

## Annual report

# INFLUENZA VIRUSES RECEIVED AND TESTED BY THE MELBOURNE WHO COLLABORATING CENTRE FOR REFERENCE AND RESEARCH ON INFLUENZA ANNUAL REPORT, 2015

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## Abstract

As part of its role in the World Health Organization's (WHO) Global Influenza Surveillance and Response System, the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne received a total of 5,557 influenza positive samples during 2015. Viruses were analysed for their antigenic, genetic and antiviral susceptibility properties. In 2015, influenza B viruses predominated over influenza A(H1)pdm09 and A(H3) viruses, accounting for a total of 58% of all viruses analysed. The vast majority of A(H1)pdm09, A(H3) and influenza B viruses analysed at the Centre were found to be antigenically similar to the respective WHO recommended vaccine strains for the Southern Hemisphere in 2015. However, phylogenetic analysis of a selection of viruses indicated that the majority of circulating A(H3) viruses were genetically distinct from the WHO recommended strain for 2015, resulting in an update to the recommended vaccine strain for the Southern Hemisphere for 2016. With an increasing predominance of B/Victoria lineage viruses over B/Yamagata lineage viruses through the course of 2015, WHO also updated the recommended influenza B strain in the trivalent influenza vaccine for 2016. Of more than 3,300 samples tested for resistance to the neuraminidase inhibitors oseltamivir and zanamivir, only 1 A(H1)pdm09 virus showed highly reduced inhibition by oseltamivir. The Centre undertook primary isolation of candidate vaccine viruses directly into eggs, and in 2015 a total of 45 viruses were successfully isolated in eggs. *Commun Dis Intell* 2017;41(2):E150–E160.

Keywords: annual report; influenza

## Introduction

The WHO Collaborating Centre for Reference and Research on Influenza in Melbourne is part of the World Health Organization Global Influenza Surveillance and Response System (WHO GISRS). GISRS is a worldwide network

of laboratories that was established in 1952 to monitor changes in influenza viruses circulating in the human population with the aim of reducing its impact through the use of vaccines and antiviral medications. The Centre in Melbourne is one of 5 such Collaborating Centres (the others being in Atlanta, Beijing, London and Tokyo) that monitor the antigenic and genetic changes in circulating human influenza viruses, and makes biannual recommendations on which influenza strains should be included in the influenza vaccine for the upcoming winter season in either the Northern or Southern Hemisphere. This report summarises the results of virological surveillance activities undertaken at the Centre in Melbourne during 2015.

Two types of influenza cause significant disease in humans: types A and B. Influenza A viruses are further classified into subtypes, based on their haemagglutinin (H) surface protein. Globally, there are currently 2 subtypes circulating in human populations: A(H1N1)pdm09 and A(H3N2). Here the N refers to the type of neuraminidase surface protein, of which there are also several types. Influenza B viruses are not classified into subtypes; however, there are 2 lineages, which currently co-circulate globally, B/Victoria/2/87 (B/Victoria) and B/Yamagata/16/88 (B/Yamagata). In addition, each year influenza C viruses are detected from humans, but these viruses tend not to cause severe disease and are not a major focus of surveillance.

## Methods

### Virus isolation

All A(H1)pdm09 and all influenza B viral isolates received at the Centre were re-passaged in cell culture (Madin-Darby Canine Kidney (MDCK) cells), whilst all A(H3) viral isolates were re-passaged in MDCK-SIAT1 cells.<sup>1</sup> Virus isolation was also attempted from a selection of original clinical specimens received. In addition, influenza positive original clinical samples were directly inoculated into eggs as potential candidate vaccine viruses.

## Antigenic analysis

The antigenic properties of influenza viral isolates were analysed using the haemagglutination inhibition (HI) assay as previously described.<sup>2</sup> In HI assays, viruses were tested for their ability to agglutinate red blood cells in the presence of ferret antisera previously raised against reference viruses. Isolates were identified as antigenically similar to the reference strain if the test samples had a titre that was no more than 4-fold different from the titre of the homologous reference strain. During 2015, results were reported by reference to the A/California/7/2009 (H1N1pdm09)-like, A/Switzerland/9715293/2013 (H3N2)-like, B/Phuket/3073/2013-like (Yamagata lineage), and B/Brisbane/60/2008-like (Victoria lineage) viruses that were recommended for the 2015 influenza vaccine. In recent years, including 2015, HI assays involving A(H3) viruses have been performed in the presence of oseltamivir carboxylate in order to reduce non-specific binding of the N protein.<sup>3</sup>

## Genetic analysis

A subset of all influenza viruses analysed at the Centre underwent genetic analysis by sequencing of viral RNA genes; usually the H and N genes as well as the matrix gene for influenza A viruses and non-structural protein genes for influenza B viruses. In addition, the full genomes (all 8 gene segments) of a smaller subset of viruses were sequenced.

For sequencing, RNA was extracted from isolates or original clinical specimens using either manual QIAGEN QIAamp spin columns method or the automated QIAGEN QIAxtractor robot, followed by reverse transcription polymerase chain reaction (PCR) using the BIORLINE MyTaq one step reverse transcription PCR kit according to the manufacturer's recommendations with gene specific primers (primer sequences available on request). Conventional Sanger sequencing was carried out on PCR product using an Applied Biosystems 3500 XL sequencer. Sequence assembly was performed using the Seqman Pro Module of DNASTAR Lasergene version 13 software (DNASTAR, Madison, WI, USA). Next generation sequencing (NGS) was performed on a selection of viruses using an Applied Biosystems Ion Torrent Personal Genome Machine System according to the manufacturer's recommendations. These sequences were analysed using a proprietary FluAnalysis pipeline. Phylogenetic analysis was performed using Geneious 9.0.4 software (Biomatters Ltd, Auckland, New Zealand) and FigTree v1.3.1.

## Antiviral drug resistance testing

As there is potential for influenza viruses to develop resistance to antiviral drugs, circulating viruses were tested for their sensitivity to the currently used neuraminidase inhibitors oseltamivir (Tamiflu) and zanamivir (Relenza). The neuraminidase inhibition (NAI) assay used was a functional fluorescence-based assay in which the susceptibility of test viruses to the antiviral drugs was measured in terms of the drug concentration needed to reduce the neuraminidase enzymatic activity by 50% ( $IC_{50}$ ), and compared with values obtained with sensitive reference viruses of the same subtype or lineage. NAI assays were performed as previously described<sup>4</sup> with the incorporation of a robotic platform by TECAN EVO200 and Infinite 200Pro for liquid handling and plate reading (Tecan Australia). For the purposes of reporting, *reduced inhibition* of influenza A viruses was defined as a 10–99-fold increase in  $IC_{50}$ , while *highly reduced inhibition* was defined as a  $\geq 100$ -fold increase in  $IC_{50}$  in a neuraminidase inhibition assay. For influenza B viruses, these figures were 5–49-fold and  $\geq 50$ -fold increases, respectively. However, it should be noted that the relationship between the  $IC_{50}$  value and the clinical effectiveness of a neuraminidase inhibitor is not well understood and reduced inhibition may not be clinically significant.

Viruses found to have highly reduced inhibition by either oseltamivir or zanamivir underwent further analysis to determine the presence of amino acid substitutions in the neuraminidase protein that were associated with the reduction of inhibition by NAIs. For example, a change from histidine to tyrosine at position 275 (H275Y) of the neuraminidase protein of A(H1N1)pdm09 viruses is known to reduce inhibition by oseltamivir, as does the H273Y neuraminidase mutation in B viruses.<sup>5</sup>

## Candidate vaccine strains

The viruses used to produce human vaccines are required to be isolated and passaged in embryonated hen's eggs or certified cell lines. The Centre undertook primary isolation of selected viruses from clinical samples directly into eggs, using previously described methods,<sup>6</sup> except for the following modifications. First, the viruses were initially inoculated into the amniotic cavity only, and once growth was established the isolates were then further passaged in the allantoic cavity. This was followed by harvest only from either the amniotic or allantoic cavity, depending on site of inoculation. Egg incubation conditions also differed slightly with A(H1)pdm09 and A(H3) viruses incubated at 35°C for 3 days, and influenza B viruses incubated

at 33°C for three days. These isolates were then analysed by haemagglutination assay, HI assay, real time (RT)-PCR and genetic sequencing.

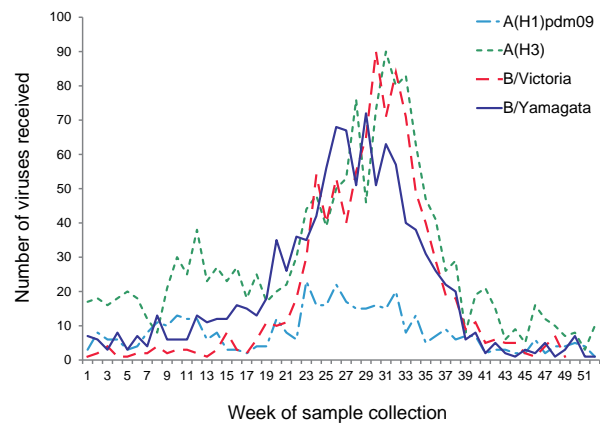
## Results

During 2015, the Centre received 5,557 clinical specimens and/or virus isolates from 34 laboratories in 14 countries (Figure 1). As in previous years, most samples were submitted by laboratories in the Asia–Pacific region, including Australian laboratories<sup>7</sup> and were received during the Southern Hemisphere influenza season. Figure 2 shows the temporal distribution of samples sent to the Centre by type or subtype and lineage.

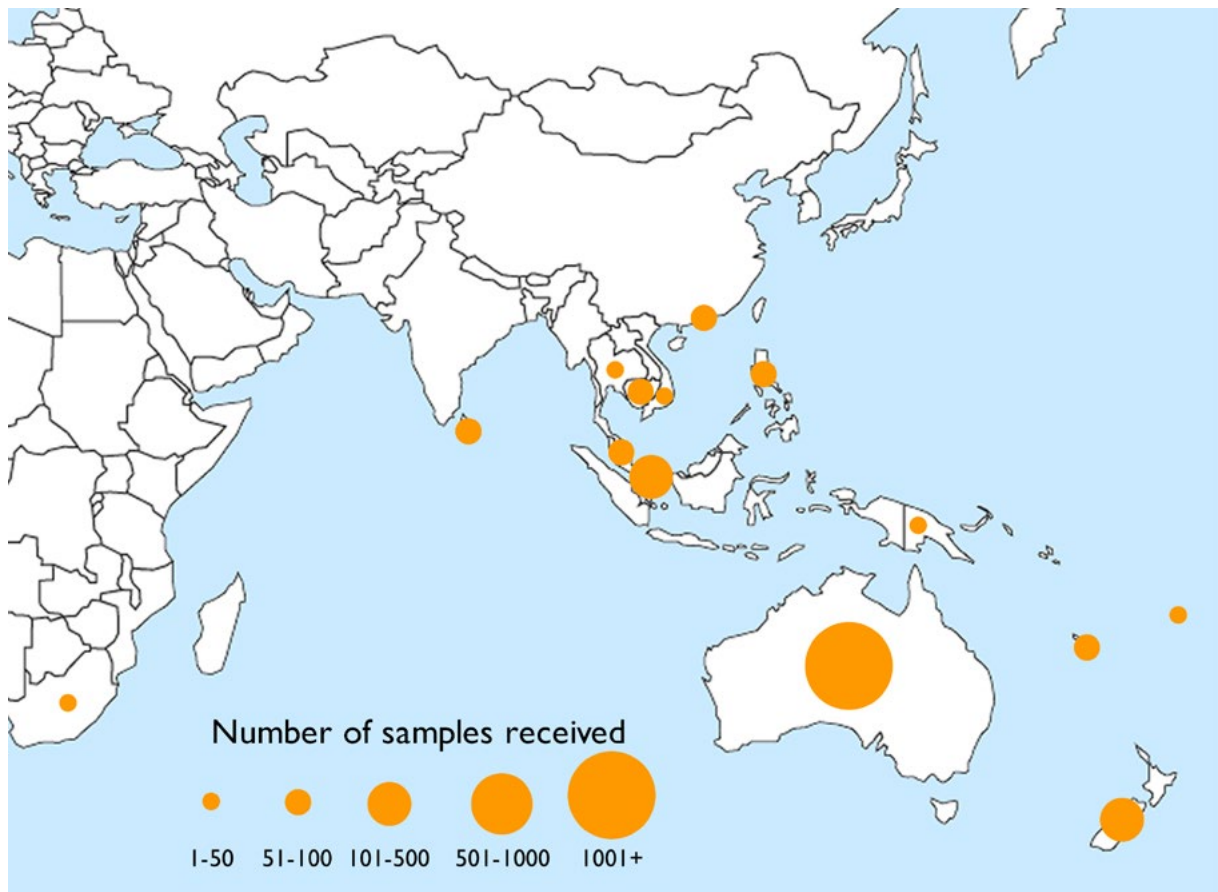
Isolation was attempted for 4,911 (88%) of the samples received and was successful in 3,360 cases (68%). Of these, 2,815 were characterised by HI assay in comparison with the 2015 vaccine strains (Table). In addition, 229 samples were characterised by RT-PCR to determine their type, subtype or lineage. Sanger sequencing and NGS techniques were used to sequence the haemagglutinin genes of 957 viruses. The full genomes of 39 viruses were sequenced using either Sanger sequencing or NGS. Of the samples for which results could be obtained

via antigenic or genetic analysis, (n=3,503), influenza B viruses predominated, comprising 58% of viruses analysed (30% B/Yamagata and 28% B/Victoria). The remaining portion of viruses were mostly A(H3) (31% of total number of viruses), followed by A(H1)pdm09 (11%). There was 1 sample with mixed A(H3)/B viruses.

**Figure 2: Number of samples received at the WHO Collaborating Centre for Reference and Research on Influenza, 2015, by week of sample collection**



**Figure 1: Geographic spread of influenza laboratories sending viruses to the WHO Collaborating Centre for Reference and Research on Influenza during 2015**



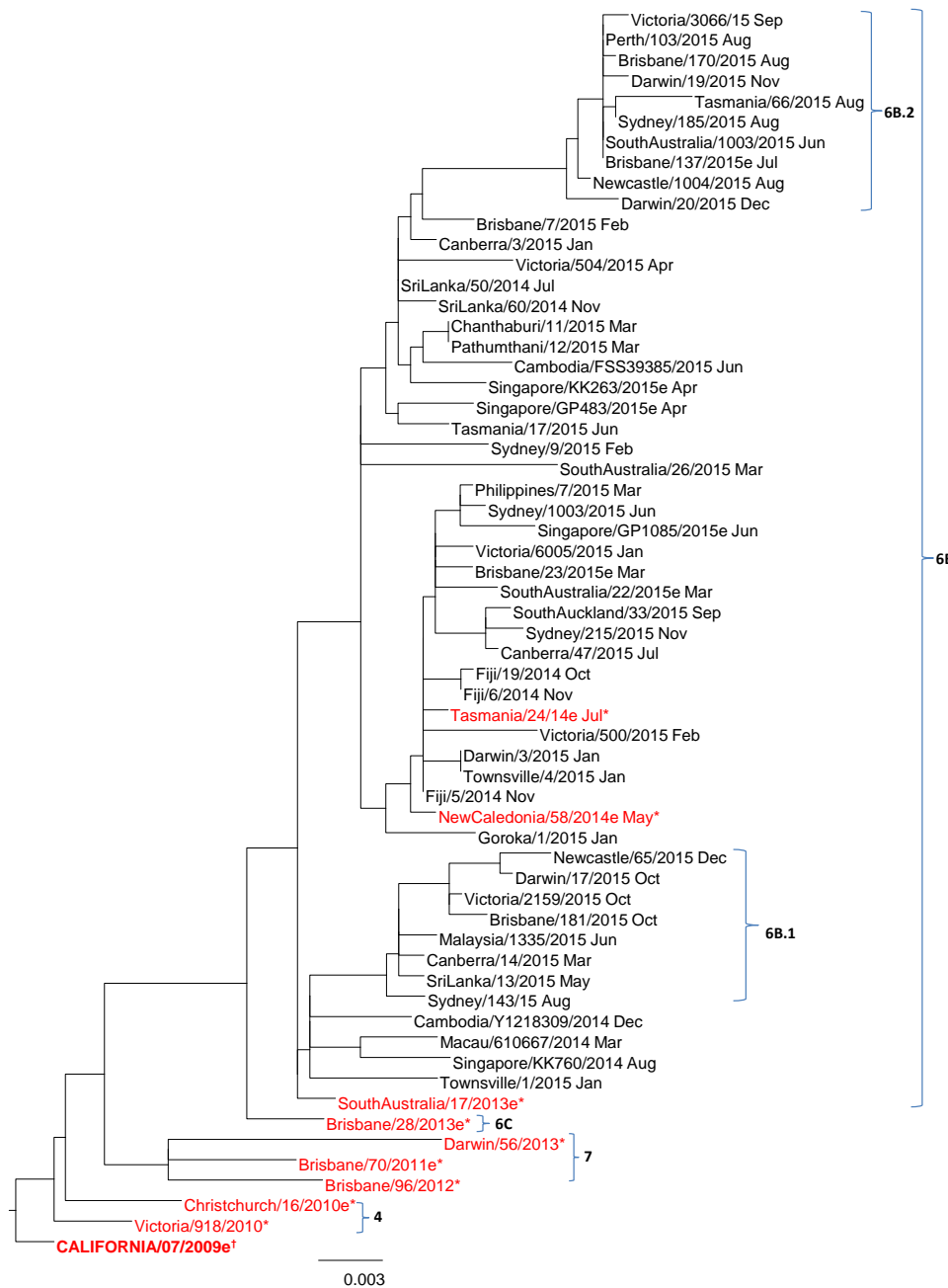
**A(H1N1)pdm09**

Of the 395 A(H1N1)pdm09 isolates analysed by HI assay using ferret antisera in 2015, the majority (98.7%) were antigenically similar to the vaccine reference strain A/California/7/2009 (Table).

Sequencing and phylogenetic analysis of haemagglutinin genes from 67 viruses showed that A(H1)

pdm09 viruses sent to the Centre during 2015 fell mainly into the 6B clade with a smaller number of viruses in subclades 6B.1 and 6B.2 (Figure 3). No antigenic differences were detectable between viruses from the 6B, 6B.1 or 6B.2 genetic clades in ferret antisera HI assays and the majority of viruses reacted in a similar manner to the reference and 2015 vaccine virus A/California/7/2009.

**Figure 3: Phylogenetic tree of representative haemagglutinin genes of A(H1N1)pdm09 viruses received by the WHO Collaborating Centre for Reference and Research on Influenza, 2015**



\* Reference virus is indicated in red text.

† 2015 Southern Hemisphere vaccine strain is presented in capital letters.

e: egg isolate

Scale bar represents 0.3% nucleotide sequence difference between viruses.

} Braces indicate clades

Twenty-three viruses were inoculated into eggs for vaccine candidate strain isolation. Of these, 9 (39%) were successfully isolated, including viruses from each of the 2 emerging subclades (6B.1 and 6B.2).

Of 380 A(H1)pdm09 viruses tested 1 exhibited highly reduced inhibition by oseltamivir. This virus was from Australia and had the H275Y mutation in the N gene, which is known to reduce inhibition by oseltamivir. No A(H1)pdm09 viruses received during 2015 showed highly reduced inhibition to zanamivir.

### A(H3N2)

Antigenic analysis of 651 A(H3) subtype isolates showed that only 1.7% were low reactors to the cell-propagated reference strain A/Switzerland/9715293/2013 (Table). However, 47% of viruses were low reactors to the egg-propagated strain A/Switzerland/9715293/2013 (data not shown). An additional 232 A(H3) viruses were inoculated and/or isolated by cell culture but did not reach sufficient titres for antigenic analysis, whilst a further 503 were successfully isolated but did not reach sufficient titres when tested by HI assay in the presence of oseltamivir.

A total of 673 haemagglutinin genes from A(H3) viruses were sequenced. Phylogenetic analysis indicated that the majority of circulating viruses fell into clade 3C.2a, which is a genetically different clade to the 2015 vaccine strain A/Switzerland/9715293/2013 (clade 3C.3a) and is represented by the A/Hong Kong/4801/2014 virus (Figure 4). A small number of H3 viruses fell into the 3C.3b clade.

Fifty-four viruses were inoculated into eggs, of which 21 (39%) grew successfully. These viruses included at least 17 viruses from clade 3C.2a and 4 viruses from clade 3C.3b.

None of the 1,339 A(H3) viruses tested had highly reduced inhibition by either oseltamivir or zanamivir.

### Influenza B

Similar proportions of viruses from both B lineages (B/Victoria and B/Yamagata) were received at the Centre during 2015. A total of 1,769 influenza B viruses were characterised by HI assay. All but 3 of the 837 B/Victoria viruses received and analysed were similar to B/Brisbane/60/2008 (Table). Only 4 of the 932 B/Yamagata lineage viruses analysed were low reactors to the B component of the 2015 vaccine, B/Phuket/3073/2013 vaccine strain (these comparisons were made to ferret antisera generated to the MDCK-isolated virus induced infections).

Sequencing was performed on 217 haemagglutinin genes from B viruses, the majority being B/Yamagata viruses. All of the viruses of B/Victoria lineage were genetically similar to the B/Brisbane/60/2008 reference virus (Figure 5). The majority of B/Yamagata lineage viruses belonged to Clade 3, which was represented by the 2015 vaccine strain B/Phuket/3073/2013 as well as B/Wisconsin/1/2010 (the 2013 Southern Hemisphere influenza vaccine strain) (Figure 6).

Egg isolation was attempted for 26 B/Victoria and 15 B/Yamagata viruses, with 8 (31%) B/Victoria viruses and 7 (47%) B/Yamagata viruses successfully isolated in eggs. At least 1 representative from the major clades of both B lineages was among the successful isolates.

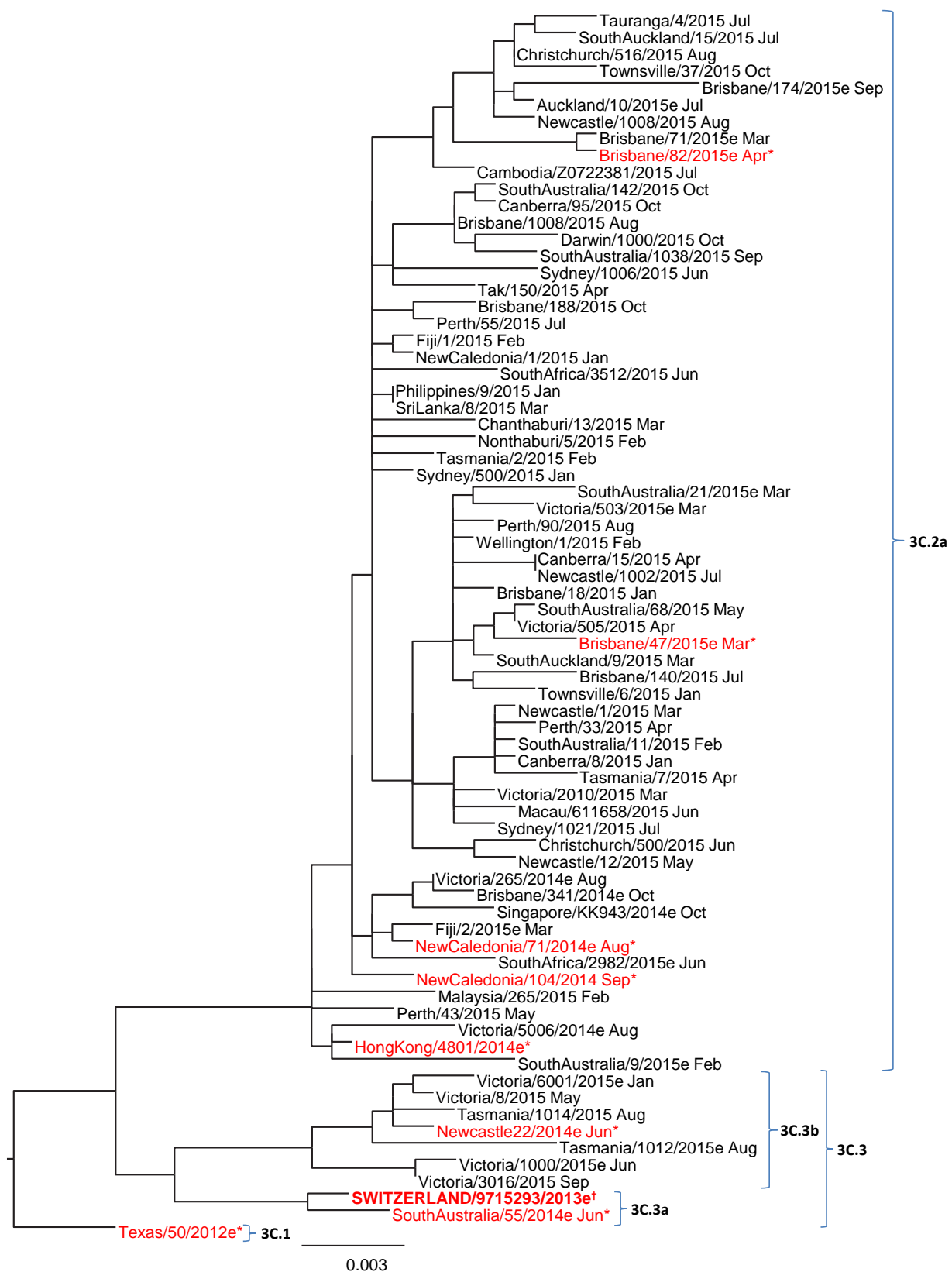
Of 780 B/Victoria and 825 B/Yamagata viruses tested, none displayed highly reduced inhibition by either oseltamivir or zanamivir.

**Table: Antigenic analysis of viruses received by the WHO Collaborating Centre for Reference and Research on Influenza, 2015, by country of origin**

Region	A(H1N1)pdm09 reference strain: A/California/7/2009 (cell)		A(H3N2)* reference strain: A/Switzerland/975193/2013 (cell)		B/Victoria reference strain: B/Brisbane/60/2008 (cell)		B/Yamagata reference strain: B/Phuket/3073/2013 (cell)	
	Like	Low reactor (%)	Like	Low reactor (%)	Like	Low reactor (%)	Like	Low reactor (%)
Australasia	224	4	459	9	809	3	766	2
Pacific	18	0	7	0	12	0	51	0
South East Asia	102	1	133	2	13	0	87	2
East Asia	14	0	25	0	0	0	18	0
South Asia	25	0	12	0	0	0	2	0
Africa	7	0	4	0	0	0	4	0
Total	390	5 (1.3%)	640	11 (1.7%)	834	3 (0.4%)	928	4 (0.4%)

\* Note that many A(H3N2) virus isolates that were obtained could not be analysed by haemagglutination inhibition assay due to low haemagglutination assay titre in the presence of oseltamivir.

**Figure 4: Phylogenetic tree of representative haemagglutinin genes of A(H3) viruses received by the Centre during 2015**



\* Reference virus is indicated in red text.

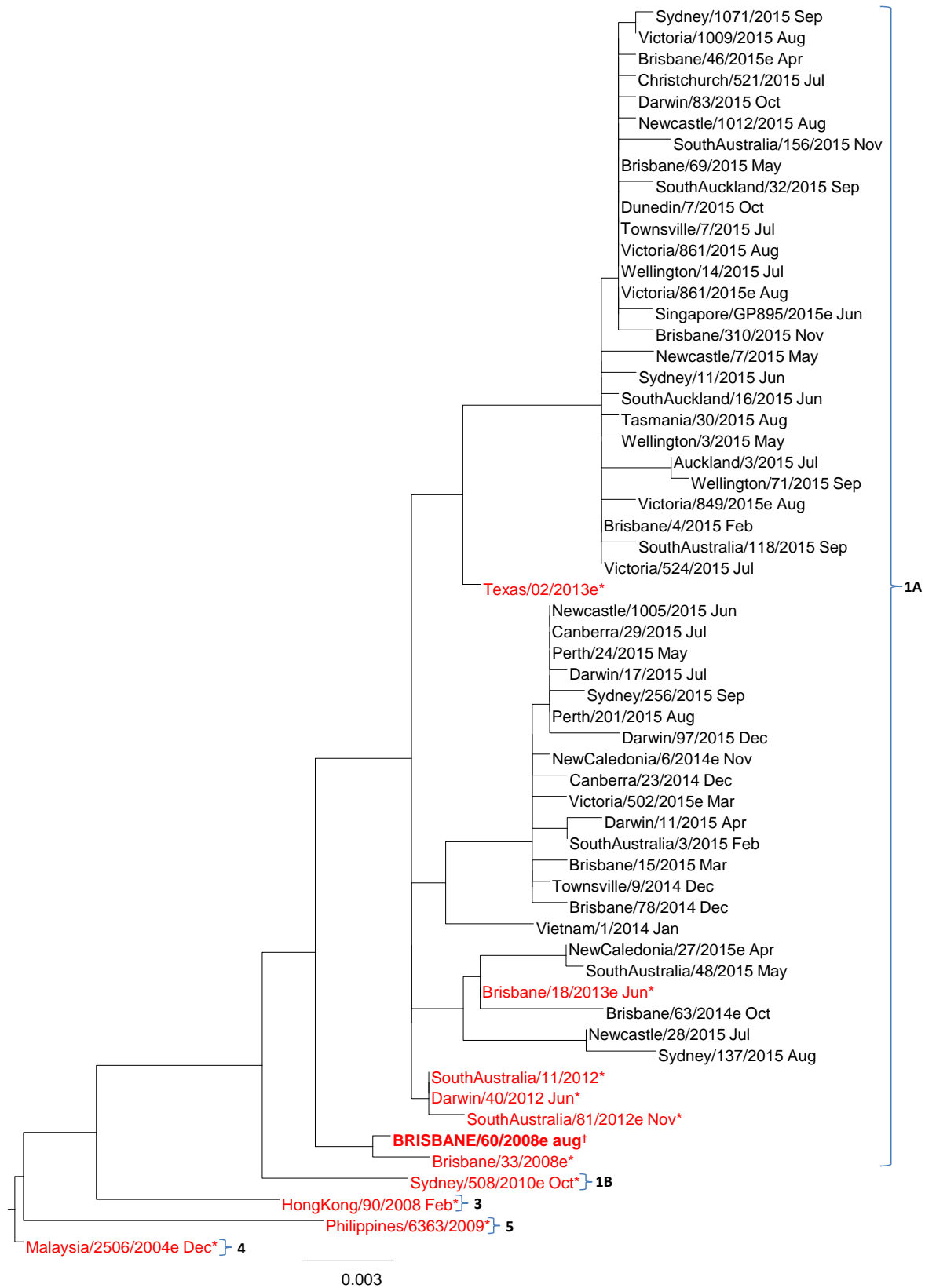
† 2015 Southern Hemisphere vaccine strain is presented in capital letters.

e: egg isolate

Scale bar represents 0.3% nucleotide sequence difference between viruses.

} Braces indicate clades

**Figure 5: Phylogenetic tree of representative haemagglutinin genes of B/Victoria viruses received by the WHO Collaborating Centre for Reference and Research on Influenza, 2015**



\* Reference virus is indicated in red text.

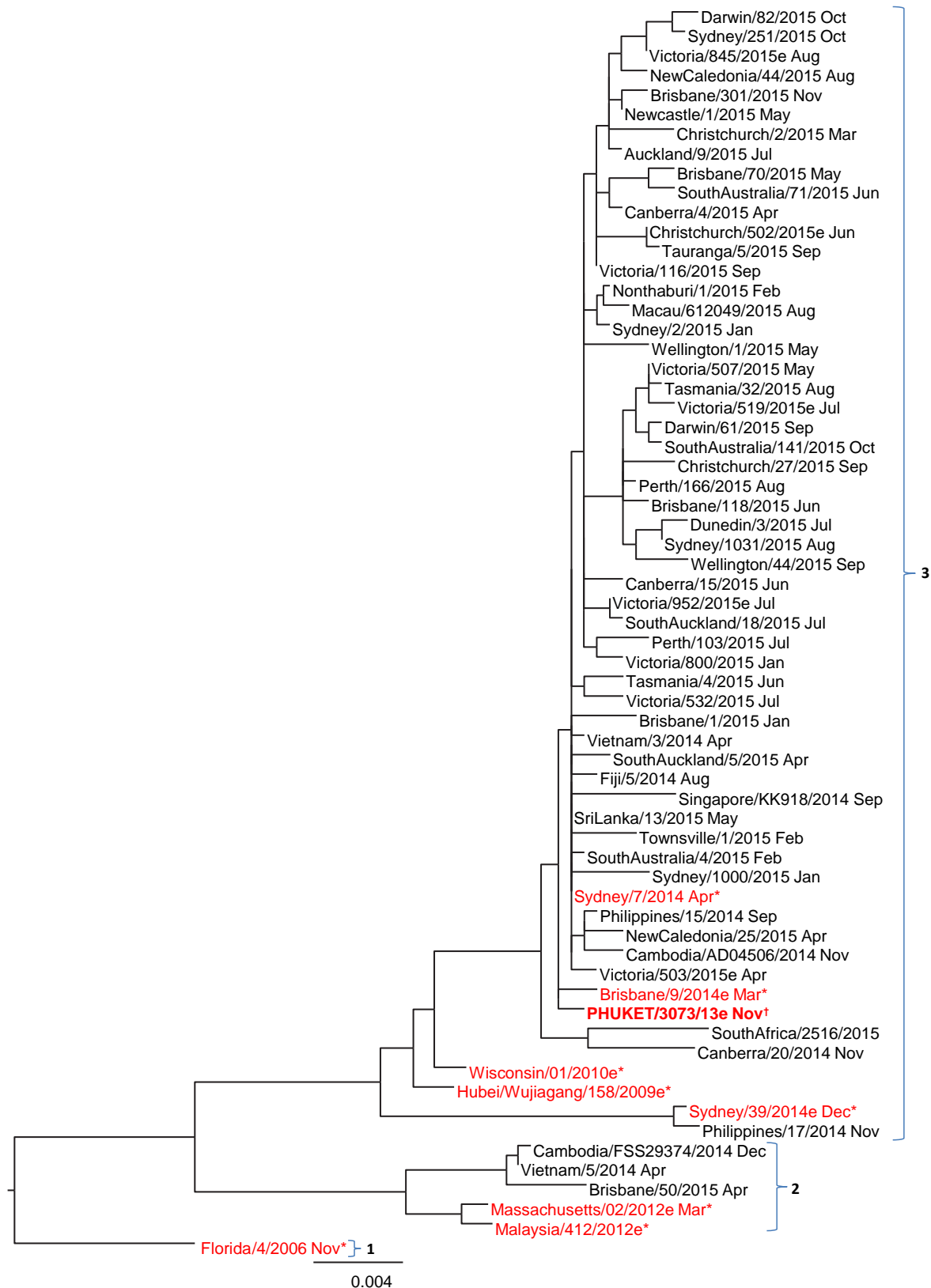
† 2015 Southern Hemisphere vaccine strain is presented in capital letters.

e: egg isolate

Scale bar represents 0.3% nucleotide sequence difference between viruses.

} Braces indicate clades

**Figure 6. Phylogenetic tree of representative haemagglutinin genes of B/Yamagata viruses received by the WHO Collaborating Centre for Reference and Research on Influenza, 2015**



\* Reference virus is indicated in red text.

† 2015 Southern Hemisphere vaccine strain is presented in capital letters.

e: egg isolate

Scale bar represents 0.4% nucleotide sequence difference between viruses

} Braces indicate clades

## Discussion

As in previous years,<sup>7</sup> the vast majority of samples received at the Centre in 2015 were from Australia. Data from the National Notifiable Diseases Surveillance System indicated that in 2015, Australia reported the highest number of notifications of laboratory-confirmed cases ( $n=100,584$ )<sup>8</sup> on record, which was at least partly due to increased influenza testing.<sup>9</sup> This was also reflected in the large quantity of samples received at the Centre in 2015, the greatest annual number since 2009, the year of the A(H1N1)pdm09 pandemic, when 6,435 samples were received. In addition, a total of 45 candidate vaccine viruses were isolated in eggs during 2015, the largest number and highest rate of successful isolations since 2010.

Data from national influenza centres indicated that influenza B predominated over influenza A viruses in Australia during 2015,<sup>8</sup> which was also reflected in the viruses analysed by the Centre. When considering only samples received from Australia and for which the lineage could be confirmed, there were slightly more B/Victoria viruses than B/Yamagata viruses (52%–48%). However, when samples submitted to the Centre from all countries were included, there were a larger number of B/Yamagata viruses (53%) compared with B/Victoria viruses (47%). This can be attributed to more B/Yamagata viruses being received from all non-Australian countries, especially New Caledonia, New Zealand and Singapore; this is also reflected in a larger number of B/Yamagata infections than B/Victoria infections recorded by the New Zealand surveillance system during 2015.<sup>10</sup>

In Australia, there was a rapid rise in the number of B/Victoria lineage viruses detected from May onwards.<sup>11</sup> The predominance of influenza B over influenza A viruses in any given year occurs relatively infrequently at a rate of approximately once a decade.<sup>12</sup> The last year in which this occurred in Australia was 2008, and a similar switch in predominance from B/Yamagata to B/Victoria viruses was also observed in that year.<sup>13</sup> The reasons for this change between the 2 lineages within the season are not clear, but may be due to subtle phylogenetic differences between the 2 lineages, differential population susceptibility or a combination of these factors.<sup>13</sup> The change in the relative predominance of B lineage viruses from B/Yamagata to B/Victoria during 2015 was also reflected in the change in recommended B strain for the 2016 Southern Hemisphere trivalent influenza vaccine (TIV) to the B/Victoria-lineage reference virus B/Brisbane/60/2008.

Almost all B/Yamagata lineage viruses tested were antigenically similar to the 2015 vaccine virus B/

Phuket/3073/2013 and this was reflected in the overall vaccine effectiveness (VE) estimates for Australia (VE 71%, 95% CI: 57–80).<sup>14</sup> Similarly, the majority of B/Victoria lineage viruses tested were antigenically similar to the reference virus B/Brisbane/60/2008. Lower VE estimates were reported for B/Victoria lineage viruses (VE 42%, 95% CI: 13–61) in Australia in 2015,<sup>14</sup> however this is unsurprising as these estimates were calculated for the trivalent influenza vaccine (TIV), which included only a B/Yamagata lineage virus. Vaccine effectiveness against both influenza B lineages may be improved through the use of the quadrivalent influenza vaccine (QIV) which was introduced in Australia in 2015, but not widely used as it was not included in the National Immunisation Program and only available for private purchase.

Despite the predominance of influenza B during 2015, influenza A(H3N2) predominated in Tasmania, and co-circulation of A(H3N2) and influenza B was observed in Western Australia.<sup>11</sup> The majority of circulating A(H3) viruses were antigenically similar to the cell derived A/Switzerland/9715293/2013 reference strain in the vaccine. However, more than half of the viruses analysed at the Centre were low reactors to the egg derived A/Switzerland/9715293/2013 reference strain. The difficulties in detecting antigenic changes in A(H3) subtype viruses using the HI assay reported in recent years<sup>7,15</sup> continue to present an ongoing challenge for surveillance. This factor and poor viral growth reduced the number of A(H3) viruses received during 2015 that could be analysed by the HI assay by 44%. The Centre is continuing to work on the development of other assays (e.g. virus neutralisation) to characterise the antigenicity of recent A(H3) viruses, however at this time such assays remain time- and labour-intensive and would most likely complement rather than replace the HI assay. In light of this, genetic analysis remains an important tool for detecting both minor and major changes in A(H3) viruses.

Genetic data from the Centre indicated that many of the A(H3) viruses in 2015 fell into clade 3C.2a. Viruses from this clade have been circulating in Australia since 2014 and are somewhat antigenically distinct to the A/Switzerland/9715293/2013 reference strain in the 2015 Southern Hemisphere vaccine, which lies in clade 3C.3a. Phylogenetic data from the Northern Hemisphere suggested that the A(H3) component of the 2015 TIV may not be ideally matched and VE estimates for Australia were indeed low to moderate for A(H3N2) (VE 44%; 95% CI: 21–60).<sup>14</sup> In the WHO recommended vaccine for the southern hemisphere TIV/QIV in 2016, the A(H3) vaccine virus was updated from A/Switzerland/9715293/2013 to A/Hong Kong/4801/2014, which lies in clade 3C.2a.

Finally, antigenic and genetic data for A(H1)pdm09 viruses analysed at the Centre in 2015 indicated an overall good match with the recommended vaccine strain A/California/7/2009. Whilst phylogenetic analysis indicated the emergence of 2 distinct subclades. These changes did not affect the antigenic behaviour of the viruses in HI assays using ferret antisera. As such, A/California/7/2009 was recommended for inclusion again in the TIV/QIV for 2016. This was also reflected in the Australian vaccine effectiveness estimates for A(H1)pdm09 viruses in 2015 (VE 79%, 95% CI: 33–93),<sup>14</sup> as well as interim vaccine effectiveness estimates from the northern hemisphere for the 2015–2016 season that indicated that vaccines containing A/California/7/2009 were also effective against viruses in the emerging 6B.1 clade (VE 64% (95% CI: 44%–77%)).<sup>16,17</sup>

With continual change and evolution in influenza viruses and the absence of a universal vaccine, there remains a need for ongoing influenza surveillance for the foreseeable future. In light of this, the work performed by the Centre in Melbourne is crucial to the efforts of the global surveillance community to ensure that viruses recommended for the influenza vaccine remain updated and as closely matched to circulating viruses as possible.

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## References

- Oh DY, Barr IG, Mosse JA, Laurie KL. MDCK-SIAT1 cells show improved isolation rates for recent human influenza viruses compared to conventional MDCK cells. *J Clin Microbiol* 2008;46(7):2189–2194.
- Hobson D, Curry RL, Beare AS, Ward-Gardner A. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. *J Hyg (Lond)* 1972;70(4):767–777.
- Lin YP, Gregory V, Collins P, Kloess J, Wharton S, Cattle N, et al. Neuraminidase receptor binding variants of human influenza A(H3N2) viruses resulting from substitution of aspartic acid 151 in the catalytic site: a role in virus attachment? *J Virol* 2010;84(13):6769–6781.
- Hurt AC, Okomo-Adhiambo M, Gubareva LV. The fluorescence neuraminidase inhibition assay: a functional method for detection of influenza virus resistance to the neuraminidase inhibitors. *Methods Mol Biol* 2012;865:115–125.
- Takashita E, Meijer A, Lackenby A, Gubareva L, Rebelo-de-Andrade H, Besselaar T, et al. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2013–2014. *Antiviral Res* 2015;117:27–38.
- WHO Global Influenza Surveillance Network. *Manual for the laboratory diagnosis and virological surveillance of influenza*. Geneva: World Health Organization; 2011.
- Sullivan SG, Chow MK, Barr IG, Kelso A. Influenza viruses received and tested by the Melbourne WHO Collaborating Centre for Reference and Research on Influenza annual report, 2014. *Commun Dis Intell* 2015;39(4):E602–E611.
- Australian Government Department of Health. National Notifiable Diseases Surveillance System. 2015. Available from: <http://www9.health.gov.au/cda/source/cda-index.cfm> Accessed on 17 August 2016.
- Fielding JE, Regan AK, Dalton CB, Chilver MB, Sullivan SG. How severe was the 2015 influenza season in Australia? *Med J Aust* 2016;204(2):60–61.
- Institute of Environmental Science and Research Ltd. Influenza Weekly Update 2015/39; 2015.
- Australian Government Department of Health. Australian Influenza Surveillance Report No 10: 26 September to 9 October 2015.
- Kaczmarek M, Owen R, Barr IG. Annual report of the National Influenza Surveillance Scheme, 2008. *Commun Dis Intell* 2010;34(1):8–22.
- Barr IG, Vijaykrishna D, Sullivan SG. Differential age susceptibility to influenza B/Victoria lineage viruses in the 2015 Australian influenza season. *Euro Surveill* 2016;21(4) doi: 10.2807/1560-7917.
- Fielding JE, Levy A, Chilver MB, Deng YM, Regan AK, Grant KA, et al. Effectiveness of seasonal influenza vaccine in Australia, 2015: An epidemiological, antigenic and phylogenetic assessment. *Vaccine* 2016;34(41):4905–4912.
- Barr IG, Russell C, Besselaar TG, Cox NJ, Daniels RS, Donis R, et al. WHO recommendations for the viruses used in the 2013–2014 Northern Hemisphere influenza vaccine: Epidemiology, antigenic and genetic characteristics of influenza A(H1N1)pdm09, A(H3N2) and B influenza viruses collected from October 2012 to January 2013. *Vaccine* 2014;32(37):4713–4725.

16. Chambers C, Skowronski DM, Sabaiduc S, Winter AL, Dickinson JA, De Serres G, et al. Interim estimates of 2015/16 vaccine effectiveness against influenza A(H1N1)pdm09, Canada, February 2016. *Euro Surveill* 2016;21(11):30168.
17. Emborg HD, Krause TG, Nielsen L, Thomsen MK, Christiansen CB, Skov MN, et al. Influenza vaccine effectiveness in adults 65 years and older, Denmark, 2015/16 – a rapid epidemiological and virological assessment. *Euro Surveill* 2016;21(14) doi: 10.2807/1560-7917.