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Biographies

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Development and testing of the Australian pandemic influenza vaccine – a timely response

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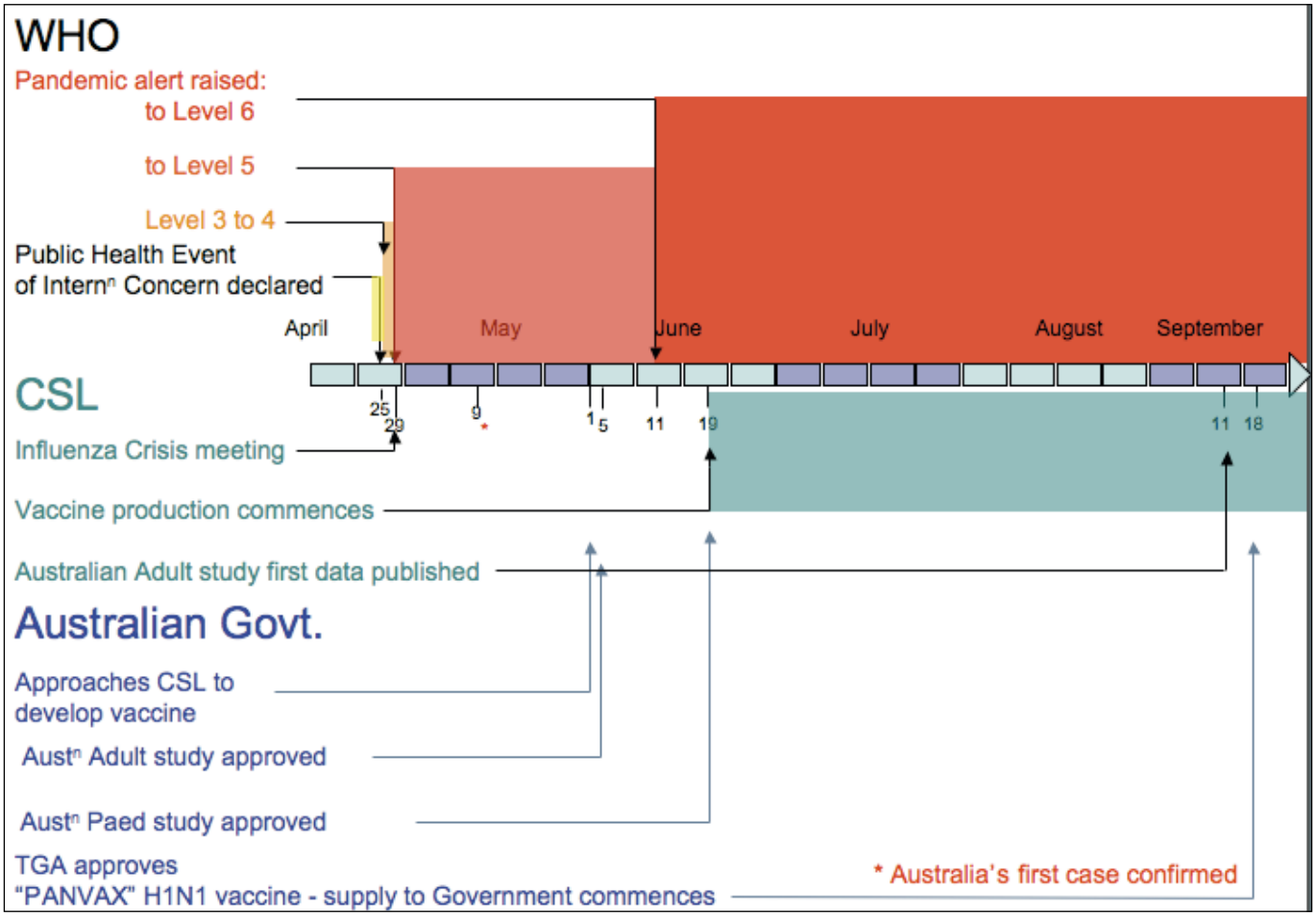
In April 2009 a novel virus strain appeared which would cause the first influenza pandemic of the 21st century. This pandemic was the first to occur in an era where bioinformatic technologies contributed to the response to this virus; still, the creation of a vaccine relied largely on existing egg-based technology. The ongoing threat of a H5N1 pandemic spurred the development of strategies to rapidly produce a pandemic vaccine. These plans were implemented and allowed CSL and Australia to conduct the first clinical trials and produce one of the first 2009 pandemic vaccines. However, new candidate influenza vaccine viruses often present challenges to manufacturing a new vaccine. This pandemic virus was no exception. Being in the post-pandemic phase, it is important to review lessons learned to improve our response to future pandemics. In hindsight, the production of a pandemic vaccine is similar to that of seasonal influenza vaccines, yet the urgency of the pandemic response compresses timelines. This report explores those timelines and implications for producing a pandemic vaccine for Australia.

Figure 1. Major event time line for the 2009 influenza pandemic outbreak in Australia.



Major influenza events of 2009 in Australia

On 25 April 2009, the World Health Organization (WHO) declared a Public Health Event of International Concern[1] with reported cases of swine-origin influenza A/H1N1 in Mexico and the United States[2] (Figure 1). On 27 April the WHO raised the pandemic alert phase from 3 to 4 due to the sustained human-to-human transmission of novel H1N1 infections. Australia reported its first case 13 days later in a 33-year-old woman. On 29 April the WHO raised the pandemic alert to phase 5 due to person-to-person spread in at least two countries within one WHO region. The Australian Government moved to a national CONTAIN phase (23 May) and Victoria escalated to Sustain on 3 June. On 11 June the WHO raised the pandemic alert phase to 6 due to outbreaks in more than one WHO region (Figure 1).

During May 2009, the Australian Government approached CSL to produce a pandemic vaccine as per the existing contract to supply pandemic vaccine as part of its biosecurity role to supply to the Australian Government and the WHO. CSL is the only influenza vaccine manufacturer located in the southern hemisphere. CSL is also unique compared to other manufacturers in that it produces two separate influenza vaccines specific to each of the northern and southern hemispheres. Vaccine production commenced on 19 June and the Australian adult clinical study protocol was approved (Figure 1). On 22 July the first trial participant received the CSL 2009 H1N1 monovalent vaccine. The completion of the study was published on 11 September and the Australian Government Therapeutic Goods Administration (TGA) approved CSL's PANVAX® vaccine one week later. By 28 September, Australia would become the second country after China to begin a pandemic vaccination program.

Vaccine development

Production of vaccine strain

On 27 April, the day the WHO raised its pandemic alert to phase 4, CSL's influenza crisis team met and decided to develop seed viruses to this new emerging strain of virus. Two different methods (classical reassorting and reverse genetics) to produce a high-growth, egg-adapted virus was implemented in parallel to maximise the chance for success. Candidate wild type virus was received from the Centers for Disease Control and Prevention, (CDC, Atlanta, GA, USA) on 4 May to produce a reassorted high-growth virus for vaccine production by classical methods[3]. The classical reassortment method requires a minimum of six passages in eggs routinely totalling up to 25 days: a mixed infection passage with an egg-adapted donor virus to provide internal genes for high-growth in eggs; two antibody selection passages, two cloning passages and a further passage to lay

down a " bulk seed" lot. This seed lot is sent for testing at WHO collaborating centres and subsequently supplied to influenza vaccine manufacturers. This seed development was undertaken under biosafety level 3 (BSL3) conditions with additional use of personnel respirators. The reassortant virus at CSL (IVR153) was ready for vaccine manufacture on 25 May, only 21 days after initiation. Reassortant seed viruses undergo numerous Quality Control (QC) tests including sterility, mycoplamsa testing, haemagglutinin (HA) and neuraminidase (NA) identity testing, genotyping and confirmation of reactivity with ferret serum prior to distribution from the WHO collaborating centres. These QC activities can take up to six weeks. In order to respond as quickly as possible to the pandemic, preparation of working seed lots and vaccine manufacture proceeded in parallel to the QC testing.

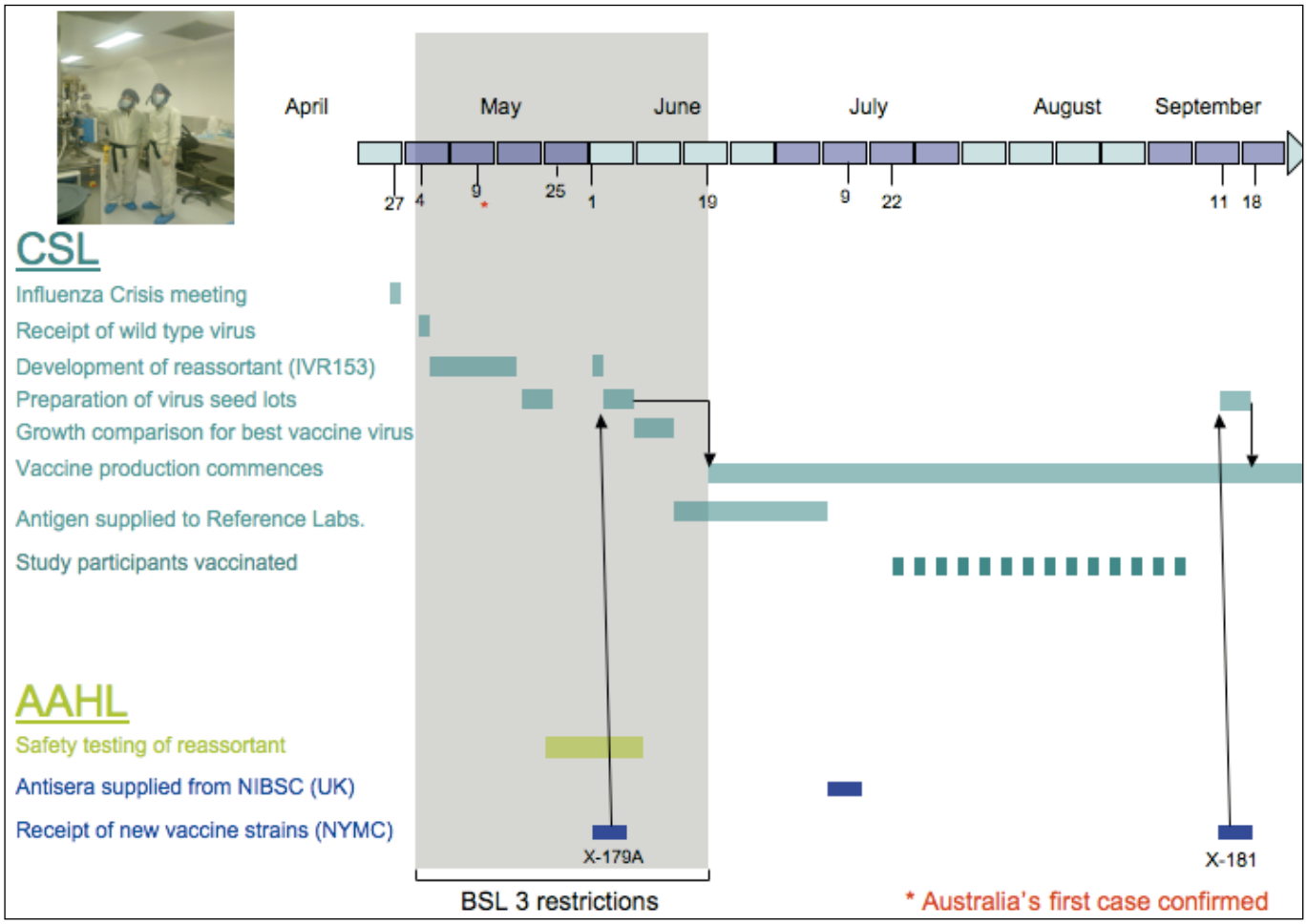
The second approach to obtain a candidate seed virus utilised reverse genetics. Reverse genetics is a technique that can generate influenza virus entirely from cloned DNA by co-transfection into appropriate cells with plasmids encoding the influenza virion RNA. To decrease the time of obtaining appropriate plasmids and therefore a candidate seed virus, a new synthetic approach was undertaken[4]. Attempts at CSL and other laboratories were unsuccessful in obtaining an A/California/07/2009 virus by the reverse genetic method before the classical reassortant was available. The reasons for this outcome is now understood in that the inability to rescue this virus was due to the requirement for egg adaptation mutations in the HA sequence[5].

Development of alternative candidate vaccine reassortant viruses occurred concurrently in a number of laboratories globally. On 1 June, CSL received a second candidate vaccine strain, that is NYMC X-179A (Figure 2). CSL was able to immediately prepare a seed lot and then perform a growth comparison study between IVR153 and X179A to determine which of the two candidates then available provided superior growth. Although both strains provided disappointingly low yields compared to seasonal H1N1 strains, NYMC X-179A was superior and was selected for use in vaccine manufacture. Subsequently, on 18 August an improved seed strain (X181) that demonstrated enhanced egg growth following multiple egg passages[5] arrived, seed prepared and introduced into vaccine production.

Production of reference antiserum and antigen

In addition to seed development, reference antigen and antisera are developed in order to establish vaccine potency necessary for vaccine formulation. Purified virus sourced from the material produced by large-scale virus manufacturing was used for the development of reference antigen for distribution to the TGA and to testing laboratories in order to establish calibrated reagents for use in the Single Radial Immuno-Diffusion vaccine potency assay. Routinely the time taken for antigen production and assay calibration is 49 days. The TGA was able to distribute

Figure 2. Vaccine development time lines for the 2009 influenza outbreak in Australia.



antigen to WHO and other manufacturers from 9 July only five weeks after the receipt of this virus.

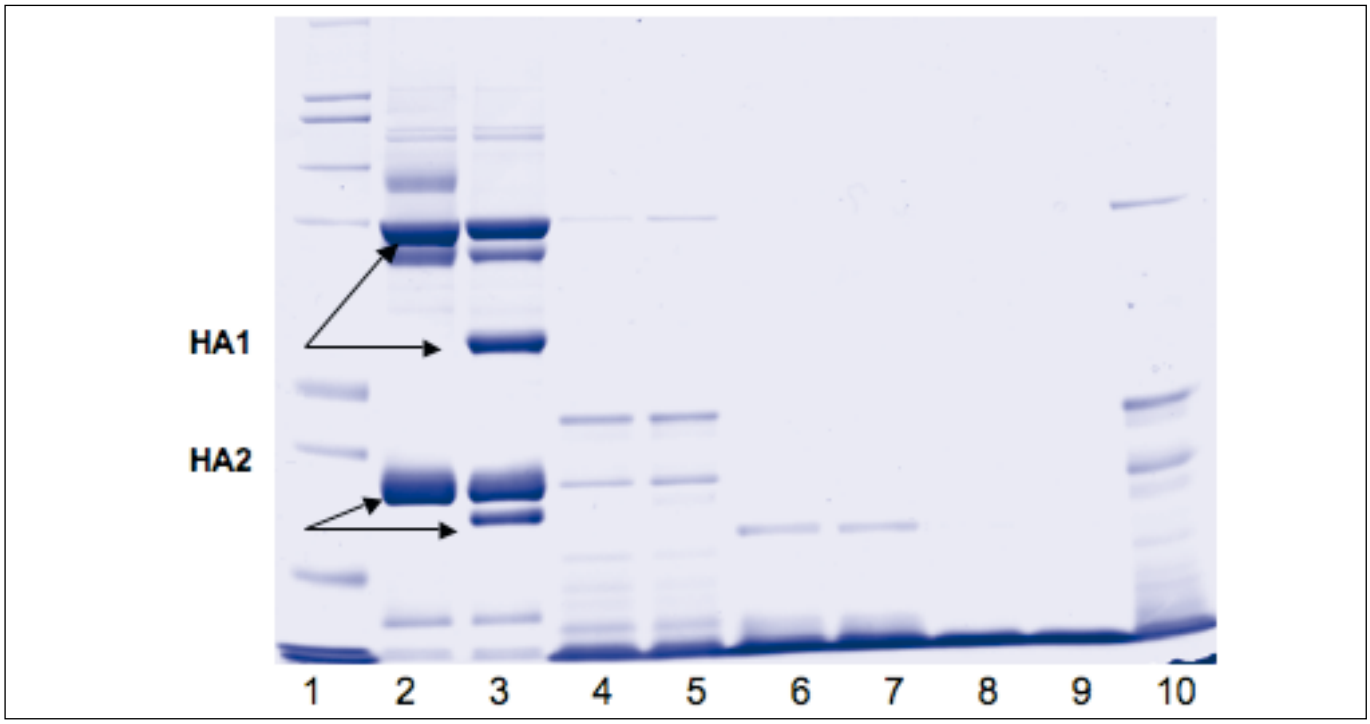
To produce reference antiserum, the HA is enzymatically cleaved from the surface of the virus using bromelain. The HA is then purified and concentrated via sucrose gradient zonal ultracentrifugation[6,7]. The purified HA is then injected into sheep, three doses over six weeks, to produce hyperimmune serum. Routine production of hyperimmune serum for antigen quantitation for vaccine formulation takes a total of 69 days. In the case of 2009 H1N1, cleavage of the HA under standard conditions from the surface of the virus resulted in degradation of the HA (Figure 3). This susceptibility for HA to be degraded by bromelain was previously encountered during development of antisera to influenza A/H5N1 viruses. Multiple attempts varying the temperature, time and level of reducing agent marginally improved the level of intact HA yet it was found that the antigen was not of sufficient quality to proceed for production of antiserum. Collaborators at the NIBSC encountered the same issue (Robert Newman, Mill Hill, UK, personal communication), but they were able to produce sufficient HA to vaccinate animals for antiserum and initial supply to WHO collaborating centres and manufacturers.

Vaccine manufacture

Vaccine manufacture began on 19 June using the reassortant virus prepared at the New York Medical Centre (X179A). [Pilot scale analysis comparing IVR153 and X179A indicated a slight improvement for antigen production, which was confirmed subsequently by other manufacturers and shown to be due to two amino acid changes[3]]. This delay was largely due to the requirement of needing to operate under BSL3 conditions as the wild type virus was believed to be more pathogenic (that is to say, virus replication in the human lower respiratory tract and lung pathology) than is typically seen with seasonal influenza infection[8]. Currently there are no large-scale, egg-based BSL3 influenza vaccine manufacturing facilities in the developed world. Therefore vaccine manufacturing was unable to proceed until this restriction was lifted following safety testing in a ferret model. Such testing is not routinely carried out prior to vaccine manufacture for seasonal influenza strains since the A strains are reassorted with a high-growth parent strain (A/Puerto rico/8/34) that is considered attenuated for humans[9]. In parallel, a submission by CSL to the WHO (Geneva) recognised that the Parkville manufacturing facilities with additional personal protection met BSL3 standards allowing early commencement of vaccine production.

In Australia, safety testing was conducted at the Australian Animal Health Laboratories in Geelong by Dr Middleton and colleagues. This was possible because of extensive experience with influenza in the ferret model made possible by earlier funding from the National Health and Medical Research Council[10-14]. In parallel, ferret studies were conducted at CDC in the US (viruses X179A and IDCDC-RG-15) and NIBSC in the UK (NIBRG-121). In each of the studies, it was concluded that the reassortant candidate vaccine viruses were attenuated in ferrets relative to the wild type H1N1 pandemic isolates. As a result of these findings, the WHO recommended on 19 June that vaccine production could proceed at enhanced BSL 2 biocontainment level using fully trained and competent staff in accordance with national safety guidelines[15].

Figure 3. Bromelain cleavage method was unsuitable for A/California/7/2009 egg adapted viruses. Time and temperature study. SDS-PAGE Coomassie stained gel Lane: 1 MW markers (Mark 12); 2 A/California /07/09 inactivated zonal pool (IZP)(X179A); 3 A/California /07/09 IZP deglycosylated; 4,5 Bromelain digestion 60 min 37˚C; 6,7 Bromelain digestion 90 min 37˚C; 8,9 Bromelain digestion 90 min 37˚C, deglycosylated; 10 Bromelain digestion 4 h RT.



Clinical trials

The Australian Health Management Plan for Pandemic Influenza (AHMPPI) was substantially revised in 2008 to include a SUSTAIN phase, where the aim is to sustain mitigation efforts while awaiting a customised pandemic vaccine (AHMPPI 2009 update p. 30). The AHMPPI does not specifically call for clinical trials to be conducted to determine safety or immunogenicity as much of the planning was based on a H5N1 scenario where many vaccines have already undergone clinical testing. The development and clinical testing of CSL's H5N1 vaccine indicated that achievement of protective antibody levels required two doses of 30 or 45 μg adjuvanted antigen[16]. A decision was made to conduct clinical trials in adults and children to determine antigen dose and the number of doses needed because genetically this was a novel virus to which the majority of the population was believed to be immunologically naive. Clinical trial protocols were approved by the TGA (Figure 1) early results were immediately communicated to regulators and public health officials in Australia and internationally. On 11 September, CSL's results were published demonstrating that a single 15 μg dose of unadjuvanted H1N1 vaccine was immunogenic and well tolerated in adults[17]. Paediatric trial data were published shortly thereafter, confirming similar results in children as young as six months of age. The vaccine was highly immunogenic after a single dose and the majority of adverse events were mild to moderate in severity. No febrile convulsions were observed in the trials. Adverse events decreased in incidence and severity following a second dose of the vaccine[18]**.** The Australian TGA recommended two doses of this vaccine for children aged six months to less than nine years of age.

Lessons learned

Invaluable lessons have been learned from the 2009 pandemic response and vaccination program. It is clear that the experience and planning that was gained from preparing to combat an avian influenza threat played a significant role in the response to 2009 H1N1. The 2009 campaign could not have been as successful without the cooperation of the WHO and influenza reference laboratories in the rapid provision of seed virus, nucleotide sequence data and reagents. While the time lines for the production of vaccine may appear to be long in the face of an ongoing pandemic, one must acknowledge the degree of development and QC required to produce a novel vaccine. A reverse genetics approach in theory should have been more efficient than one using classical reassortant methods. We now understand why this method did not prevail. Hopefully this should not be an issue in the future and could yield a time saving of up to two weeks. Production of vaccine was delayed by the requirement for containment levels at manufacturing scale due to uncertainties in the pathogenic potential of the virus. Our experience with pandemic viruses has continued to progress and it was fortunate that the infection was mild in the majority of cases though severe in some. CSL's adult clinical trial in Australia provided data within four months and established an effective and safe dose which had implications for pandemic plans worldwide**.** If we review the time taken from public notification of a health event of international concern to the time where vaccine was being manufactured for the 2009 campaign; this was within eight weeks. For this pandemic, Australia responded quickly and an egg-based manufacturer of an influenza vaccine was able to meet the needs of Australia. The question that remains is how to improve this to combat a threat more pathogenic than the pandemic virus of 2009.

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Biography

Dr Steven Rockman is currently the head of the Influenza Innovation department at CSL Ltd (Parkville). The Influenza Innovation department role is to optimise the development of influenza vaccines for both seasonal and pandemic influenza. Earlier in his career he completed a Bachelor of Applied Science in Microbiology at RMIT (Melb), followed by a Master of Science in Immunology in the Department of Surgery, University of Melbourne and subsequently a PhD in Molecular Biology in the Department of Pathology, University of Melbourne. A large proportion of his work (18 years) was in diagnostic oncology at the Peter MacCallum Hospital and Royal Melbourne Hospital. For the past 10 years he has held several positions in the Research and Development Division at CSL Ltd, specialising in the areas of adjuvanted vaccines and more recently influenza. His current interest is the molecular biology and immunology of influenza.