influenza B type in most parts of the world, however this trend was reversed again in 2003 and 2004 with the

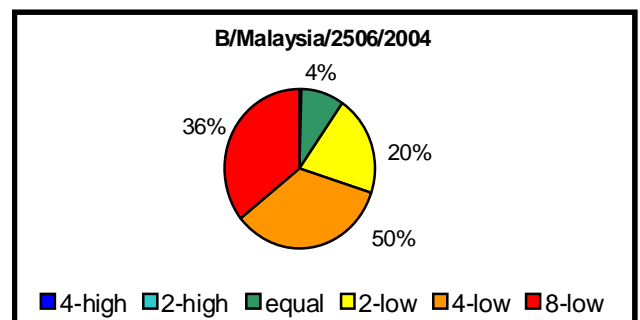
B/Yamagata lineage predominating and since then both lineages have co-circulated.

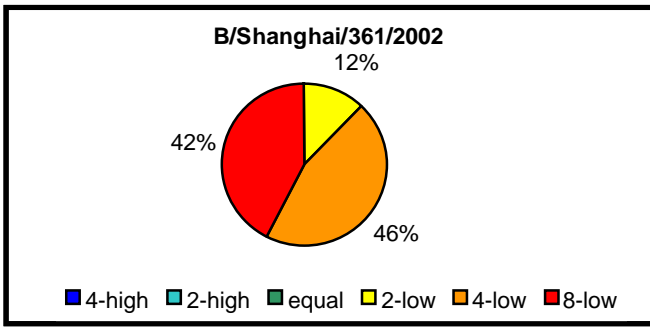
In 2006, of the 434 influenza B isolates analysed, 362 (83.4%) were B/Malaysia/2506/2004-like viruses and 72 (16.6%) were B/Shanghai/361/2002-like viruses. Most countries (eg Australia, Malaysia, Philippines) had mainly B/Malaysia/2506/2004-like viruses circulating with a small proportion of B/Shanghai/361/2002-like viruses with only Thailand having a higher proportion of B/Shanghai/361/2002-like viruses.

Country	B/Shanghai/361/2002-like strains	B/Malaysia/2506/2004-like strains	TOTAL
Australia	15	216	231
Macau	1	14	15
Malaysia	5	59	64
New Caledonia	0	7	7
New Zealand	1	4	5
Philippines	3	10	13
Singapore	1	3	4
South Africa	1	5	6
Taiwan	2	7	9
Thailand	43	35	78
Other	0	2	2
<b>TOTAL</b>	<b>72</b>	<b>362</b>	<b>434</b>

Summary of influenza B strains in 2006

Reactivity with post-infection ferret antisera showed that 36% of recent isolates of the B/Victoria/304/2006 lineage showed lower reactivity (8 - fold or greater) with B/Malaysia/2506/2004-like reference strain. Better reactivity was seen with antisera to the reference strain A/Victoria/304/2006 or the antisera raised to the cell grown virus B/Malaysia/174/2006. Recent isolates of the B/Shanghai lineage continued to react well with antisera to B/Shanghai-like reference strains such as B/Florida/7/2004. A summary of the HI reactivity of B isolates is shown below. Representative HI tables for a number of 2006 B viruses are contained in Appendix 2



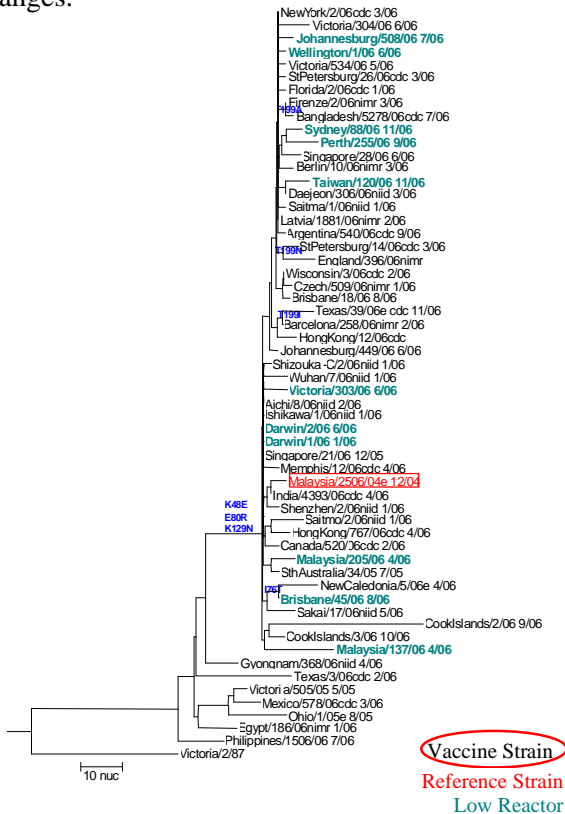


Summary of B HI fold differences compared to their respective homologous titre

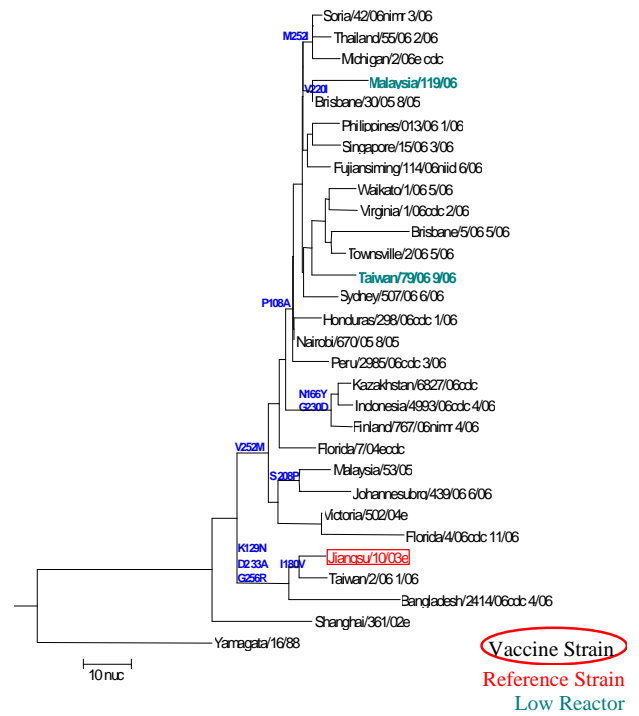
## B Haemagglutinin

Sequence analysis of the HA1 region of the viral haemagglutinin gene from 80 isolates was performed. These isolates were selected based on their reduced reactivity with reference antisera in the HI test, their origin, or date of isolation.

By phylogenetic analysis B/Malaysia/2506/2004 and B/Shanghai/361/2002 lineage viruses were clearly distinguishable. 2006 viruses with a B/Victoria-like HA1 were similar to B/Malaysia/2506/2004 with minor amino acid changes. Viruses of the B/Yamagata lineage that were sequenced were broadly similar to B/Florida/7/2004 with only a few amino acid changes.



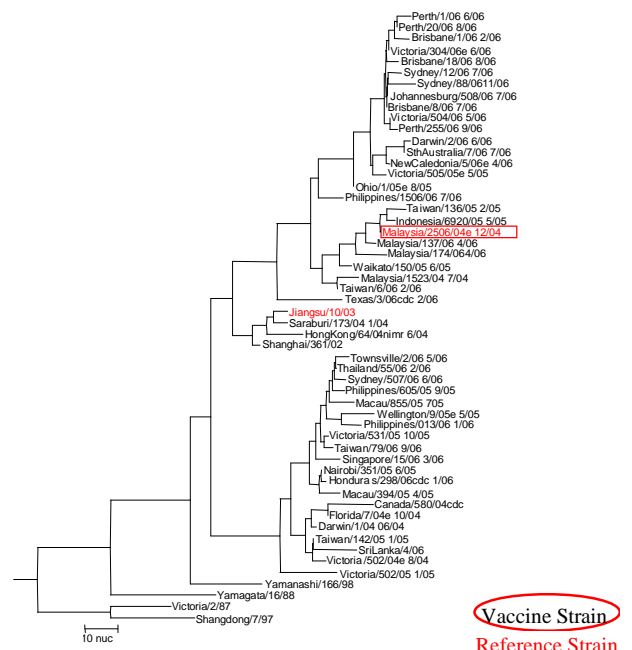
Phylogenetic tree for influenza B haemagglutinin (Victoria lineage)



Phylogenetic tree for influenza B haemagglutinin (Yamagata lineage)

## B Neuraminidase

Neuraminidase sequence analysis of 27 influenza B isolates was performed. A phylogenetic tree showing the two lineages of neuraminidases is shown below. All the 2006 viruses had a B/Sichuan/379/99-like NA regardless of whether they were of B/Yamagata or B/Victoria lineage. This continues the circulation of the B/Victorian reassortants seen in previous years. The majority of the 2006 viruses grouped with B/Victoria/304/2006 or with B/Malaysia/2506/2004



Phylogenetic tree for influenza B neuraminidase

## SEROLOGICAL STUDIES

A further assessment of the significance of antigenic changes in recent influenza isolates is made by measuring the degree to which they are inhibited by antibodies generated in subjects immunised with current influenza vaccines. These studies are undertaken on a collaborative basis within the WHO network by international exchange of serum panels and analysis in a number of laboratories. The results obtained by the Australian Centre for the Southern Hemisphere vaccine formulation consultation, September 2006, are reported as follows.

Human serum panels from four sources were utilised in this analysis. Note that sera from subjects who had responded to immunisation were selected for use in the Australian, European and US panels while the Japanese panel was unselected.

### Australian Serum Panel:

Two panels of pre- and post-immunisation sera were assayed, one from younger adults (18-60 years), the other from an older (60-75 years) population immunised in February 2006 with vaccine containing the formulation recommended by the Australian Therapeutic Goods Administration (TGA) for the 2006 winter.

The vaccine contained 15µg of HA antigen from each of:

an A/New Caledonia/20/99 (H1N1)-like strain  
an A/California/7/2004 (H3N2)-like\* strain  
a B/Malaysia/2506/2004 strain

(\*actual strain A/New York/55/2004)

### European Serum Panel:

Two panels of pre- and post-immunisation sera were assayed, one from younger adults (18-60 years), the other from an older (60-75 years) population immunised during May 2006 with vaccine containing the formulation below.

The European panel was sourced from the National Institute for Biological Standards and Control, UK.

The vaccine contained 15µg of HA antigen from each of:

an A/New Caledonia/20/99 (H1N1)-like strain  
an A/Wisconsin/67/2005 (H3N2)-like\* strain  
a B/Malaysia/2506/2004\*\*-like strain

(\*actual strain A/Hiroshima/52/2005

\*\*actual strain B/Jiangsu/10/2003)

### Japanese Serum Panels:

A panel of sera from Japan from young and older adults receiving vaccine of the WHO recommended formulation for the 2005/2006 Northern Hemisphere winter was assayed.

The vaccine contained 15µg of HA antigen from each of:

an A/New Caledonia/20/99 (H1N1)-like strain  
an A/Wisconsin/67/2005 (H3N2)-like\* strain  
a B/Malaysia/2506/2004\*\*-like strain

(\*actual strain A/Hiroshima/52/2005

\*\*actual strain B/Jiangsu/10/2003)

### US Serum Panels:

A panel of sera from American young and older adults receiving vaccine of the WHO recommended formulation for the 2005/2006 Northern Hemisphere winter was assayed.

The vaccine contained 15µg of HA antigen from each of:

an A/New Caledonia/20/99 (H1N1)-like strain  
an A/Wisconsin/67/2005 (H3N2)-like\* strain  
a B/Malaysia/2506/2004\*\*-like strain

(\*actual strain A/Hiroshima/52/2005

\*\*actual strain B/Jiangsu/10/2003)

Sera were assayed by haemagglutination-inhibition for antibodies to the vaccine strains and selected recent isolates. For the influenza A strains, whole virus cultivated in eggs or MDCK cells was used as the antigen. For influenza B, egg grown virus disrupted by treatment with ether was used as the antigen.

## Results

The criteria applied by the WHO collaborative studies for assessing antibody responses to recent isolates in comparison with current vaccine strains are:

- percentage of subjects demonstrating a 4-fold rise in antibody
- changes in overall antibody titre (geometric mean titre)
- percentage of subjects achieving an antibody titre of at least 1 in 40.

These criteria are consistent with those used by the Committee for Proprietary Medicinal Products, European Agency for the Evaluation of Medicinal Products in their requirements for clinical trials of influenza vaccines for annual licensing.

## INFLUENZA A(H1N1)

All subjects were immunised with the same H1N1 vaccine strain (A/New Caledonia/20/99). Australian and European subjects showed significant rises in titre to recent A/New Caledonia/20/99-like viruses. As is commonly seen older subjects demonstrated lower antibody responses than younger vaccinees. Lower titres were seen with some recent strains including A/Hong Kong/2652/2006 and A/Solomon Islands/3/2006.

Population	N	Antigen	Passage History	% Rise	GMT		%≥40		%≥160	
					Pre	Post	Pre	Post	Pre	Post
Australian Younger Adult	24	A/New Caledonia/20/99	E5	21	25.9	53.4	42	67	8	25
		A/Hong Kong/2652/2006	E4	17	6.5	12.2	4	17	0	0
		A/Solomon Islands/3/2006	E3	13	20.6	32.7	46	63	4	8
		A/Fukushima/141/2006	E5	21	13.3	29.1	29	28	4	4
		A/New Caledonia/20/99	E5	83	9.2	106.8	8	83	0	50
European Younger Adult	24	A/Hong Kong/2652/2006	E4	67	5.6	33.6	0	50	0	21
		A/Solomon Islands/3/2006	E3	79	10.9	87.2	13	88	0	38
		A/Fukushima/141/2006	E5	83	6.9	69.2	4	79	0	42
		A/New Caledonia/20/99	E5	33	34.6	73.3	54	79	21	38
		A/Hong Kong/2652/2006	E4	29	6.7	14.1	0	25	0	0
American Younger Adult	24	A/Solomon Islands/3/2006	E3	29	23.1	43.6	50	54	13	29
		A/Fukushima/141/2006	E5	25	15	32.7	4	17	0	0
		A/New Caledonia/20/99	E5	20	9.5	23	7	27	0	3
		A/Hong Kong/2652/2006	E4	17	6.2	11	0	10	0	0
Japanese Younger Adult	30	A/Solomon Islands/3/2006	E3	20	6.8	13.2	0	13	0	3
		A/Fukushima/141/2006	E5	10	7.6	14.5	3	20	0	3
		A/New Caledonia/20/99	E5	20	9.5	23	7	27	0	3
		A/Hong Kong/2652/2006	E4	17	6.2	11	0	10	0	0

Haemagglutination inhibition antibody responses influenza type A(H1) vaccine component – young adults

Population	N	Antigen	Passage History	% Rise	GMT		%≥40		%≥160	
					Pre	Post	Pre	Post	Pre	Post
Australian Older Adult	24	A/New Caledonia/20/99	E5	38	8.4	21.8	13	29	0	4
		A/Hong Kong/2652/2006	E4	25	6.1	12.6	4	8	0	0
		A/Solomon Islands/3/2006	E3	25	6.5	14.6	0	21	0	0
		A/Fukushima/141/2006	E5	17	5.9	13.3	0	13	0	0
European Older Adult	24	A/New Caledonia/20/99	E5	38	13.3	36.7	21	54	4	17
		A/Hong Kong/2652/2006	E4	21	6.7	14.6	4	21	0	8
		A/Solomon Islands/3/2006	E3	29	7.7	16.8	4	33	0	4
		A/Fukushima/141/2006	E5	29	7.5	18.3	4	33	0	8
American Older Adult	24	A/New Caledonia/20/99	E5	33	10.6	22.4	21	42	0	4
		A/Hong Kong/2652/2006	E4	13	6.1	10.3	4	8	0	0
		A/Solomon Islands/3/2006	E3	17	8.7	16.3	4	25	0	0
		A/Fukushima/141/2006	E5	13	7.3	11.9	0	17	0	0
Japanese Older Adult	30	A/New Caledonia/20/99	E5	23	29.6	68	47	77	20	3
		A/Hong Kong/2652/2006	E4	10	6.4	9.1	3	3	0	0
		A/Solomon Islands/3/2006	E3	23	25.2	50.4	47	67	20	33
		A/Fukushima/141/2006	E5	27	16.2	37.3	30	60	7	20

Haemagglutination inhibition antibody responses influenza type A(H1) vaccine component – older adults

## INFLUENZA A(H3N2)

Australian subjects receiving the A/New York/55/2004 containing influenza vaccine responded well to the homologous virus but these sera did not inhibit a number of viruses isolated in 2006 eg. A/Santiago/7981/2006. There was some improvement when the vaccine A(H3) component was replaced with A/Hiroshima/52/2005 as was seen with the European sera.

Population	N	Antigen	Passage History	% Rise	GMT		%≥40		%≥160	
					Pre	Post	Pre	Post	Pre	Post
Australian younger Adult	24	A/Wisconsin/67/2005	SPFCK3E5	75	16.3	87.2	29	88	8	42
		A/Brisbane/9/2006	E3	67	10.3	43.6	17	63	4	13
		A/Sendai-H/F131/2006	E5	71	18.3	87.2	29	88	8	42
		A/Lyon/1292/2006	MDCK2	58	10.9	32.7	8	58	0	0
		A/Santiago/7981/2006	E3/E2/E1	58	7.3	23.8	8	42	0	0
European Younger Adult	24	A/Wisconsin/67/2005	SPFCK3E5	96	8.4	160.0	4	92	0	71
		A/Brisbane/9/2006	E3	92	5.8	63.5	0	88	0	21
		A/Sendai-H/F131/2006	E5	96	9.4	155.4	4	92	0	63
		A/Lyon/1292/2006	MDCK2	88	6.5	41.2	0	79	0	4
		A/Santiago/7981/2006	E3/E2/E1	75	5.0	29.1	0	54	0	13
		A/Hiroshima/52/2005	E4	88	10.0	155.4	17	83	4	63
		A/Wisconsin/67/2005	SPFCK3E5	83	8.4	113.1	13	88	4	46
American Younger Adult	24	A/Lyon/1292/2006	E5	50	5.9	21.8	4	46	0	0
		A/Wisconsin/67/2005	SPFCK3E5	75	21.2	151.0	50	96	8	63
		A/Brisbane/9/2006	E3	83	11.9	84.7	21	83	0	29
		A/Sendai-H/F131/2006	E5	75	29.1	169.5	54	100	17	67
		A/Lyon/1292/2006	MDCK2	75	12.2	54.9	8	83	0	17
Japanese Younger Adult	30	A/Santiago/7981/2006	E3/E2/E1	67	7.3	35.6	4	54	0	13
		A/Wisconsin/67/2005	SPFCK3E5	33	21.9	65.0	30	53	7	30
		A/Brisbane/9/2006	E3	37	10.0	29.5	7	37	0	13
		A/Sendai-H/F131/2006	E5	48	19.1	65.0	27	57	7	27
		A/Lyon/1292/2006	MDCK2	23	10.2	24.1	7	23	0	10
		A/Santiago/7981/2006	E3/E2/E1	16	7.1	14.5	3	20	0	7

Haemagglutination inhibition antibody responses influenza type A(H3) vaccine component – young adults

Population	N	Antigen	Passage History	% Rise	GMT		%≥40		%≥160	
					Pre	Post	Pre	Post	Pre	Post
Australian Older Adult	24	A/Wisconsin/67/2005	SPFCK3E5	83	14.6	92.4	33	79	0	42
		A/Brisbane/9/2006	E3	71	9.2	44.9	8	63	0	29
		A/Sendai-H/F131/2006	E5	88	15.9	97.9	33	79	0	42
		A/Lyon/1292/2006	MDCK2	58	8.4	30.0	4	50	0	13
		A/Santiago/7981/2006	E3/E2/E1	50	6.3	21.8	0	42	0	13
European Older Adult	24	A/Wisconsin/67/2005	SPFCK3E5	83	21.8	127.0	38	83	4	54
		A/Brisbane/9/2006	E3	63	13.7	63.5	13	71	4	42
		A/Sendai-H/F131/2006	E5	71	26.7	130.7	42	88	4	54
		A/Lyon/1292/2006	MDCK2	67	11.9	46.2	8	75	0	8
		A/Santiago/7981/2006	E3/E2/E1	58	7.9	32.7	8	54	0	17
		A/Hiroshima/52/2005	E4	88	13.0	226.2	25	92	0	83
		A/Wisconsin/67/2005	SPFCK3E5	75	10.6	164.6	21	92	4	67
American Older Adult	24	A/Lyon/1292/2006	E5	71	7.1	38.9	8	63	0	13
		A/Wisconsin/67/2005	SPFCK3E5	54	24.5	95.1	46	96	21	42
		A/Brisbane/9/2006	E3	67	14.1	53.4	21	88	0	4
		A/Sendai-H/F131/2006	E5	54	24.5	97.9	50	96	13	42
		A/Lyon/1292/2006	MDCK2	42	11.2	31.7	17	54	0	4
Japanese Older Adult	30	A/Santiago/7981/2006	E3/E2/E1	42	8.7	21.8	13	33	0	0
		A/Wisconsin/67/2005	SPFCK3E5	50	21.9	65.0	37	67	7	37
		A/Brisbane/9/2006	E3	23	9.5	19.1	7	30	3	3
		A/Sendai-H/F131/2006	E5	37	17.8	43.9	37	73	3	13
		A/Lyon/1292/2006	MDCK2	20	10.0	17.8	7	27	0	0
Japanese Older Adult	30	A/Santiago/7981/2006	E3/E2/E1	17	6.9	11.0	3	13	0	0

*Haemagglutination inhibition antibody responses influenza type A(H3) vaccine component – older adults*

## INFLUENZA B

All vaccinees received B/Malaysia/2506/2004. This resulted in significantly increased titres against B/Malaysia/2506/2004 and other B/Victoria-like viruses. HI titres to a B/Yamagata lineage viruses (B/Michigan/2/2006) were lower as expected but these were also boosted following vaccination.

Population	N	Antigen	Passage History	% Rise	GMT		%≥40		%≥160	
					Pre	Post	Pre	Post	Pre	Post
Australian Younger Adult	24	B/Malaysia/2506/2004*	E4	54	16.3	65.3	29	83	0	33
		B/Bangladesh/5278/2006 <sup>†</sup>	E3	42	22.4	75.5	38	83	13	38
		B/Texas/39/2006 <sup>†</sup>	E4	63	18.9	82.3	29	83	8	38
		B/Michigan/2/2006 <sup>†</sup>	E4	8	47.6	84.7	63	75	21	42
European Younger Adults	24	B/Malaysia/2506/2004*	E4	88	12.2	113.1	21	79	4	54
		B/Bangladesh/5278/2006 <sup>†</sup>	E3	88	15.0	142.5	33	88	4	63
		B/Texas/39/2006 <sup>†</sup>	E4	79	15.0	142.5	33	88	4	63
		B/Michigan/2/2006 <sup>†</sup>	E4	63	11.2	54.9	21	67	0	38
American Younger Adults	24	B/Malaysia/2506/2004*	E4	56	27.5	151.0	48	88	16	60
		B/Bangladesh/5278/2006 <sup>†</sup>	E3	67	32.7	226.2	50	96	21	71
		B/Texas/39/2006 <sup>†</sup>	E4	71	28.3	213.5	21	71	0	0
		B/Michigan/2/2006 <sup>†</sup>	E4	36	80.0	190.2	80	84	40	68
Japanese Younger Adults	30	B/Malaysia/2506/2004*	E4	7	18.7	44.9	27	57	0	7
		B/Bangladesh/5278/2006 <sup>†</sup>	E3	20	23.5	66.5	40	60	3	13
		B/Texas/39/2006 <sup>†</sup>	E4	33	20.9	66.5	30	60	3	17
		B/Michigan/2/2006 <sup>†</sup>	E4	3	16.6	24.6	27	43	0	3

(\*B/Victoria strains, \*\*B/Yamagata strains)

*Haemagglutination inhibition antibody responses influenza type B vaccine component – young adults*

Population	N	Antigen	Passage History	% Rise	GMT		%≥40		%≥160	
					Pre	Post	Pre	Post	Pre	Post
Australian Older Adult	24	B/Malaysia/2506/2004*	E4	58	10.9	38.9	17	71	0	8
		B/Bangladesh/5278/2006 <sup>†</sup>	E3	63	11.2	53.4	17	83	0	13
		B/Texas/39/2006 <sup>†</sup>	E4	71	11.2	51.9	17	79	0	13
		B/Michigan/2/2006 <sup>†</sup>	E4	21	23.8	50.4	29	75	13	25
European Older Adults	24	B/Malaysia/2506/2004*	E4	67	18.9	77.7	38	83	8	38
		B/Bangladesh/5278/2006 <sup>†</sup>	E3	67	21.2	103.7	42	92	13	46
		B/Texas/39/2006 <sup>†</sup>	E4	75	20.0	113.1	42	92	8	50
		B/Michigan/2/2006 <sup>†</sup>	E4	13	37.7	67.3	50	83	17	29
American Older Adults	24	B/Malaysia/2506/2004*	E4	50	25.9	82.3	50	83	8	42
		B/Bangladesh/5278/2006 <sup>†</sup>	E3	42	37.7	109.9	50	100	25	50
		B/Texas/39/2006 <sup>†</sup>	E4	50	34.6	106.8	58	96	17	46
		B/Michigan/2/2006 <sup>†</sup>	E4	17	61.7	119.8	79	92	25	50
Japanese Older Adults	30	B/Malaysia/2506/2004*	E4	33	27.0	74.6	50	87	7	30
		B/Bangladesh/5278/2006 <sup>†</sup>	E3	40	31.7	87.7	47	90	27	50
		B/Texas/39/2006 <sup>†</sup>	E4	43	30.3	94.0	53	93	20	47
		B/Michigan/2/2006 <sup>†</sup>	E4	13	89.8	133.0	90	97	47	63

(\*B/Victoria strains, \*\*B/Yamagata strains)

*Haemagglutination inhibition antibody responses influenza type B vaccine component – older adults*

## SURVEILLANCE OF INFLUENZA STRAINS FOR RESISTANCE TO ANTIVIRAL DRUGS

### ADAMANTANES (M2 BLOCKERS)

The adamantanes (amantadine and rimantadine) were the first antivirals licensed for use against influenza A viruses and have been used in some countries to control seasonal influenza. While increasing resistance of A(H3) viruses to this class of drug has been reported in recent years, only low levels of resistance were seen with A(H1) viruses until the 2005-6 influenza season in the USA. We analysed 200 human influenza A viruses isolated in 2006 that were referred to the WHO Collaborating Centre for Reference and Research in Melbourne, from Australia and the surrounding regions, for evidence of resistance to adamantanes by sequencing of the M2 gene. We found that whereas previously A(H1) resistant viruses were rare, 22% of the 2006 viruses had a resistant genotype. By comparison, 52% of influenza A(H3) viruses isolated in 2006 that were tested at the Centre, had a resistant genotype. The A(H1) resistance was not as widespread as A(H3) resistance with several countries appearing to have no resistant viruses amongst the samples tested. Interestingly practically all resistant viruses of both A(H3) and A(H1) subtype had the same S31N mutation in the M2 gene. Clearly both the A(H1) and A(H3) resistant viruses have no lack of viral fitness and are easily transmissible in man. Ongoing surveillance and monitoring will be required for a number of years in order to determine if adamantanes

can be used effectively in individual countries in the future.

Country	2006		2006	
	H3	Totals	H1	Totals
Macau	1/1	100%	9/11	82%
Taiwan	2/3	66%	1/5	20%
Thailand	6/10	60%	1/10	10%
Malaysia	2/14	14%	0/10	0%
Cambodia	-	-	0/3	0%
Singapore	0/1	0%	1/10	10%
Philippines	1/1	100%	0/14	0%
Indonesia	-	-	-	-
Australia	21/41	50%	8/20	40%
New Zealand	9/12	75%	1/7	14%
New Caledonia	0/5	0%	0/5	0%
South Africa	10/10	100%	1/3	33%
Solomon Islands	-	-	0/3	0%
Sri Lanka	0/2	0%	-	-
China	-	-	-	-
France	-	-	-	-
<b>Totals</b>	<b>52/100</b>	<b>52%</b>	<b>22/100</b>	<b>22%</b>

*Resistance of Influenza A viruses to adamantanes as predicted by M2 sequencing*

## NEURAMINIDASE INHIBITORS

During 2006, a total of 503 isolates were tested by the Centre for their sensitivity to the NA inhibitor drugs zanamivir (Relenza, GSK) and oseltamivir carboxylate (Tamiflu, Roche) using a fluorescence-based NA enzyme inhibition assay. The isolates tested were collected through the WHO global influenza surveillance program from Australia, New Zealand, Singapore, USA, Malaysia, Thailand, Macau, Hong Kong, New Caledonia and Taiwan. The following table is a summary of the mean 50% inhibitory concentration (IC<sub>50</sub>) and standard deviation of the viruses tested during 2006 for each of the NA inhibitors.

Type / subtype	No. Tested	Zanamivir Mean IC <sub>50</sub> (nM) ± SD	Oseltamivir carboxylate Mean IC <sub>50</sub> (nM) ± SD	Peramivir Mean IC <sub>50</sub> (nM) ± SD
A(H1N1) A(H1N2)	146	0.26 ± 0.08	0.53 ± 0.33	0.16 ± 0.09
A(H3N2)	266	0.81 ± 0.52	0.33 ± 0.41	0.15 ± 0.04
B	91	1.95 ± 0.98	14.66 ± 9.76	1.59 ± 1.00

Of the viruses tested, 2 had a 5-fold or greater reduction in sensitivity. Literature reports

describe reductions in sensitivity of 'resistant' strains identified following treatment with either zanamivir or oseltamivir, in the range of 40 to 7200-fold. Based on these values, none of the viruses tested by the Centre during 2006 demonstrated clinically significant resistance to the NA inhibitor drugs.

## PREPARATION & DISTRIBUTION OF DIAGNOSTIC REAGENTS

Kits of diagnostic reagents containing polyclonal sera and viral antigens for relevant influenza strains are prepared annually and distributed by the Centre to regional and reference laboratories. These kits allow basic characterisation of current strains of influenza and presumptive identification of variants for submission to WHO Collaborating Centres for further analysis. Viruses must be first propagated in tissue culture or in eggs. A list of the laboratories receiving these kits is given in Appendix 1.



*The Centre's 2006 kit for influenza diagnosis*

## Contents of the 2006 Kit

1x10mL each of the reference antigens:

Antigens	HA Titre with RBC Type*			
	Turkey	Fowl	Guinea Pig	Human
A/New York/55/2004 (H3N2)-like	1600	800	1600	1200
A/New Caledonia/20/99 (H1N1)-like	3200	4000	1200	1600
B/Malaysia/2506/2004-like	4800	10000	1600	2800
B/Shanghai/361/2002-like	4000	6400	1600	3200

\*Approximate guide only.

## HAI Reactions of the 2006 Reagents

Reference Antigens		Reference Antisera			
		1 A/New York	2 A/New Cal	3 B/Malay	4 B/Shang
1	A/New York/55/2004 - like	128	<1	<1	<1
2	A/New Caledonia/20/99 - like	<1	128	<1	<1
3	B/Malaysia/2506/2004 - like	<1	<1	128	<1
4	B/Shanghai/361/2002 - like	<1	<1	<1	64
<b>Field Isolates</b>					
	A/Brisbane/3/2006 (H3)	64	<1	<1	<1
	A/Thailand/504/2005 (H3)	64	<1	<1	<1
	A/Macau/925/2005 (H1)	<1	64	<1	<1
	A/Taiwan/135/2006 (H1)	<1	64	<1	<1
	B/Malaysia/3327/2005 (B/Malaysia/2506/2004 - like)	<1	<1	128	<1
	B/Thailand/7/15/2005 (B/Shanghai/361/2002 - like)	<1	<1	<1	64

*HAI titres of the 2006 reagents*

## IFA Reagents – Monoclonal Antibodies

Two broadly-reactive monoclonal antibodies are provided on request which react with the internal antigens of influenza A and most influenza B viruses and are suitable for use in indirect fluorescent antibody detection of influenza viruses in clinical specimens or cell cultures or in other rapid detection techniques. The lack of reactivity with some recent B viruses with the B monoclonal has led to the Centre removing these items from the kit and the provision of these antibodies is recommended for research purposes only.

1.0 mL each of monoclonal antibodies available:  
Anti-influenza type A nucleoprotein, Lot MA03-1  
Anti-influenza type B nucleoprotein, Lot MB03-1

## CANDIDATE VACCINE STRAINS

An increasingly important role of the Centre is the primary isolation of reference viruses into embryonated eggs which are then offered through the WHO network for development as candidate vaccine strains. These isolates are obtained from original clinical isolates under conditions acceptable to regulatory authorities. Only viruses obtained exclusively from clinical samples and passaged in eggs or primary egg-derived cells are currently acceptable to regulatory agencies for production of influenza vaccines. The tables below list isolates passaged from viruses received as egg isolates and viruses directly isolated from clinical samples into eggs at the Centre in 2006.

Type	Strain	No.
H1	A/KENTUCKY/1/2005	8
	A/SHENZHEN/141/2005	
	A/TOKYO/6708/2005	
	A/ST.PETERSBURG/6/2006	
	A/KENTUCKY/2/2006	
H3	A/NOVI SAD/150/2006	7
	A/EGYPT/39/2005	
	A/CONCEPCION/7195/2006	
	A/HIROSHIMA/52/2005	
	A/ANHUI/1239/2005	
	A/ANHUI/1238/2005	
	A/WISCONSIN/67/2005	
B	A/BRAZIL/1742/2005	4
	A/KENTUCKY/03/2006	
	A/HIROSHIMA/33/2006	
	B/OHIO/1/2005 X B/LEE/40	
	B/PERU/4101/2006	4
	B/PERU/4051/2006	
	B/MICHIGAN/02/2006	19
	TOTAL	

*Potential candidate vaccine strains sourced from overseas and passaged in eggs at the Centre*

Type	Strain	No.
H1	A/WELLINGTON/14/2005	7
	A/VICTORIA/561/2005	
	A/VICTORIA/562/2005	
	A/VICTORIA/500/2006	
	A/MALAYSIA/100/2006	
	A/SOLOMON ISLANDS/3/2006	
H3	A/SOLOMON ISLANDS/1/2006	3
	A/VICTORIA/503/2006	
	A/BRISBANE/11/2006	
B	A/BRISBANE/9/2006	4
	B/BRISBANE/5/2005	
	B/NEW CALEDONIA/5/2006	
	B/VICTORIA/304/2006	
	B/VICTORIA/312/2006	14
	TOTAL	

*Potential candidate vaccine strains isolated in eggs at the Centre*



## 4. RESEARCH STUDIES AND OTHER ACTIVITIES

### Rapid Diagnostic Tests

Over recent years a number of 'near patient' tests have been developed principally with a view to assisting in patient management and the prescribing of antiviral drugs. Because such tests also offer the potential to conduct surveillance for influenza outbreaks and in remote regions, the Centre regularly conducts assessment of commercially-available tests and, on occasions, prototypes under development. In 2006 six kits were evaluated in conjunction with the Royal Children's Hospital (RCH), Melbourne. This evaluation is contained in a forthcoming publication.

### Genetic Studies

#### Reverse Genetics

During the last decade a number of research laboratories have developed procedures for reconstituting influenza viruses from cloned DNA copies of the viral genes. These methods, referred to as 'reverse genetics', provide opportunities for directed modification of influenza viruses useful both in research activities and, potentially, the rapid generation of candidate vaccine strains, particularly in the case of potential pandemic viruses which are highly pathogenic for poultry. This technique was used extensively in the studies undertaken by our 2006 BSc (Honours) student as outlined below.

### BSc (Honours) Students



Ms Hui Ting HO from Monash University, Gippsland Campus was the Centre's 2006 Honours student. Her thesis title was: The Use of Reverse Genetics to Investigate Neuraminidase Inhibitors Resistance in Influenza A(H1N1) viruses.

The project used reverse genetics to modify selected residues in the neuraminidase of influenza A(H1) strains to investigate the role these residues might play in the generation of antiviral drug resistance.

Neuraminidase (NA) inhibitors, a new class of antivirals designed to target the conserved residues at the influenza NA active site, are currently available for the control of influenza. However, viruses with resistance to NA inhibitors, conferred by mutations at key residues in the NA active site, have been identified. To investigate the role of other NA active site residues in NA inhibitor resistance, amino acid mutations at residues Glu227 and Glu276 in NA of an influenza A(H1N1) virus were studied. Site-directed mutagenesis was used to generate three alternative amino acids at positions 227 and 276; recombinant mutant viruses were then generated using the reverse genetics system. These NA mutant viruses were characterised in terms of their growth, NA activity and sensitivity to NA inhibitors. Of the six recombinant viruses expressing NA with mutations at either Glu227 or Glu276, most showed significantly compromised growth and all had a significant reduction in NA activity. The growth of mutant Glu227Asp and Glu276Asp viruses was the least impaired. The Glu276Asp mutation demonstrated no significant role in resistance to NA inhibitors. The mutant Glu227Asp virus demonstrated significantly reduced sensitivity to zanamivir; however the potential clinical significance of this mutation in N1 subtype viruses needs further investigation.

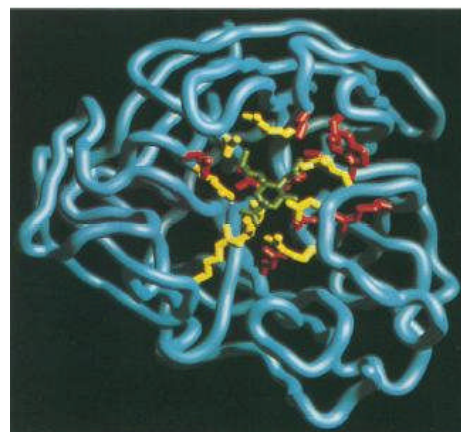


Figure 1.3: Active site structure of influenza A virus neuraminidase (Colman et al., 1993). (A) Yellow residues (N2 numbering: Arg118, Arg152, Arg224, Glu276, Arg292, Arg371 and Tyr406) are defined as catalytic in that they make contact with the substrate (sialic acid) (green) and are involved directly in enzymatic catalysis. Red residues (Glu119, Arg156, Trp178, Ser179, Asp198, Ile222, Glu227, Asn294 and Glu425) are defined as framework in that they make no direct contact with the substrate but support the catalytic residues to hold them in place for binding and catalysis. All these amino acids are conserved across all known strains of types A and B influenza except for Asp197, which is Asn in N7 and N9 subtype.(From Colman et al., 1993)

## SEQUENCE ANALYSIS FOR RESEARCH PURPOSES

A number of sequence analyses were undertaken for various research projects carried out at the Centre. A breakdown of these is shown in the table below. A number of neuraminidase and hemagglutinin genes were sequenced for NA inhibitor sensitivity assay comparisons. A large number of sequences of the matrix gene were analysed for Adamantane resistance. See page 15.

Influenza Gene	Number of Sequences
HA	147
NA	121
MP	283
NP	6
NS	7
PB1	8
PB2	9
PA	8
TOTAL	589

## EXCHANGE OF MATERIALS WITH OTHER WHO COLLABORATING CENTRES

Influenza strains and data were exchanged with the WHO Collaborating Centres for Reference and Research on Influenza in Atlanta, Mill Hill and Tokyo.

Centre	Atlanta	Mill Hill	Tokyo
Viruses received from	34	9	9
Viruses sent to	19	19	19
Ferret antisera sent to	6	6	6
Human sera sent to*	48	48	48

\* Panels of 48 sera from these shipments were also sent to NIBSC and CBER

## REGIONAL COLLABORATION

During 2006 the Centre has continued to support the activities of the Pacific Public Health Surveillance Network ([www.spc/int/phs/PPHSN/index.htm](http://www.spc/int/phs/PPHSN/index.htm)) in their work to improve influenza surveillance diagnosis and awareness in the Pacific. The Centre supported an application for funding via the CDC program "Development of

Influenza Surveillance Networks" funding number: AA011, to improve influenza surveillance in 2005-6 and this grant for US\$250,000 was successfully renewed for 2006-2007.

## WEB SITE

During 2006 our Web site ([www.influenzacentre.org](http://www.influenzacentre.org)) was continually updated and improved. Throughout the influenza season fortnightly updates of influenza type and subtype were added for Australia and the region. The site averages around 1000 hits per month.

Information carried by the site includes:

- information regarding the Centre
- general information on influenza
- vaccine formulation information
- regular bulletins on developments and research
- links to the principal sites carrying information on influenza
- annual reports and diagnostic methods for use with the reagents the Centre supplies are available from the site for download as .pdf files.
- Centre publication listings.

## THE WILD SYSTEM

In 2000 the staff of the Centre designed a proprietary database the WHO Influenza Laboratory Database (WILD) using a Microsoft Access platform. Since that time the staff have continued to develop the networked database, progressively increasing the volume of data stored, as well as providing more accurate and rapid means of interrogating the data. As a WHO Reference Centre it is essential that our database provides complete and secure record keeping relating to the receipt, storage and test results of all the influenza viruses received by the Centre. The WILD currently contains extensive details on over 26,000 influenza viruses.

## INFLUENZA AWARENESS PROGRAMS

Staff of the Centre participated with the Australian Influenza Specialist Group (ISG) and Australian State and National Departments of Health to encourage vaccination amongst the priority risk groups defined by the Australian National Health and Medical Research Council (Australian Immunisation Handbook, 8<sup>th</sup> Edition) and also the use of influenza antiviral drugs.

A 2004 survey conducted by the Australian Institute of Health and Welfare indicated that vaccine coverage in the group aged 65 years or older was 79% (<http://www.aihw.gov.au/publications>).

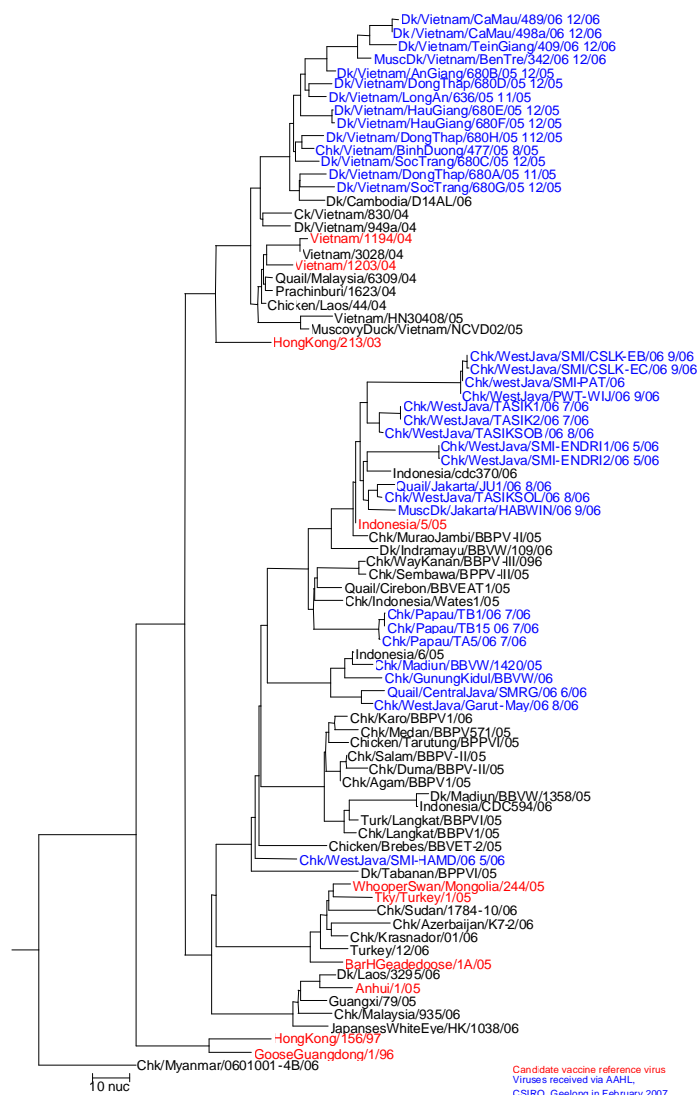
## PANDEMIC PREPAREDNESS

During 2006, human influenza cases due to highly pathogenic avian influenza A(H5N1) occurred in a number of countries and resulted in more deaths than occurred in 2004 and 2005 combined. The Centre in collaboration with VIDRL imported further A(H5) viruses from human cases into Australia. These viruses were used to extract RNA to allow molecular assays to be developed and tested to ensure that Australian laboratories can detect A(H5) cases efficiently and accurately.

An ongoing collaboration with Dr Paul Selleck at AAHL, CSIRO, Geelong, gave the Centre access to a large number of avian A(H5) isolates (either inactivated or RNA extracted) for sequencing, antiviral susceptibility testing, HI analysis and serology studies. Sequences from A(H5) viruses were added to publicly available databases. Some of the sequences generated are displayed below.

## MIGRATORY BIRD STUDY

Continuing the Centre's interest in avian influenza viruses, Centre staff have in collaboration with the Victorian Institute of Animal Science, Attwood, and the University of Newcastle, continued a program of swabbing migratory wading birds for evidence of influenza virus carriage in 2006. Birds were sampled from a number of field studies conducted by the Victorian Waders Study Group (VWSG) and from birds sampled around Victoria Newcastle, and elsewhere. Over 1000 samples have been collected during the 2006 season in Victoria and tested at the Attwood facility.



## INFLUENZA VACCINE STUDIES

During 2006 the Centre was involved in a number of NHMRC - funded grants for urgent research on pandemic avian influenza. Details of these grants are outlined below and in Appendix 3. Many of the outcomes of these studies will be incorporated into future publications and presentations.

### 1. (401314) Surveillance and analysis of avian influenza viruses in migratory birds in Australia

Birds are commonly infected with bird flu viruses but most of these viruses do not cause disease. However, certain types of bird flu viruses, such as the H5N1 strain, can cause severe illness and death in 100% of infected birds. There is currently an epidemic of H5N1 bird flu occurring in Asia. Occasionally humans become infected by bird to human

transmission. There are 3 ways in which dangerous strains of the virus may be brought to Australia, by infected people, domestic birds and migratory wading birds. In order to monitor the import of these viruses by migratory birds we have caught and taken samples from large numbers of these birds in important areas where they congregate close to humans and poultry farms. There is an additional concern that non-disease causing viruses could mutate in ducks and become pathogenic and pass from bird to bird or even develop human to human transmission which could cause a major human epidemic of bird flu in Australia. This study has sampled local ducks that occur in the same areas as the wading birds and tested them for influenza viruses. Our results showed that very low levels of influenza virus are carried by wader birds and only slightly higher levels are carried in ducks.

#### **2. (365240) Mucosal vaccine for influenza based on inactivated virus and mannan**

This project looked at improving vaccines for influenza which is important because of the possibility of an avian influenza pandemic. The vaccine could be given via the nose and may be a better alternative than injection. The mannan adjuvant vaccines were tested in mice and ferrets and while marked enhancements were seen in mouse influenza models little enhancement was seen in initial trials in ferrets.

#### **3. (382917) Development of National Protocols for the Detection of Influenza A H5N1**

This project developed various H5N1 panels to assess influenza H5N1 RT-PCR, tests for detection of influenza proteins including immunofluorescence, and rapid point of care influenza A detection tests available in Australia.

Recommendations for standard protocols for influenza A H5N1 are now being developed and will be submitted for review and endorsement by Commonwealth ministerial advisory committees.

#### **4. (404189) Spatial Simulation Modelling of Containment Strategies for Pandemic Influenza**

This project has developed a spatial simulation model to predict the spread of pandemic influenza within Australia. This prototype software program

can predict the effect of various containment measures on the size, rate and location of disease spread, through a city, state or the nation. Specifically, this project will apply the simulation model to determine optimal use of limited resources such as the “when and where” targeting of antiviral drugs and initial supplies of vaccine. The initial data set has used Albany as its model population.

#### **5. (400595) Assessment of development of resistance to neuraminidase inhibitors in A(H5N1) influenza viruses using a ferret model**

The neuraminidase (NA) inhibitors are considered the most effective anti-influenza drugs available for both prevention and treatment of influenza virus infection including A(H5N1) viruses. The drugs are effective against all subtypes of influenza A, making them ideal for use in the early months of a pandemic prior to an appropriate vaccine being produced. As a result many countries around the world, including Australia, have stockpiled these drugs (mainly Tamiflu) as part of their pandemic preparedness plans. However, of concern is the increasing number of recent reports of a higher than expected level of resistance in epidemic influenza being generated against these drugs.

The aim of the project was to determine the levels, mode and type of resistance that may occur when ferrets are experimentally infected with HP A(H5N1) virus and then treated with NA inhibitors drugs such as Tamiflu. This work was still being performed at the end of 2006.

In addition a number of H5N1 viruses have been assessed for sensitivity to NIs and this work is contained in a forthcoming publication.

#### **6. (400584) Assessment of alpha-galactosylceramide as a novel adjuvant for pandemic influenza A virus vaccines**

This proposal aimed to evaluate the efficacy of a novel vaccine strategy using alpha-galactosylceramide as a way to promote immune protection against a potential pandemic influenza strain. This work was still ongoing at the end of 2006.

#### **7. (400583) Establishing the capacity for H5N1 challenge of ferrets within Australia & optimising pandemic vaccines in this model**

It is not possible to test vaccines in people for their effectiveness against avian influenza infection prior to a disease outbreak, using deliberate virus challenge, so an animal model for the disease has been used to assist in optimising the formulation of flu vaccines and in testing their efficacy in preventing infection or reducing the severity of disease. Ferrets are an excellent host for influenza viruses, and have similar responses to vaccination as people and also develop a similar disease to humans when infected with influenza. This work was showing promising results and was ongoing at the end of 2006.

#### **8. (381735) Rapid, point-of-care diagnostic tests to differentiate HA subtypes in patient samples**

A number of rapid, point-of-care tests are available for the detection of human and avian influenza types, but they vary greatly in sensitivity. In particular, these tests are based on current strains of H5 (avian) influenza, and may be unable to detect variant or pandemic strains, and negative results can give the false impression that patients do not have H5 influenza.

This project aimed at developing rapid, point-of-care tests based on highly conserved parts of the virus, so that all H5 strains (current and future) that can be detected with equal sensitivity, along with the H1 and H3 strains that are currently found in man to provide a definitive diagnosis. These tests would be valuable in the identification and differentiation of pandemic influenza cases, allowing better use of public health resources, especially against a background of continuing “standard” (H1 and H3) influenza infections. This work was still being evaluated at the end of 2006.

#### **9. (381734) Chimeric virus-like particles (VLPs) displaying H1, H3 and H5 haemagglutinins – construction and immunogenicity**

Virus-like particles (VLPs) provoke strong immune responses in the body. We have developed a novel VLP system that allows the production of VLPs containing foreign vaccine antigens of much larger size than previously possible, and have shown that these VLPs provoke strong immune responses in mice without the use of adjuvants. The capacity of these VLPs is large enough to accommodate the most important vaccine antigen of influenza, the

haemagglutinin (HA) molecule. We have tested whether VLPs can be produced containing each of the three most important HA types – H1 and H3 that are currently circulating in man, and H5 (avian) that is considered a pandemic threat. VLPs have been tested for their ability to induce neutralizing antibody and cellular immune responses in mice, and for their ability to protect ferrets from influenza infection. Further work was continuing on this project at the end of 2006.

#### **10. (401178) Avian influenza – improvement of serological & molecular diagnostics using quality assurance.**

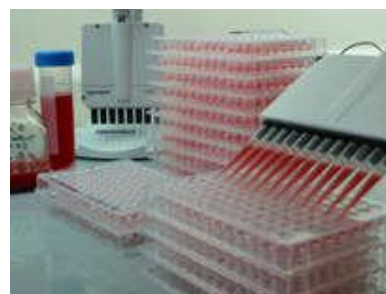
This project aimed to produce Quality Assurance (QA) algorithms to ensure accurate diagnosis of highly pathogenic avian influenza (HPAI) using serological and molecular techniques. The two key elements of the project were:

- Development of quality assurance processes to ensure accurate diagnosis and to evaluate laboratory testing procedures, accuracy and interpretation of results.
- Production of suitable antigenic material by the molecular virology research group as targets for HPAI antibody and molecular diagnosis. This will thereby allow large quantities of non-infectious material for HPAI QA assessment of serological and molecular assays around Australia, using baculovirus expressed antigens and cloned gene targets respectively.

This program was instituted and a report on the outcomes is available to participants of the study at <http://www.rcpaqap.com.au/serology/>

#### **11. (400589) Are routine Healthcare worker hand hygiene protocols (soap/water, alcohol based hand rubs) effective against influenza?**

This project tested a number of common hand washing protocols including soap and water and several alcohol based products. All performed well in removing infectious influenza virus from hands. A manuscript is in preparation.



## 5. TRAINING, VISITORS & OTHER

### Visiting Scientists

The following people visited the Centre for training or discussions during 2006:

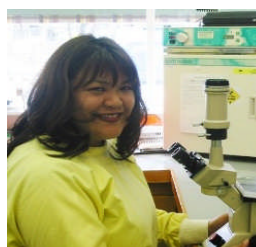
Four research scientists from Japan visited the Centre on March 22: Masao Matsuoka, Institute for Virus Research, Kyoto University; Yasuhiro Morita, Research Associate, Research Institute for Microbial Diseases, Osaka University; Assoc. Prof. Ayako Sano, Research Centre for Pathogenic Fungi and Microbial Toxicoses, Chiba University; and Prof. Osamu Nakagomi, Nagasaki University Graduate School of Biomedical Sciences, Department of Molecular Microbiology and Immunology.

Two scientists from ABI, Foster City, CA, USA visited the Centre on 28 February.

Two overseas scientists visited the Centre. Dr Glenn Marsh from Peter Palese's laboratory in New York visited the Centre on 16 May to discuss matters of mutual interest. Ms Sarah Fischer, a recent MPH graduate from the UCLA School of Public Health visited the Centre on 19 June to discuss the role of the Centre in influenza surveillance and the WHO Global Influenza program.



Andy Derijk from Roche visited the Centre on 23<sup>rd</sup> February to make available some stock of Tamiflu to the Centre staff



Three overseas scientists visited the Centre for training in detection of influenza by immunofluorescence from 21 – 25 August. Clockwise from above; Mr March Kloulubak from Palau (left), Ms Claire Baradi from Guam and Mr Urai Rabuatoka from Fiji



Dr Takeshi Kasai, Regional Adviser Communicable Disease Surveillance and Response and Dr Koji Nabae, Avian and Human Influenza Regional Coordinating Officer, both from WHO WPRO visited the Centre on 25 September to get a better understanding of the Centre's operations and functions and to discuss future opportunities.



Samira H Al Mahruqy visited the Centre from 27 November – 15 December for training in Avian Influenza Molecular Diagnosis



A/Prof Kevin Downard from University of Sydney visited the centre for an overview on serology and molecular strain analysis techniques from 30 October – 1 November.



*A visit to Taiwan by Ian Gust.*

## 6. PUBLICATIONS AND MEETINGS

### WHO Sponsored Meetings:

Professor Gust participated in:

- The WHO Northern Hemisphere consultation on the composition of influenza vaccines, 13 – 15 February 2006
- WHO consultation for the development of a global action plan for increasing pandemic vaccine supply, 2 - 3 May 2006.
- WHO meeting on clinical trials of influenza pandemic vaccines, 4 - 5 May 2006.
- The WHO Southern Hemisphere consultation on the composition of influenza vaccines, Geneva 18 – 20 September 2006

Dr Ian Barr participated in:

- The WHO Northern Hemisphere consultation on the composition of influenza vaccines, 13 – 15 February 2006
- A WHO Influenza Workshop, Hong Kong, 17 – 24 February 2006
- A WPRO meeting to promote influenza surveillance in Siem Reap, Cambodia 14 - 15 August 2006
- The WHO Southern Hemisphere consultation on the composition of influenza vaccines, Geneva 18 – 20 September 2006
- A WHO Avian influenza meeting, Beijing 4 – 8 December 2006

### Papers and Articles Published:

Ayob AE, Selviendran N, **Hampson AW, Barr, IG**, Selvaraja AL, Chua KB. Outbreak of Influenza amongst Residential School Students in Malaysia. *Med. J. Malaysia*. 2006 61: 168-72.

**Barr IG**, McCaig M, **Durrant C, Shaw R**. The rapid identification of influenza neuraminidase N1 and N2 subtypes by ELISA. *Vaccine*. 2006, 24:6675-8.

**Barr I.G, Komadina N, Durrant C, Sjogren H, Hurt, A, Shaw R.P**. Circulation and antigenic drift in human influenza B viruses in SE Asia and Oceania since 2000. *Comm. Dis. Intell.* 2006, 30:350-7.

Cook IF, **Barr IG**, Hartel G, Pond D, **Hampson AW**. Reactogenicity and immunogenicity of an inactivated influenza vaccine administered by intramuscular or subcutaneous injection in elderly adults. *Vaccine* 2006 24(13):2395-402.

Dwyer DE, Smith D, Catton M, **Barr I**. Laboratory diagnosis of human seasonal and pandemic influenza virus infection. *Med. J. Aust.* 2006; 185, S48-S53.

Firestone S, Roche P, **Barr IG**, Walker JC. Annual report of the National Influenza Surveillance Scheme, 2005. *Comm. Dis. Intell.* 2006;30:189-200.

Haaheim LR, **Tomasov CC, Barr IG**, Hampson AW, **Komadina N**. Identification of genetic diversity by cultivating influenza A(H3N2) virus in vitro in the presence of post-infection sera from small children. *Vaccine*. 2006, 10;24:6708-11.

**Hurt AC, Iannello P**, Jachno, **Komadina N, Hampson AW, Barr IG**, McKimm-Breschkin JL. Naturally occurring neuraminidase inhibitor resistant and sensitive influenza B viruses in a human clinical sample. *Antiviral Research*. 2006 50(5):1872-4.

**Hurt AC**, Hansbro PM, Selleck P, Olsen B, Minton C, Hampson AW, **Barr IG**. Isolation of avian influenza viruses from two different transhemispheric migratory shorebird species in Australia. *Arch. Virol.* 2006 151:2301-9.

**Hurt A, Barr I**. Rapid diagnostic test kits for influenza. *Microbiology Australia*. May 1 2006.

Ng LFP, **Barr I**, Nguyen T, Noor SM, Tan RSP, Agathe LV, Gupta S, Khalil H, To TL, Hassan, Ren EC. Specific detection of H5N1 avian influenza A virus in field specimens by a one-step RT-PCR assay. *BMC Infect Diseases* 2006, 6:40.

Stephenson I, **Gust I**, Kieny MP, Pervikov Y. Development and evaluation of influenza pandemic vaccines. *Lancet Infect Dis.* 2006 6(2):71-2.

Stephenson I, **Gust I**, Pervikov Y, Kieny MP. Development of vaccines against influenza H5. *Lancet Infect Dis.* 2006 6(8):458-60.

## Oral Presentations:

### International Meetings:

**Aeron Hurt**, Isolation of avian influenza viruses from two different transhemispheric migratory shorebird species in Australia. Presented at Steamboat Springs Influenza Conference, Colorado, USA, April.

**Aeron Hurt**, Research activities down South (Southern Hemisphere). Presented at the St Jude's Children's Hospital, Memphis, USA, 3 April.

**Aeron Hurt**, Influenza virus susceptibility to the NA inhibitors, Melbourne, WHO Centre. Presented at the NISN meeting, Geneva, 23 October.

**Aeron Hurt**, Influenza activities down under. Presented at the Erasmus Medical Centre, Rotterdam, Netherlands, 25 October.

**Ian Barr**, What's new at the Melbourne influenza Centre. Presented at the St Jude's Childrens Hospital, Memphis, USA, 3 April.

**Ian Barr**, Influenza testing at the Melbourne WHO Influenza Centre. Presented at PPHSN Labnet meeting, Noumea, 3 August.

**Ian Barr**, Update on the influenza surveillance for 2005-season in the Western Pacific. Presented at a WHO workshop, Sihanoukville, Cambodia 14 August.

**Ian Barr**, Influenza surveillance system in Australia. Presented at a WHO workshop, Sihanoukville, Cambodia 14 August.

**Ian Barr**, Influenza surveillance and research activities at WHO Collaborating Centre, Melbourne, Australia. Presented at the Shanghai Public Health Centre, Shanghai, 31 October.

**Ian Gust**, Australia's Surveillance System for Influenza. Presented at Academia Sinca, Taipei, 25 September.

**Ian Gust**, Influenza Strain Selection. Presented at CDC, Taipei, 26 September.

### Local Meetings:

**Aeron Hurt**, From Birds to Bedside. Presented at Avian Influenza Wild Bird Surveillance Workshop, Adelaide 7 September.

**Ian Barr**, Influenza update 2005-2006. Presented at the Influenza Specialist Group (ISG) meeting, Melbourne 30 Jan.

**Ian Barr**, Human epidemic influenza, pandemics and the current risk from "bird flu." Presented at Melbourne University, Melbourne 10 April.

**Ian Barr**, Influenza and HCW (nursing & influenza). Presented to nurses at Epworth Hospital, Melbourne 21 March.

**Ian Barr**, The evolution of H5N1 avian influenza and the pandemic risk. Presented at the Australian Veterinary Association meeting, Hobart 24 May.

**Ian Barr**, Seasonal and pandemic influenza: The human and economic consequences. Presented at the Business Continuity Planning group, Sydney 29 May.

**Ian Barr**, Bird flu: Should we be alert, alarmed or preparing for Armageddon? Presented at the GTAC VCE teachers series, Melbourne 7 June.

**Ian Barr**, UPDATE: The global spread of avian influenza and the threat of a human pandemic. Presented at AFMA, Sydney 21 June.

**Ian Barr**, The evolution of H5N1 avian influenza and the pandemic risk. Presented at ASM, Gold Coast 3 July.

**Ian Barr**, Influenza. Presented at the Virology Master Class, Adelaide University, Adelaide 21 July.

**Ian Barr**, Bird flu and pandemics. Presented to Young Presidents, Melbourne 27 July.

**Ian Barr**, Influenza: A disease worth controlling. Presented at Monash University, Clayton 1 August.

**Ian Barr**, Health care workers in influenza. Part of the problem or part of the solution? Presented to the Nurses group, VIDRL, Melbourne, 20 October.

**Ian Barr**, H5N1 and the pandemic threat.  
Presented to the Eastern Ranges GP Association,  
Melbourne 22 November.

**Ian Barr**, Influenza H5N1. Presented at Biota, 24  
November.

### Other Presentations:

### Poster Presentations:

Cook IF, **Barr IG**, Hartel G, Pond D, Hampson  
AW. Reactogenicity and immunogenicity of an  
inactivated influenza vaccine administered by  
intramuscular or subcutaneous injection in elderly  
adults. Presented at the Springs Influenza  
Conference, Colorado, USA March/April 2006

**Hurt A**, Hanbro P, Selleck P, Olsen, Minton,  
Hampson AW and Barr IG. Isolation of avian  
influenza viruses from two different  
transhemispheric migratory shorebird species in  
Australia. Presented at the Springs Influenza  
Conference, Colorado, USA March/April 2006

**Komadina N**, Tomasov C, Iannello P, Deed N,  
Hurt AC, Barr IG. Increased resistance to the  
influenza antivirals amantadine and rimantidine  
with A(H3) viruses in Australia and regionally in  
2005. Presented at the 13th Negative Strand  
Viruses 2006, 17-22 June 2006, Salamanca,  
Spain.

### Other Relevant Meetings:

**Aeron Hurt** attended the Avian Influenza Wild  
Bird Steering Group, Adelaide 6 – 7 September  
2006.

**Ian Barr and Aeron Hurt** attended the  
Steamboat Springs Influenza Conference,  
Colorado, USA March/April 2006.

**Ian Barr and Aeron Hurt** visited WHO  
Collaborating Centre for Animal Influenza in  
Memphis on 3 April 2006.

**Ian Barr** visited the WHO Collaborating Centre  
for influenza in CDC Atlanta on 5 April 2006.

**Ian Barr** attended the AVID (Australasian  
Vaccines and Immunotherapy Development)  
meeting in Melbourne, May 31 - June 1, 2006.

**Ian Barr** attended a PPHSN Labnet meeting,  
Noumea 2 – 4 August 2006.

**Ian Barr** attended a SINO Australian meeting on  
Infectious Disease Research in Shanghai, 30 – 31  
October 2006.

**Ian Gust** took part in a World Bank mission on avian  
influenza in Indonesia from 27 March to 7 April  
2006.

**Ian Gust and Ian Barr** attended a Workshop on  
Australian technical assistance for avian influenza in  
Canberra, 14 - 15 June, 2006.

**Ian Gust** attended the Influenza Consultation with  
Taiwan Academia Sinica and CDC, Taiwan 25 - 26  
Sept 2006.

**Ian Gust and Ian Barr** attended the AIVC meeting  
in Canberra, 4 October 2006.

**Ian Gust, Ian Barr, Aeron Hurt and Karen Laurie**  
attended the 2<sup>nd</sup> Influenza Symposium, Canberra 5 –  
6 October 2006.

**Karen Laurie** attended the Virology Master class,  
Adelaide 9 – 21 July 2006.



*The WHO Influenza Centre along with the TGA, held the 2<sup>nd</sup>  
Influenza Symposium, at the TGA, Canberra 5 – 6 October 2006.*

*Above: Dr Ian Barr (top) and Prof John Horvath*

## APPENDIX 1

### LABORATORIES SUPPLYING INFLUENZA SAMPLES FOR ANALYSIS & RECEIVING WHO KITS IN 2006

#### AUSTRALIA

The Victorian Infectious Disease Reference Laboratory, North Melbourne, Victoria;  
Monash Medical Centre, Clayton, Victoria\*;  
Royal Children's Hospital, Melbourne, Victoria;  
Queensland Health Scientific Services, Coopers Plains, Queensland;  
Townsville General Hospital, North Ward, Queensland;  
Institute of Medical and Veterinary Science, Adelaide, South Australia;  
Virology Division, SEALS Microbiology, Prince of Wales Hospital, Randwick, New South Wales;  
Institute for Clinical Pathology and Medical Research, Westmead Hospital, Sydney, New South Wales;  
QEII Medical Centre, PathCentre, Nedlands, Western Australia;  
Princess Margaret Hospital for Children, Subiaco, Western Australia;  
Royal Darwin Hospital, Darwin, Northern Territory\*

#### ARGENTINA

Dr. Carlos G. Malbran National Institute of Microbiology, Buenos Aires†

#### CAMBODIA

Institute Pasteur du Cambodge, Phnom Penh\*

#### CHINA

Department of Health, Macau

#### COOK ISLANDS

Rarotonga Hospital, Rarotonga

#### FIJI

National Centre for Scientific Services for Virology and Vector Borne Diseases, Suva†

#### FRANCE

WHO National Influenza Centre, Lyon

#### FRENCH POLYNESIA

Institute Malarde Clinical Laboratory, Tahiti

#### INDONESIA

Viral Diseases Program, US NAMRU 2, Jakarta

#### KENYA

KEMRI Centre for Virus Research, Nairobi

#### MALAYSIA

Institute of Medical Research, Kuala Lumpur  
University of Malaya, Kuala Lumpur  
WHO National Influenza Centre, University of Malaysia, Kuala Lumpur

#### NEW CALEDONIA

Institute Pasteur, Noumea

#### NEW ZEALAND

Institute of Environmental Science & Research (ESR), Communicable Disease Group, Porirua  
Christchurch Hospital, Christchurch  
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#### SINGAPORE

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#### SOLOMON ISLANDS

National Referral Hospital, Honiara

#### SOUTH AFRICA

National Institute for Communicable Diseases, Sandringham

#### SRI LANKA

Medical Research Institute, Colombo

#### TAIWAN, PROVINCE OF CHINA

Centre for Disease Control, Department of Health, Taipei

#### THAILAND

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\* did not receive kit      † did not supply isolates

# APPENDIX 2

## Haemagglutination-Inhibition Reactions of Influenza A(H1N1)

Compilation		Haemagglutination Inhibition Assay - WHO influenza Centre, Melbourne													
ANTISERA NO.	A	B	C	D	E	F	G	H	I	J	K	Human	Passage	Sample	
REF. Ag	NC/20	SING/14	NC/9	BRI/193	SHE/141	VIC/500	MAL/100	PHIL/673	SOL/3	HK/2562	FUK/141	2006	History	Date	
A	A/NEW CALEDONIA/20/99	640	160	80	80	320	320	320	40	80	80	320	160	E5	
B	A/SINGAPORE/14/2004	640	640	320	320	640	640	1280	320	160	160	80	320	E3	
C	A/NEW CALEDONIA/9/2004	1280	640	320	320	640	640	1280	320	160	160	320	320	E3	
D	A/BRISBANE/193/2004	1280	1280	320	640	640	640	1280	320	160	160	80	320	E3	
E	A/SHENZHEN/14/2005	1280	1280	640	1280	>2560	1280	1280	640	160	160	160	640	E6	
F	A/VICTORIA/500/2006	320	320	160	160	320	640	640	160	160	160	160	160	3	
G	A/MALAYSIA/100/2006	640	640	320	160	640	640	640	160	160	80	320	320	E4	
H	A/PHILIPPINES/673/2006	160	160	80	<20	40	40	80	320	160	640	1280	80	MDCKX,MDCK3	
I	A/SOLOMON ISLANDS/3/2006	160	320	80	40	80	160	320	640	320	640	160	160	E3	
J	A/HONGKONG/2652/2006	160	80	40	<20	<20	20	40	40	80	640	1280	40	E4	
K	A/FUKUSHIMA/14/2006	320	160	80	20	20	40	80	80	160	640	>2560	160	E5	
TEST ANTIGENS															
1	A/THAILAND/655/2006	640	320	160	80	320	320	320	80	80	160	160	160	MDCK3	13/09/06
2	A/THAILAND/657/2006	640	320	160	160	320	320	320	160	80	80	80	160	MDCK3	
3	A/THAILAND/600/2006	640	640	320	320	640	640	640	160	80	80	80	160	MDCK4	15/09/06
4	A/THAILAND/617/2006	640	640	320	640	640	1280	1280	320	160	160	160	320	MDCK3	14/09/06
5	A/PHILIPPINES/768/2006	640	640	160	160	320	320	640	1280	320	640	640	320	MDCK3	20/07/06
6	A/PHILIPPINES/681/2006	640	640	320	320	40	640	1280	320	160	320	80	160	MDCK4	10/07/06
7	A/TAIWAN/772/2006	320	160	80	80	40	80	320	20	40	80	80	80	MDCK4	17/09/06
8	A/MALAYSIA/419/2006	320	160	80	<20	40	40	80	160	160	640	>2560	160	X,MDCK1	11/10/06
9	A/THAILAND/632/2006	320	320	80	80	80	160	320	640	160	320	320	80	MDCK3	7/09/06
10	A/THAILAND/645/2006	320	160	80	40	80	80	160	320	160	320	320	80	MDCK3	7/09/06
11	A/THAILAND/654/2006	320	320	80	80	160	160	320	640	160	320	320	80	MDCK3	9/08/06
12	A/SINGAPORE/62/2006	320	320	80	<20	40	80	160	320	160	640	640	160	MDCKX,mdck1	12/07/06
13	A/PHILIPPINES/769/2006	320	320	80	20	40	80	160	640	320	1280	>2560	160	MDCK3	20/07/06
14	A/PHILIPPINES/731/2006	320	160	80	40	80	80	160	320	160	640	640	160	MDCK3	17/07/06
15	A/PHILIPPINES/746/2006	320	160	160	40	40	80	160	160	320	640	1280	160	MDCK3	18/07/06
16	A/MALAYSIA/416/2006	160	80	40	<20	20	20	40	80	320	1280	80	X,MDCK1	11/09/06	
17	A/THAILAND/664/2006	160	160	40	<20	40	80	80	160	160	320	640	80	MDCK4	12/09/06
18	A/THAILAND/651/2006	160	80	40	20	20	40	80	80	160	640	1280	80	MDCK3	11/09/06
19	A/THAILAND/652/2006	160	160	40	40	40	80	160	320	160	160	160	80	MDCK4	11/09/06
20	A/THAILAND/658/2006	160	160	40	<20	40	40	80	160	160	320	320	80	MDCK3	18/09/06
21	A/SINGAPORE/69/2006	160	160	40	<20	20	40	80	160	160	640	1280	160	MDCK2	17/07/06
22	A/SINGAPORE/61/2006	160	80	40	<20	20	40	40	80	160	320	1280	160	MDCK2	5/07/06
23	A/SINGAPORE/72/2006	160	160	80	<20	40	80	160	320	160	320	640	160	MDCKX,MDCK1	21/08/06
24	A/SINGAPORE/73/2006	160	160	80	20	40	80	80	160	160	320	640	160	MDCKX,MDCK1	23/08/06
25	A/SYDNEY/72/2006	160	160	80	80	80	160	160	320	160	320	160	80	MDCK1	20/08/06
26	A/PHILIPPINES/659/2006	160	80	80	20	20	40	40	80	160	320	>2560	160	MDCK3	6/07/06
27	A/PHILIPPINES/660/2006	160	80	80	20	20	40	80	80	80	320	1280	80	MDCK3	6/07/06
28	A/HONGKONG/2652/2006	80	80	40	<20	<20	20	40	80	80	640	640	40	MDCKX,MDCK1	
29	A/MALAYSIA/459/2006	80	80	40	<20	20	80	80	80	80	320	80	80	X,MDCK1	
30	A/SINGAPORE/70/2006	80	80	40	<20	20	40	40	80	80	320	1280	160	MDCK2	25/07/06

Compilation: 3 & 6 Nov, 4 & 5 Dec 06		Haemagglutination Inhibition Assay - WHO influenza Centre, Melbourne													
HA1 Sequenced	A	B	C	D	E	F	G	H	I	Human	Passage	Sample			
ANTISERA NO.	F861	F862	F830	F837	F869	F895	F894	F892	F914	Pool	History	Date			
REF. Ag	NC/20	SING/14	NC/9	BRI/193	SHE/141	VIC/500	MAL/100	PHIL/673	SOL/3	2006	History	Date			
A	A/NEW CALEDONIA/20/99	640	320	160	80	320	320	160	20	80	160	E6			
B	A/SINGAPORE/14/2004	640	640	320	640	1280	1280	1280	320	160	320	E4			
C	A/NEW CALEDONIA/9/2004	1280	640	320	320	640	640	640	160	160	160	E3			
D	A/BRISBANE/193/2004	640	640	320	320	640	640	640	160	80	160	E3			
E	A/SHENZHEN/14/2005	1280	1280	640	1280	1280	1280	>2560	640	320	320	E6			
F	A/VICTORIA/500/2006	320	640	320	320	640	640	640	320	160	320	E4			
G	A/MALAYSIA/100/2006	640	640	320	320	640	640	640	160	80	160	E4			
H	A/PHILIPPINES/673/2006	80	80	40	<20	<20	20	20	320	160	40	MDCKX,MDCK3			
I	A/SOLOMON ISLANDS/3/2006	80	160	80	40	80	160	320	640	320	160	E6			
TEST ANTIGENS															
1	A/CHRISTCHURCH/63/2006	1280	640	320	640	1280	1280	1280	320	160	320	MDCKX,MDCK1	3/09/2006		
2	A/OTAGO/26/2006	640	640	320	640	1280	1280	1280	320	160	320	MDCKX,MDCK1	21/09/2006		
3	A/OTAGO/23/2006	640	640	160	320	640	640	640	160	80	160	MDCKX,MDCK1	5/09/2006		
4	A/OTAGO/24/2006	640	320	160	320	640	320	320	160	80	160	MDCKX,MDCK1	3/09/2006		
5	A/CHRISTCHURCH/78/2006	640	640	320	640	1280	640	640	320	160	320	MDCKX,MDCK1	28/09/2006		
6	A/SRI LANKA/11/2006	320	1280	640	640	1280	1280	1280	320	160	320	MDCK1			
7	A/OTAGO/20/2006	320	1280	320	640	1280	1280	1280	320	160	320	MDCKX,MDCK1	30/08/2006		
8	A/OTAGO/21/2006	320	640	320	320	640	640	640	160	80	160	MDCKX,MDCK1	30/08/2006		
9	A/WELLINGTON/84/2006	320	1280	640	1280	1280	1280	1280	640	160	320	MDCKX,MDCK1	11/09/2006		
10	A/CHRISTCHURCH/66/2006	320	320	160	320	640	320	320	160	80	160	MDCKX,MDCK1	13/09/2006		
11	A/CHRISTCHURCH/100/2006	160	320	160	320	320	320	320	160	80	320	MDCKX,MDCK1	30/09/2006		
12	A/BRISBANE/213/2006	160	640	320	320	640	640	640	320	80	160	MDCKX,MDCK1	30/08/2006		
13	A/MALAYSIA/366/2006	160	320	160	160	320	320	640	80	80	80	X,MDCK1			
14	A/SRI LANKA/16/2006	160	640	320	320	640	1280	640	320	160	320	X,MDCK1			
15	A/SRI LANKA/17/2006	160	640	320	320	1280	1280	1280	320	160	320	X,MDCK1			
16	A/WELLINGTON/81/2006	160	640	320	640	1280	1280	1280	320	160	320	MDCKX,MDCK1	1/09/2006		
17	A/WELLINGTON/82/2006	160	640	320	640	640	640	1280	320	160	320	MDCKX,MDCK1	5/09/2006		
18	A/OTAGO/25/2006	160	640	320	320	640	640	640	320	160	320	MDCKX,MDCK1	18/09/2006		
19	A/MALAYSIA/369/2006	80	320	160	80	160	320	320	1280	320	160	X,MDCK1			
20	A/MALAYSIA/392/2006	80	40	40	<20	20	20	40	80	160	80	X,MDCK1	20/09/2006		
21	A/CHRISTCHURCH/65/2006	80	160	80	80	160	160	160	20	40	80	MDCKX,MDCK1	12/09/2006		
22	A/MALAYSIA/365/2006	40	20	20	<20	<20	20	20	80	160	80	X,MDCK1			
23	A/MALAYSIA/362/2006	40	20	20	<20	<20	20	20	40	80	40	X,MDCK1	8/08/2006		
24	A/CHRISTCHURCH/49/2006	40	40	40	20	<20	40	40	80	40	40	MDCKX,MDCK1	29/07/2006		
25	A/MALAYSIA/356/2006	20	40	40	40	20	80	40	80	80	40	X,MDCK1	27/07/2006		

## Haemagglutination-Inhibition Reactions of Influenza A(H3N2) Isolates

Compilation: 29/8, 23/8		Haemagglutination Inhibition Assay - WHO influenza Centre, Melbourne												Human	Passage	Sample
HA1 Sequenced	A	B	C	D	E	F	G	H	I	J	Human	Passage	Sample			
ANTISERA NO.	F780	F812	F821	F831	F833	F838	F848	F851	F855	F864	Pool	Passage	Sample			
REF. Ag	FUJ/411	WEL/1	SIN/37	VIC/523	CAL/7	NY/55	BRIS/3	VIC/512	MAL/753	WIS/67	2006	History	Date			
A	A/FUJIAN/411/2002	1280	640	160	160	320	640	80	320	320	160	640	C2,MDCK8			
B	A/WELLINGTON/1/2004	160	1280	640	320	640	320	160	320	640	320	640	E5			
C	A/SINGAPORE/37/2004	320	640	1280	640	640	1280	640	640	1280	320	640	E4			
D	A/VICTORIA/523/2004	160	320	1280	640	320	1280	320	640	1280	160	640	E7			
E	A/CALIFORNIA/7/2004	320	640	320	320	1280	640	160	640	640	320	640	SPFCK3E.E5			
F	A/NEWYORK/55/2004	160	640	640	640	1280	640	320	640	640	160	640	SPFCK3E.E5			
G	A/BRISBANE/3/2005	320	1280	2560	2560	2560	>5120	1280	2560	2560	320	640	E4			
H	A/VICTORIA/512/2005	320	640	640	320	640	640	160	640	1280	320	640	E5			
I	A/MALAYSIA/753/2005	40	80	80	80	160	80	80	80	80	40	80	MDCKX,MDCK4			
J	A/WISCONSIN/67/2005	80	320	320	320	640	320	80	320	320	640	320	E4			
<b>TEST ANTIGENS</b>																
1	A/BRISBANE/49/2006	1280	1280	2560	640	2560	640	160	1280	1280	2560	1280	MDCKX, MDCK1	1/08/2006		
2	A/VICTORIA/170/2006	160	640	2560	320	1280	320	160	640	640	640	640	MDCK1	3/08/2006		
3	A/VICTORIA/156/2006	80	320	320	160	640	320	160	320	320	640	320	MDCK2	21/07/2006		
4	A/SYDNEY/13/2006	160	320	320	160	640	160	80	320	320	640	320	MDCKX,MDCK1			
5	A/SYDNEY/15/2006	80	320	160	160	640	160	80	160	320	640	320	MDCKX,MDCK1			
6	A/SYDNEY/16/2006	80	320	320	160	640	160	160	320	320	640	640	MDCKX,MDCK1			
7	A/VICTORIA/149/2006	40	160	160	80	320	160	80	80	160	160	320	MDCK2	18/07/2006		
8	A/VICTORIA/153/2006	40	80	80	80	160	80	40	80	160	160	160	MDCK2	20/07/2006		
9	A/SINGAPORE/57/2006	80	160	640	80	320	80	80	160	160	160	160	MDCK2	6/07/2006		
10	A/SINGAPORE/57/2006	80	160	640	80	320	80	80	160	160	160	160	MDCK2	6/07/2006		
11	A/SOUTH AUSTRALIA/3/2006	40	160	320	80	160	80	<40	160	160	160	160	mdckx,MDCK1	3/07/2006		
12	A/JOHANNESBURG/177/2006	40	80	320	80	160	80	80	80	160	80	80	mdckx,mdck2	1/05/2006		
13	A/JOHANNESBURG/429/2006	40	80	80	40	160	80	80	80	80	80	80	mdckx,mdck2	5/06/2006		
14	A/VICTORIA/148/2006	40	80	80	40	160	80	40	80	80	80	80	MDCK2	18/07/2006		
15	A/SYDNEY/17/2006	80	80	80	80	160	80	80	80	80	80	80	MDCKX,MDCK1			
16	A/BRISBANE/46/2006	80	160	40	80	160	80	80	80	80	40	80	mdckx,mdck1	22/07/2006		
17	A/PERTH/16/2006	40	80	<40	40	160	40	80	40	80	40	80	MDCKX,MDCK2	24/07/2006		
18	A/JOHANNESBURG/501/2006	40	40	160	40	160	80	40	40	80	40	80	mdckx,mdck3	29/06/2006		
19	A/THAILAND/86/2006	80	160	320	80	160	160	160	80	80	40	80	MDCK3	10/04/2006		
20	A/AUCKLAND/47/2006	<40	40	160	40	80	40	40	40	80	40	40	mdckx,MDCK1	9/07/2006		
21	A/SOUTH AUSTRALIA/4/2006	40	80	<40	40	160	80	40	40	80	<40	40	MDCKX,MDCK2	19/07/2006		
22	A/AUCKLAND/61/2006	40	80	40	40	160	80	40	40	80	<40	80	MDCKX,MDCK1	13/07/2006		
23	A/AUCKLAND/75/2006	40	40	<40	40	160	40	40	40	80	<40	80	MDCKX,MDCK1	13/07/2006		
24	A/JOHANNESBURG/510/2006	40	40	<40	40	80	40	40	40	40	<40	40	mdckx,mdck2	11/07/2006		
25	A/SYDNEY/18/2006	40	40	<40	40	40	40	40	40	40	<40	20	MDCKX,MDCK1			
26	A/CHRISTCHURCH/20/2006	<40	40	<40	<40	80	40	40	<40	40	<40	20	MDCKX,MDCK2	7/07/2006		
27	A/SINGAPORE/29/2006	<40	<40	320	<40	40	40	<40	<40	40	<40	20	MDCK3	16/06/2006		

Compilation		Haemagglutination Inhibition Assay - WHO influenza Centre, Melbourne												Human	Passage	Sample
HA1 Sequenced	A	B	C	D	E	F	G	H	I	J	K	Human	Passage	Sample		
ANTISERA NO.	F834	F858	F833	F838	F851	F866	F864	F896	F897	NIID	NIID	CDC	Pool	Passage	Sample	
REF. Ag	WELL/1	VIC523	CAL/7	NY/55	VIC512	MAL/753	WIS/67	VIC503	BRIS/9	Hirosh/33	Send/131	San/7981	2006	History	Date	
A	A/WELLINGTON/1/2004	1280	320	1280	320	640	640	80	320	320	320	640	640	640	E5	
B	A/VICTORIA/523/2004	320	1280	640	640	640	1280	320	80	160	320	160	160	320	E4	
C	A/CALIFORNIA/7/2004	320	320	640	320	640	320	320	160	160	320	320	320	320	SPFCK3.E5	
D	A/NEWYORK/55/2004	640	1280	1280	1280	1280	640	320	320	1280	640	1280	640	640	SPFCK3.E5	
E	A/VICTORIA/512/2005	320	320	320	320	640	640	320	80	320	320	320	320	320	E4	
F	A/MALAYSIA/753/2005	160	80	320	160	160	320	160	320	640	320	320	160	160	MDCKX,MDCK4	
G	A/WISCONSIN/67/2005	160	80	640	320	320	320	640	640	640	640	640	320	320	SPFCK.E5	
H	A/VICTORIA/503/2006	160	40	320	80	160	160	320	320	320	320	320	160	160	E4	
I	A/BRISBANE/9/2006	80	<40	160	80	160	160	320	320	320	160	320	80	80	E3	
J	A/HIROSHIMA/33/2006	160	40	320	160	320	160	320	640	320	640	160	1280	160	E6	18/12/2006
K	A/SENDI-H/F13/1/2006	320	80	320	320	640	320	1280	1280	1280	1280	1280	1280	640	E4	18/12/2006
L	A/SANTIAGO/7981/2006	320	80	320	160	320	320	320	640	320	640	320	1280	1280	E3/E2	12/12/2006
<b>TEST ANTIGENS</b>																
1	A/MARSEILLE/1215/2006	160	40	160	80	160	80	320	160	320	320	80	160	160	MDCK, MDCK1	22/11/2006
2	A/LYON/1292/2006	320	80	320	160	320	320	320	320	640	160	640	160	160	MDCK, MDCK1	18/12/2006
3	A/LYON/1313/2006	80	40	160	80	160	160	320	160	320	320	320	80	80	MDCK, MDCK1	20/12/2006
4	A/THAILAND/763/2006	80	40	160	160	160	160	160	640	320	320	320	80	80	MDCK3	8/11/2006
5	A/THAILAND/797/2006	40	40	160	80	80	40	160	160	160	80	160	40	40	MDCK2	28/11/2006
6	A/TOULON/1244/2006	40	40	80	80	80	80	160	160	320	160	80	160	80	MDCK, MDCK1	6/12/2006
8	A/LYON/1312/2006	80	40	320	80	160	160	160	320	320	160	320	80	80	MDCK, MDCK1	20/12/2006
11	A/THAILAND/770/2006	40	<40	80	80	80	80	80	80	160	160	80	160	80	MDCK2	9/11/2006
12	A/THAILAND/785/2006	40	<40	160	80	80	80	80	40	320	160	80	160	80	MDCK3	13/11/2006
13	A/THAILAND/775/2006	80	<40	160	80	80	80	80	80	320	160	80	160	40	MDCK2	15/11/2006
14	A/BORDEAUX/1276/2006	40	<40	160	40	40	40	80	<40	160	160	80	160	80	MDCK, MDCK1	30/11/2006
15	A/LYON/1324/2006	80	<40	160	80	80	40	80	80	160	160	80	160	80	MDCK, MDCK1	20/12/2006
16	A/LYON/1305/2006	40	40	160	160	80	80	80	80	160	160	80	160	80	MDCK, MDCK1	19/12/2006
17	A/LYON/1331/2006	40	<40	80	40	40	40	80	40	160	160	80	160	40	MDCK, MDCK1	22/12/2006
18	A/LYON/1359/2006	40	<40	80	40	40	40	80	40	160	160	160	320	40	MDCK, MDCK1	27/12/2006
19	A/THAILAND/805/2006	40	<40	80	40	40	<40	80	40	160	80	80	160	40	MDCK2	28/11/2006
20	A/THAILAND/784/2006	40	40	80	80	40	80	40	40	320	80	80	160	80	MDCK3	10/11/2006
21	A/THAILAND/809/2006	<40	<40	80	80	40	40	40	40	160	80	40	80	40	MDCK2	28/11/2006
22	A/LYON/CHU/52.58/2006	40	40	160	80	40	80	40	80	160	80	80	160	80	MDCK, MDCK1	25/12/2006
23	A/LYON/1288/2006	40	40	80	40	40	40	40	40	160	80	80	160	40	MDCK, MDCK1	16/12/2006
24	A/LYON/1308/2006	<40	<40	40	40	<40	<40	<40	80	40	40	80	20	MDCK, MDCK1	16/12/2006	
25	A/LYON/1320/2006	<40	<40	80	40	<40	40	<40	<40	160	80	80	40	40	MDCK, MDCK1	21/12/2006

## Haemagglutination-Inhibition Reactions of Influenza B Isolates

Compilation: 10 & 16 August		Haemagglutination Inhibition Assay - WHO influenza Centre, Melbourne										
HA1 Sequenced		A	B	C	D	E	F	G	H	I		
ANTISERA NO.		F778	F836	F865	F856	F884	F806	F866	F841	F843	Passage	Sample
REF. Ag											History	Date
A	B/BRISBANE/32/2002	320	320	640	640	320	<20	<20	<20	<20	E2	
B	B/HAWAII/33/2004	320	640	1280	640	640	<20	<20	<20	<20	E4	
C	B/MALAYSIA/2506/2004	320	160	640	320	320	<20	<20	<20	<20	E4	
D	B/OHIO/1/2005	160	320	640	320	160	<20	<20	<20	<20	E4	
E	B/MALAYSIA/174/2006	40	20	40	80	640	<20	<20	<20	<20	MDCK2	
F	B/SHANGHAI/361/2002	<20	40	80	<20	640	20	640	320	20	E7	
G	B/JIANGSU/10/2003	<20	<20	<20	<20	<20	320	1280	80	160	E6	
H	B/VICTORIA/502/2004	<20	20	40	<20	<20	640	<20	640	320	E7	
I	B/FLORIDA/7/2004	<20	20	40	<20	<20	640	<20	640	320	E5	
<b>TEST ANTIGENS</b>												
1	B/SOUTH AUSTRALIA/17/2006	160	320	1280	640	320	<20	<20	<20	<20	mdckx.MDCK1	18/07/06
2	B/JOHANNESBURG/449/2006	160	320	1280	320	320	<20	<20	<20	<20	mckx.mdck1	12/06/06
3	B/SOUTH AUSTRALIA/22/2006	160	320	640	320	320	<20	<20	<20	<20	mdckx.MDCK1	18/07/06
6	B/SYDNEY/10/2006	320	320	320	320	320	<20	<20	<20	<20	MDCK2	18/07/06
7	B/SYDNEY/12/2006	320	320	320	320	160	<20	<20	<20	<20	MDCK2	24/07/06
8	B/VICTORIA/304/2006	80	160	320	320	320	<20	<20	<20	<20	E2	
9	B/SYDNEY/6/2006	40	40	160	80	320	<20	<20	<20	<20	MDCKX.MDCK1	
10	B/SYDNEY/1/2006	40	40	160	80	320	<20	<20	<20	<20	MDCKX.MDCK1	
11	B/SYDNEY/14/2006	160	160	160	160	640	<20	<20	<20	<20	MDCK2	24/07/06
12	B/PHILIPPINES/519/2006	40	40	160	80	320	<20	<20	<20	<20	MDCK3	15/06/06
13	B/BRISBANE/11/2006	20	40	80	80	640	<20	<20	<20	<20	MDCKX.MDCK1	25/07/06
14	B/PHILIPPINES/1412/2006	<20	20	80	40	640	<20	<20	<20	<20	MDCK3	3/07/06
15	B/PHILIPPINES/1506/2006	<20	20	80	40	320	<20	<20	<20	<20	MDCK3	5/07/06
16	B/SINGAPORE/10/2006	<20	20	80	40	320	<20	<20	<20	<20	MDCK	25/02/06
17	B/JOHANNESBURG/480/2006	20	20	40	40	40	<20	<20	<20	<20	mdckx.mdck1	29/06/06
18	B/JOHANNESBURG/517/2006	40	40	40	40	40	<20	<20	<20	<20	mdckx.mdck1	5/07/06
19	B/WELLINGTON/1/2006	20	20	40	40	40	<20	<20	<20	<20	mdckx.mdck1	13/06/06
21	B/SOUTH AUSTRALIA/20/2006	20	40	40	80	320	<20	<20	<20	<20	mdckx.MDCK1	16/07/06
22	B/SOUTH AUSTRALIA/21/2006	<20	20	40	40	640	<20	<20	<20	<20	mdckx.MDCK1	19/07/06
23	B/PERTH/12/2006	<20	20	40	40	640	<20	<20	<20	<20	mdckx.MDCK1	9/07/06
24	B/PERTH/14/2006	40	<20	<20	40	640	<20	<20	<20	<20	mdckx.MDCK1	14/07/06
25	B/BRISBANE/8/2006	20	20	20	40	40	<20	<20	<20	<20	mdckx.mdck1	4/07/06
26	B/JOHANNESBURG/508/2006	20	20	20	40	40	<20	<20	<20	<20	mdckx.mdck1	11/07/06
27	B/CHRISTCHURCH/4/2006	20	20	20	40	40	<20	<20	<20	<20	MDCKX.MDCK1	8/03/06
28	B/JOHANNESBURG/425/2006	80	20	20	40	40	<20	<20	<20	<20	mckx.mdck1	7/06/06
29	B/SOUTH AUSTRALIA/7/2006	20	20	20	40	40	<20	<20	<20	<20	mdckx.MDCK1	5/07/06
30	B/JOHANNESBURG/439/06	<20	<20	<20	<20	<20	160	20	80	160	mdckx.mdck1	18/06/06
31	B/WAIKATO/1/2006	<20	<20	<20	<20	<20	160	20	160	160	mdckx.mdck1	24/05/06
32	B/SYDNEY/3/2006	<20	<20	<20	<20	<20	160	20	80	320	MDCKX.MDCK1	
33	B/PHILIPPINES/521/2006	<20	<20	<20	<20	<20	160	20	80	320	MDCK3	15/06/06
34	B/THAILAND/290/2006	<20	<20	<20	<20	<20	160	20	40	320	MDCK2	31/01/06
35	B/SINGAPORE/15/2006	<20	<20	<20	<20	<20	160	40	80	160	MDCKX.MDCK1	25/03/06

Compilation: 20 & 22 Nov, 19 Dec		Haemagglutination Inhibition Assay - WHO influenza Centre, Melbourne										
HA1 Sequenced		A	B	C	D	E	F	G	H	I	Human	
ANTISERA NO.		F865	F856	F884/	F898	F899	F806	F866	F841	F843	Sera	Passage
REF. Ag		MAL/2506	OHIO/1	MAL/174	NC/5	VIC/304	SHAN/361	JIAN/10	VIC/502	FLOR/7	Pool	History
												Date
A	B/MALAYSIA/2506/2004	640	1280	640	1280	1280	<20	<20	<20	<20	80	E4
B	B/OHIO/1/2005	640	1280	640	640	1280	<20	<20	<20	<20	40	E5
C	B/MALAYSIA/174/2006	160	320	320	320	1280	<20	<20	<20	<20	40	X.MDCK3
D	B/NEW CALEDONIA/5/2006	160	640	160	640	640	<20	<20	<20	<20	40	E5
E	B/VICTORIA/304/2006	320	640	320	640	1280	<20	<20	<20	<20	40	E3
F	B/SHANGHAI/361/2002	<20	20	<20	40	40	1280	40	1280	320	40	E9
G	B/JIANGSU/10/2003	<20	<20	<20	<20	<20	160	640	40	80	20	E7
H	B/VICTORIA/502/2004	<20	20	<20	40	40	640	20	640	320	20	E6
I	B/FLORIDA/7/2004	<20	<20	<20	20	20	640	20	640	320	20	E5
<b>TEST ANTIGENS</b>												
1	B/BRISBANE/89/2006	320	640	320	640	1280	<20	<20	<20	<20		X.MDCK1
2	B/PERTH/248/2006	320	320	320	640	1280	<20	<20	<20	<20		mdck1
3	B/SYDNEY/71/2006	160	320	320	320	640	<20	<20	<20	<20	40	MDCK1
4	B/SYDNEY/79/2006	160	320	160	640	320	<20	<20	<20	<20	20	MDCK1
5	B/BRISBANE/80/2006	160	160	640	320	1280	<20	<20	<20	<20		MDCKX.MDCK1
6	A/PERTH/41/2006	160	160	640	160	1280	<20	<20	<20	<20		MDCKX.MDCK1
7	A/WELLINGTON/85/2006	80	80	320	160	640	<20	<20	<20	<20	20	MDCKX.MDCK1
8	B/CHRISTCHURCH/75/2006	80	80	640	80	640	<20	<20	<20	<20	20	MDCKX.MDCK1
9	B/TAWAN/85/2006	80	80	320	80	640	<20	<20	<20	<20	20	MDCK3
10	B/TAWAN/115/2006	80	80	640	80	640	<20	<20	<20	<20	20	MDCK3
11	B/TAWAN/103/2006	80	80	640	80	640	<20	<20	<20	<20	20	MDCK3
12	B/TAWAN/120/2006	80	80	320	80	640	<20	<20	<20	<20	20	MDCK3
13	B/TAWAN/106/2006	80	80	640	160	640	<20	<20	<20	<20	20	MDCK3
14	B/SYDNEY/74/2006	80	80	640	160	640	<20	<20	<20	<20	20	MDCK1
15	B/SYDNEY/88/2006	80	160	320	160	640	<20	<20	<20	<20	20	MDCK1
16	B/BRISBANE/87/2006	80	80	640	80	640	<20	<20	<20	<20		X.MDCK1
17	B/BRISBANE/90/2006	80	160	640	160	640	<20	<20	<20	<20		X.MDCK1
18	B/SYDNEY/83/2006	40	80	160	80	320	<20	<20	<20	<20	20	MDCK1
19	B/BRISBANE/88/2006	40	80	320	80	640	<20	<20	<20	<20		X.MDCK1
20	B/PERTH/203/2006	40	40	640	80	640	<20	<20	<20	<20		mdck1
21	B/PERTH/217/2006	40	40	640	80	640	<20	<20	<20	<20		mdck1
22	B/PERTH/263/2006	40	40	640	80	1280	<20	<20	<20	<20		mdck1
23	B/SRI LANKA/4/2006	<20	<20	<20	<20	<20	160	40	80	160		MDCK1
24	B/SYDNEY/511/2006	<20	<20	<20	<20	<20	160	20	80	320	20	X.MDCK2
25	B/TAWAN/79/2006	<20	<20	<20	<20	<20	160	20	80	320	20	MDCK3

## APPENDIX 3

### NHMRC avian induced pandemic Influenza A urgent grants in which the Centre participated

Project	NO	Title	Investigators	\$ Grant
1	401314	Surveillance and analysis of avian influenza viruses in migratory birds in Australia	<b>Chief:</b> Dr Philip Hansbro, Dr Ian Barr, Dr Aeron Hurt, Dr Irene Peroulis, Dr Simone Warner, Dr John Curran, Dr Paul Selleck, Dr Peter Wark, Prof Peter Gibson, Prof Paul Foster	\$248,854
2	365240	Mucosal vaccine for influenza based on inactivated virus and mannan	<b>Chief:</b> Prof Geoffrey Pietersz, Dr Ian Barr, Prof Mark Hogarth, Dr David Anderson	\$131,600
3	382917	Development of National Protocols for the Detection of Influenza A H5N1	<b>Chief:</b> Dr Mike Catton, Dr Chris Birch, Dr Ian Barr, Dr Paul Selleck, Dr David Smith, Dr Dominic Dwyer <b>Assoc:</b> Prof John Mackenzie, Dr Peter Kirkland	\$248,230
4	404189	Spatial Simulation Modelling of Containment Strategies for Pandemic Influenza	<b>Chief:</b> Prof George Milne, Dr Ian Barr, Assoc Prof Heath Kelly <b>Assoc:</b> Mr Alan Hampson, Dr Joel Kelso, Dr Jodie McVernon, Prof John Matthews, Dr David Smith	\$99,534
5	400595	Assessment of development of resistance to neuraminidase inhibitors in A(H5N1) influenza viruses using a ferret model	<b>Chief:</b> Mr Aeron Hurt, Dr Ian Barr, Dr Paul Selleck, Dr Deb Middleton <b>Assoc:</b> Mr Alan Hampson, Dr Michael Johnson	\$163,940
6	400584	Assessment of alpha-galactosylceramide as a novel adjuvant for pandemic influenza A virus vaccines	<b>Chief:</b> Dr Stephen Turner, Dr Dale Godfrey, Dr Ian Barr, Dr Aeron Hurt <b>Assoc:</b> Prof Peter Doherty, Dr Richard Webby	\$218,010
7	400583	Establishing the capacity for H5N1 challenge of ferrets within Australia & optimising pandemic vaccines in this model	<b>Chief:</b> A/Prof Lorena Brown, Dr Deborah Middleton, Mr Paul Selleck, Mr David Ryan, Dr Ian Barr, Prof Peter Doherty, A/Prof David Jackson. <b>Assoc:</b> Ms Suzanne Lowther, Dr John Bingham, Dr Stephen Turner, Dr Steven Rockman, Mr Alan Hampson	\$400,000
8	381735	Rapid, point-of-care diagnostic tests to differentiate HA subtypes in patient samples	<b>Chief:</b> A/Prof David A Anderson, Ms Mary L Garcia, Dr Ian Barr <b>Assoc:</b> Dr Fan Li, Dr Aeron Hurt	\$167,900
9	381734	Chimeric virus-like particles (VLPs) displaying H1, H3 and H5 haemagglutinins – construction and immunogenicity	<b>Chief:</b> A/Prof David A Anderson, Dr Elizabeth VL Grgacic, A/Prof Rosemary A French, Dr Ian Barr <b>Assoc:</b> Prof Mark Hogarth, Prof Geoff Pietersz, A/Prof Lorena Brown, Dr Fan Li, Dr Aeron Hurt	\$207,150
10	401178	Avian influenza – improvement of serological & molecular diagnostics using quality assurance	<b>Chief:</b> William David Rawlinson	\$248,626
11	400589	Are routine healthcare worker hand hygiene protocols (soap/water, alcohol based hand rubs) effective against influenza	<b>Chief:</b> Lindsay Grayson	\$99,950