Australian Government
Department of Health
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR 137

Commercial supply of
genetically modified live attenuated influenza vaccines

Applicant: AstraZeneca Pty Ltd

January 2016

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Summary of the Risk Assessment and Risk Management Plan

for

Licence Application DIR 137

Decision

The Gene Technology Regulator (the Regulator) has decided to issue a licence for this application. The licence authorises import, transport, storage and disposal of the attenuated genetically modified (GM) influenza vaccines, known as FluMist®, for the purposes of their commercial supply as therapeutic products.

A Risk Assessment and Risk Management Plan (RARMP) for this application was prepared by the Regulator in accordance with requirements of the *Gene Technology Act 2000* (the Act) and corresponding state and territory legislation. A wide range of experts, agencies and authorities, and the public were also consulted before the RARMP was finalised. The RARMP concludes that this commercial release poses negligible risks to human health and safety and to the environment, and consequently, no specific risk treatment measures are proposed. However, general licence conditions have been imposed to ensure that there is ongoing oversight of the licence.

Before these GM influenza vaccines can be used as therapeutic agents, AstraZeneca must also obtain regulatory approval from the Therapeutic Goods Administration (TGA). Medicines and other therapeutic goods for sale in Australia are required to be assessed for quality, safety and efficacy under the *Therapeutic Goods Act 1989* and must be included in the Australian Register of Therapeutic Goods (ARTG). The TGA consult the OGTR during the assessment of applications for therapeutic products that are, or contain, genetically modified organism (GMOs). AstraZeneca will also need approval from the Department of Agriculture and Water Resources for import of the GM vaccines.

The application

Application number	DIR 137
Applicant	AstraZeneca Pty Ltd (AstraZeneca)
Project title	Commercial supply of attenuated GM influenza vaccines[1]
Parent organism	Human *influenza A virus* and *influenza B virus*
Introduced genes and modified trait	Hemagglutinin and neuraminidase genomic segments from influenza strains recommended by the Australian Influenza Vaccine Council (AIVC) for seasonal influenza vaccines (antigen expression)
Proposed release date	Ongoing from the date of approval
Proposed locations	Locations throughout Australia where vaccines are normally stored and administered (subject to registration by the Therapeutic Goods Administration)

[1] The title of the project as supplied by the applicant is 'Quadrivalent live attenuated influenza vaccine (Q/LAIV) to prevent seasonal influenza (FluMist® Quadrivalent) and monovalent pandemic live attenuated influenza vaccine (P/LAIV) suspensions for nasal administration.'

Proposed activities	Import, transport, storage and disposal of the GM influenza vaccines for the purpose of their commercial supply as therapeutic products (administration is subject Therapeutic Goods Administration approval)

AstraZeneca proposes the commercial supply of attenuated GM influenza vaccine strains for use as vaccines. Two types of influenza (flu) vaccines are proposed. The first is a seasonal flu vaccine which would contain a mixture of four GM vaccine strains to target currently circulating flu viruses. The second is a contingency flu vaccine which would contain a single GM vaccine strain to target a pandemic flu strain, should one arise.

Subject to approval by the Therapeutic Goods Administration (TGA), the GM vaccines would be manufactured overseas and imported into Australia. They would be administered as a nasal spray by qualified healthcare professionals at locations where flu vaccines would generally be dispensed, such as medical practices and pharmacies.

Influenza A and influenza B viruses are highly infectious pathogens which are endemic in Australia. These viruses transmit predominantly through aerosol droplets generated when a carrier coughs or sneezes, and flu infections peak during the winter months.

Symptoms usually present as a sudden onset of mild respiratory illness. In healthy individuals, infection normally resolves in under two weeks but the elderly, young children, pregnant women and the immunocompromised can suffer more severe symptoms.

In infected individuals, the main immune response is induced by two viral surface proteins, hemagglutinin and neuraminidase. The initials of these two proteins are used when naming influenza A virus subtypes (eg H1N1 or H3N2).

The parent organisms of the GM vaccine strains are attenuated influenza A or influenza B strains that are temperature-sensitive, and therefore do not grow well at body temperature. As a result, GM vaccine strains based on these parent organisms are weakened and less virulent than naturally occurring flu strains.

In the proposed GM vaccine strains, the attenuated parental flu strains would be modified by incorporation of antigens from the current circulating flu strains to provide protection against these flu strains. As flu viruses change rapidly, every year the World Health Organisation (WHO) issues advice on the antigen composition of flu vaccines for the coming flu season. The WHO's recommendations are evaluated by the Australian Influenza Vaccine Committee (AIVC) which provides advice to the TGA on the composition of the seasonal flu vaccine to be supplied each year in Australia. Seasonal flu vaccines, including those currently approved for use in Australia, are reformulated every year based on this advice. The application is for GM flu vaccines which are modified with the recommended antigens for each flu season.

These GM vaccines have not previously been released commercially in Australia. However, vaccines based on the attenuated, temperature-sensitive influenza parent strains are currently approved for use in the United States of America (USA), Canada and the European Union (EU). These GM vaccines were first released in the USA during the 2003/2004 flu season (as FluMist®) and in the EU during the 2012/2013 flu season (as Fluenz®). They are currently authorised in the USA and Canada as FluMist® Quadrivalent, and in the EU as Fluenz Tetra. The authorisations for these vaccines are held by either AstraZeneca or MedImmune, a subsidiary of AstraZeneca.

Risk assessment

The risk assessment concludes that risks to the health and safety of people, or the environment, from the proposed dealings are negligible.

The risk assessment process considers how the genetic modifications and proposed activities conducted with the GM vaccines might lead to harm to people or the environment. Risks are characterised in relation to both the seriousness and likelihood of harm, taking into account information in the application (including proposed controls), relevant previous approvals and current scientific/technical knowledge. Both the short and long term impact are considered.

To avoid duplication of regulatory oversight, the Regulator does not assess risks to people receiving or administering the GMO as a therapeutic. However, import, transport and disposal are regulated under the *Gene Technology Act 2000* (the Act), and the Regulator has assessed risks posed to people and to the environment associated with these activities.

Credible pathways to potential harm that were considered included whether or not expression of the introduced genes and genetic modifications could alter characteristics that may impact on the disease burden from the GM vaccine strains, or produce unintended changes in viral characteristics. The opportunity for gene transfer to other organisms, and its effects if it were to occur, was also considered.

The principal reasons for the conclusion of negligible risks are that:

- exposure to the proposed GM flu vaccines would be minimised by well-established clinical, import, transport, storage and disposal procedures
- influenza virus survival outside of a host is limited to short periods, and it is susceptible to common chemical decontaminants
- the proposed GM flu vaccine strains would contain a number of naturally occurring mutations that lead to their attenuation, reducing their ability to replicate, persist and be transmitted
- all of the genes and genome segments in the GM flu vaccines would be derived from existing non-GM flu strains
- there would be limited opportunity for the GM vaccine strains and circulating flu strains to reassort, and resulting reassortants would not be expected to be more virulent.

Risk management plan

The risk management plan describes measures to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan is given effect through licence conditions.

As the level of risk is assessed as negligible, specific risk treatment is not required. However, the Regulator has imposed licence conditions under post-release review (PRR) to ensure that there is ongoing oversight of the release and to allow the collection of information to verify the findings of the RARMP. The licence also contains a number of general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements, which include an obligation to report any unintended effects.

Table of contents

TABLE OF FIGURES

Abbreviations

the Act	the Gene Technology Act 2000
APVMA	Australian Pesticides and Veterinary Medicines Authority
ARTG	Australian Register of Therapeutic Goods
cDNA	complementary DNA
cRNA	complementary RNA
DAWR	Department of Agriculture and Water Resources
DIR	Dealings involving Intentional Release
EID_{50}	median egg infectious dose
flu	influenza
FSANZ	Food Standards Australia New Zealand
GM	genetically modified
GM flu vaccines	live attenuated GM influenza vaccines
GMO	genetically modified organism
GTTAC	Gene Technology Technical Advisory Committee
HID_{50}	median human infectious dose
IgA	immunoglobulin A
LAIV	live attenuated influenza vaccine
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
OGTR	Office of the Gene Technology Regulator
pandemic GM flu vaccine	pandemic live attenuated GM influenza vaccine
PCK	primary chick kidney
PI	Product information
Q/LAIV	quadrivalent live attenuated influenza vaccine
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
seasonal GM flu vaccine	seasonal GM live attenuated influenza vaccine
SPF	specific pathogen free
T/LAIV	trivalent live attenuated influenza vaccine
$TCID_{50}$	median tissue culture infective dose
TGA	Therapeutic Goods Administration
UV	ultraviolet
vRNA	viral RNA

Chapter 1 Risk assessment context

Section 1 Background

1. Australia's national regulatory system for gene technology comprises the *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 (the Regulations), an inter-governmental agreement and corresponding legislation that is being enacted in each State and Territory.

2. The objective of the Act is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or because of gene technology, and by managing those risks through regulating dealings with genetically modified organisms (GMOs).

3. An application has been submitted under the Act for a licence to conduct Dealings involving the Intentional Release (DIR) of GMOs into the Australian environment.

4. Since risks posed by GMOs are subject to regulation under the Act, before a licence can be issued, the Gene Technology Regulator (the Regulator) must assess potential risks to the health and safety of people or the environment presented by the proposed release. Decisions on licence applications are based on the Risk Assessment and Risk Management Plan (RARMP). This chapter of the RARMP describes the risk assessment context as established within the regulatory framework and application-specific parameters (Figure 1).

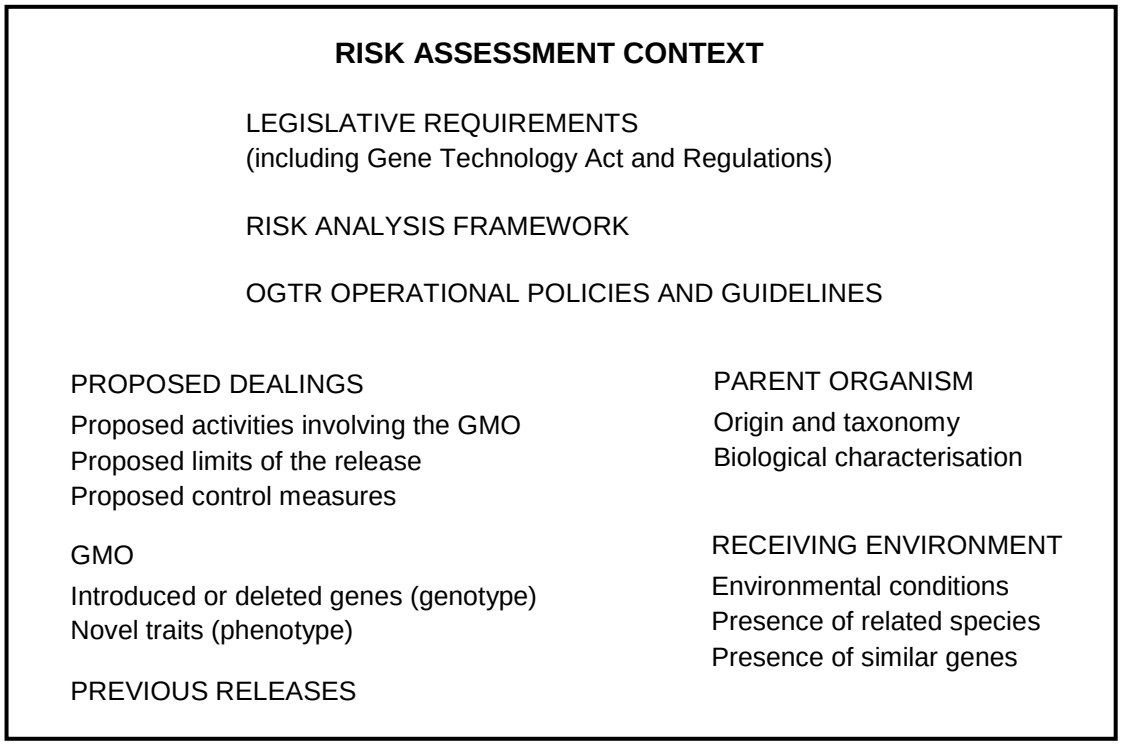


Figure 1. Summary of parameters used to establish the risk assessment context

Section 2 Regulatory framework

5. The Regulations and Sections 50, 50A and 51 of the Act outline the matters that the Regulator must consider and who must be consulted when preparing the RARMP.

6. This application is for commercial purposes and as such, it cannot be considered as limited and controlled release application under Section 50A of the Act. This means that two rounds of consultation are required:

- In the first round, required by Section 50(3), the Regulator must seek advice from prescribed experts, agencies and authorities on matters relevant to the preparation of the RARMP. Advice was sought from the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government authorities/agencies prescribed in the Regulations, all Australian local councils and the Minister for the Environment. The issues raised in their submissions are summarised in Appendix A.
- In the second round, required by Section 52 of the Act, the Regulator must seek advice on the RARMP from the aforementioned groups as well as the public. Advice from the prescribed experts, agencies and authorities for the second round of consultation, and how it was taken into account, is summarised in Appendix B. No submissions were received from members of the public.

7. The Risk Analysis Framework (OGTR 2013) explains the Regulator's approach to the preparation of RARMPs in accordance with the legislative requirements. The Office of the Gene Technology Regulator (OGTR) has also developed several operational policies and guidelines that are relevant to DIR licences. These documents are available from the OGTR website.

2.1 Interface with other regulatory schemes

8. Gene technology legislation operates in conjunction with other regulatory schemes that regulate GMOs or genetically modified (GM) products in Australia. Dealings conducted under a licence issued by the Regulator may also be regulated by the Therapeutic Goods Administration (TGA), Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority (APVMA), the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and the Department of Agriculture and Water Resources (DAWR). Dealings may also be subject to the operation of State legislation declaring areas to be GM, GM-free, or both, for marketing purposes.

9. DAWR administers Australian biosecurity conditions for importation of biological products under the *Biosecurity Act* 2015, and the importation of GM vaccines requires a permit from DAWR.

10. Medicines and other therapeutic goods for use in Australia are required to be assessed for quality, safety and efficacy under the *Therapeutic Goods Act 1989* and must be included in the Australian Register of Therapeutic Goods (ARTG). The TGA is responsible for administering the provisions of this legislation. The labelling, handling, sale and supply of scheduled medicines are regulated through the *Scheduling Policy Framework for Medicines and Chemicals* (AHMAC 2015).

11. Where a GMO is proposed to be a registered therapeutic, the TGA has regulatory responsibility for quality, efficacy and patient safety. To avoid duplication of regulatory oversight, administration/use of the GMO as a therapeutic is not regulated under gene technology legislation. The Regulator notes that the TGA would assess risks to patients, vaccine administrators, vaccine recipients and their carers who may be present during administration of the GM flu vaccines. The TGA's assessment would also consider viral shedding after vaccination. The Regulator has assessed risks posed to other people and to the environment associated with import, transport, storage and disposal of the GM flu vaccines. These activities are subject to regulation under the *Gene Technology Act 2000*.

12. Requirements for the safe transport, storage and distribution of Schedule 4 medicines such as vaccines are specified through the *Australian Code of good wholesaling practice for medicines in schedules 2, 3, 4 & 8* (NCCTG 2011). It also specifies practices for dealing with

damaged or spilled stock. This Code applies to persons and organisations that store or supply the GM flu vaccines, including importers and distribution agents for the manufacturer. The provisions of this Code are applied through applicable State and Territory therapeutic goods/drugs and poisons legislation, and/or State or Territory wholesaler licensing arrangements. Destruction of unused or outdated vaccines is regulated by the States and Territories. These procedures appropriately manage risks that may be associated with unintentional exposure to vaccines and other medicines.

13. Quality aspects assessed by the TGA include batch-to-batch consistency in vaccine composition, purity and potency. For influenza vaccines, the production and quality control data for each batch are assessed prior to supply in Australia through a batch release program operated by the TGA in accordance with recommendations of the World Health Organization. Quality aspects assessed by DAWR include the potential presence of organisms other than the GMOs in the vaccine. The Regulator has assessed the genetic stability of GMOs and risks from persistence of the GMOs or the introduced genes in the environment in the context of transport, storage and disposal.

14. The TGA would also consider the toxicological and allergenicity profile of the whole vaccine, including excipients, by-products and impurities from flu vaccine manufacture. The Regulator does not assess excipients and would not assess manufacturing by-products and impurities unless they are GM products.

Section 3 The proposed dealings

15. AstraZeneca Pty Ltd (AstraZeneca) has proposed the commercial supply of two types of flu vaccines based on live attenuated GM influenza viruses (the GMOs). The first is a seasonal flu vaccine, known as FluMist® Quadrivalent (FluMist), which would contain a mixture of four GM live attenuated flu vaccine strains to target currently circulating influenza viruses. The seasonal flu vaccine would be formulated and supplied annually. The second vaccine is a pandemic flu vaccine which would contain a single GM vaccine strain to target a pandemic influenza strain. Formulation and supply of the pandemic vaccine is contingent on the emergence of a pandemic strain.

16. The GM vaccines would be imported and provided as a sterile liquid contained in single-use 0.5 mL nasal sprayers for administration as a nasal spray by qualified healthcare professionals at locations where flu vaccines would normally be dispensed, such medical practices and some pharmacies. The sprayer, which delivers a high speed vaccine aerosol, comes with a dose divider. The first half-dose is delivered to one nostril, the dose divider is removed and the second half-dose is delivered into the other nostril. The vaccine recipient is in an upright position during vaccine delivery.

17. These GMOs are not proposed to be manufactured in Australia.

18. The therapeutic use of vaccines is regulated by the TGA. For the ongoing commercial supply of GM flu vaccines, the dealings assessed by the Regulator are:

- import;
- transport;
- disposal; and
- the possession (including storage), supply or use of the GMOs for the purposes of, or in the course of, any of the above.

3.1 Details of the proposed activities

19. The GM flu vaccines would be imported, in the same way as other live vaccines, from MedImmune manufacturing facilities in the United States of America (USA) and the United Kingdom (UK). Import of the GM flu vaccines into Australia requires a permit from DAWR.

20. AstraZeneca propose to distribute the GM flu vaccines to healthcare facilities, such as pharmacies and medical practices, where flu vaccines are normally available.

21. AstraZeneca proposes to supply the vaccine in single dose dispensers sealed in tamper proof packaging. These packs would be placed in single unit cartons or multipack cartons and the cartons packed in corrugated cardboard shipping cartons for distribution.

22. Storage, handling and transport would be in accordance with the *Australian Code of good wholesaling practice for medicines in schedules 2, 3, 4 & 8* (NCCTG 2011) and the World Health Organisation's (WHO) *Good distribution practices for pharmaceutical products* (WHO 2010).

23. Storage and transport within Australia would be conducted by a commercial courier experienced in the distribution of pharmaceutical products, such as live vaccines, which must be handled securely and maintained within a strict temperature regime (2°C to 8°C).

24. The GM flu vaccines would be held in central storage facilities in Sydney, and distribution sites in Brisbane, Melbourne and Perth before supply to healthcare facilities.

25. At healthcare facilities, the GM flu vaccines would be stored according to the National vaccine storage guidelines. Storage would be in a secure temperature controlled freezer with access limited to authorised personnel.

26. Following administration, all residual vaccine and associated waste, such as sprayers, would be discarded into appropriate clinical waste containers according to institutional procedures for the disposal of clinical waste. This would be disposed of by healthcare staff in accordance with the requirements of the Work Health and Safety Act 2011 and related state and territory legislation.

27. Unused expired vaccine would be disposed of at the healthcare or storage facilities in accordance with the relevant state and territory legislation procedures for medical waste disposal. The vaccine would be inactivated by clinical waste disposal methods, such as high temperature incineration, approved by the Environmental Protection Agency of each State/Territory.

28. Secondary and tertiary packaging will be disposed of in normal waste streams.

Section 4 The parent organism

29. Human influenza A and B viruses are highly infectious viruses that cause human influenza (flu), a contagious disease of the respiratory system. Flu viruses generally transmit through large aerosol droplets that are generated when a carrier coughs, sneezes or talks. They are also transmitted when contaminated surfaces, such as hands or tissues, make contact with the mucous membranes.

30. In temperate climates, the annual influenza epidemic peaks during winter while in the tropics, it can occur throughout the year. The annual attack rate or proportion of people who become ill after exposure is estimated at 5%–10% in adults and 20%–30% in children (WHO 2014). Influenza viruses are endemic in Australia, and each year they cause about 13,500 hospitalisations and over 3,000 deaths among Australians aged over 50 years (Department of Health 2015).

31. The onset of flu is sudden and it is accompanied by malaise, persistent runny nose, cough, headache, sore throat and high fever. Infection normally resolves in less than two weeks without the need for treatment in healthy individuals. Symptoms may be reduced if antiviral drugs are administered within 48 hours of initial symptoms (Stiver 2003). Fatalities can occur when individuals who are weakened by influenza develop pneumonia and bronchitis from a secondary bacterial or viral infection.

32. Those at highest risk of the more severe symptoms include the elderly, young children, pregnant women and the immunocompromised. Influenza generally aggravates respiratory conditions such as asthma.

33. The shedding of detectable amounts of influenza virus begins the day before symptoms appear. Viral replication peaks approximately 48 hours after infection and declines slowly from there. Shedding continues for a further three to five days in adults and up to seven days in young children (Wright et al. 2007).

34. Cells infected with influenza viruses undergo apoptosis (Mori et al. 1995; Fesq et al. 1994) and are susceptible to phagocytosis. In response to the infection, the host increases the production of protective but pro-inflammatory cytokines. These attract leucocytes to the site of infection, resulting in tissue damage and the associated respiratory symptoms. The inflammatory cells also release pyrogenic cytokines inducing fever (Brydon et al. 2005).

4.1 Naming of influenza viruses

35. The naming of influenza viruses is based on the antigenic type (e.g. A for influenza A virus, B for influenza B virus), host of origin, geographical origin, isolate number and year of isolation. Influenza A virus names also include the subtype (based on the classification of haemagglutinin and neuraminidase). For human influenza viruses, the host of origin is omitted (Figure 2).

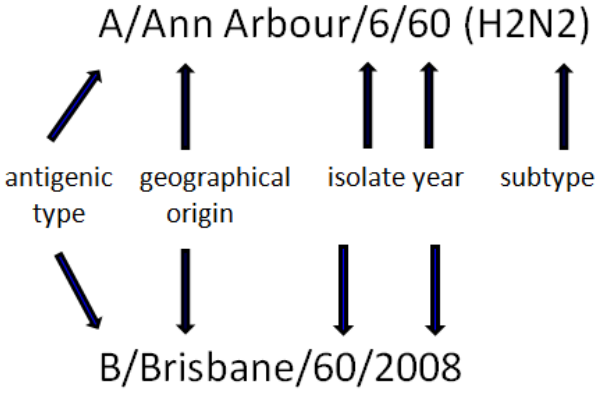


Figure 2. Naming of human influenza viruses

4.2 Use of cold adapted influenza viruses for GM flu vaccine production

36. The parent organisms for the proposed GM vaccines are:

- a stable attenuated strain of influenza A virus derived from A/Ann Arbor/6/60(H2N2)
- a stable attenuated strain of influenza B virus derived from B/Ann Arbor/1/66.

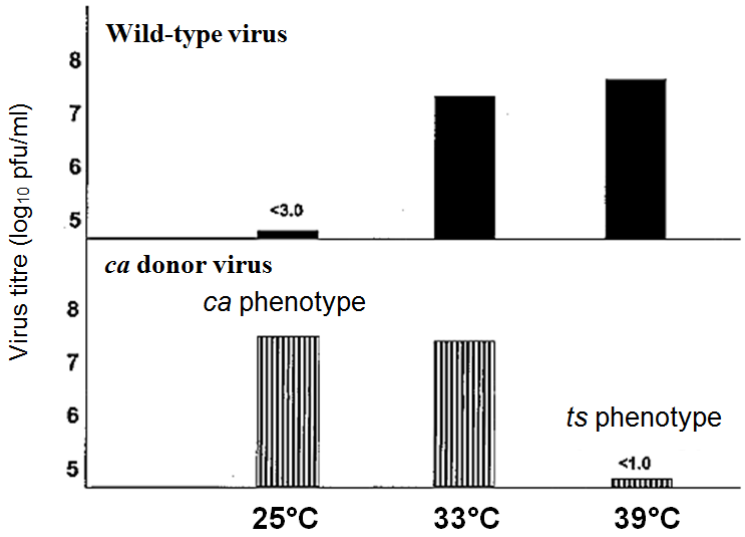
37. These strains were first reported in 1968 by Hunein Maassab, who adapted them for growth at 25°C by the serial passaging of the above influenza virus isolates in primary chick kidney (PCK) cells at sequentially lower temperatures (Maassab 1968). When tested in ferrets, which are a model organism for the study of human influenza (see paragraph 100), these strains

heightened the antibody response but did not cause disease (Maassab 1969; Maassab et al. 1969).

38. During vaccine production, these strains are called 'Master Donor Viruses' (MDVs) and they will be referred to as A-MDV (for type A) and B-MDV (for type B). They would be used for the construction of the GM flu vaccine strains.

39. Cold-adaptation is characterised by more efficient replication at 33°C than at human body temperature (37°C). It results from several mutations that confer three selectable phenotypes, cold-adapted (*ca*), temperature sensitive (*ts*) and attenuated (*att*), which are simultaneously carried by the attenuated strains, defined as follows:

- *ca* phenotype – growth at low temperature (25°C) is not more than 100-fold different than at the normal permissive temperature (33°C), as measured by the virus' median tissue culture infective dose ($TCID_{50}$) observed in PCK cell cultures (Figure 3)
- *ts* phenotype – growth rate at 39°C is at least 100-fold less than at 33°C, as measured by the virus $TCID_{50}$ observed in PCK cell cultures (Figure 3)
- *att* phenotype – does not produce classic influenza-like illness in a ferret, with replication detected in the nasal turbinates, but not in the lungs, as determined by the median Egg Infectious Dose (EID_{50}) assay.



Adapted from Murphy & Coelingh 2002

Figure 3. Comparison of replication of wild-type and cold-adapted A/Ann Arbor/6/60 at different temperatures

4.3 Genome and virion structure of influenza A and B viruses

40. Influenza virions are characterised by distinctive spikes of haemagglutinin and neuraminidase, the two surface glycoproteins. The virions range in shape from ovoid to filamentous (Chu et al. 1949). Freshly isolated influenza virus is largely filamentous but the ovoid form dominates when grown in eggs in the laboratory.

41. The genome of influenza A and B viruses is made up of eight single-stranded, negative-sense RNA segments. As shown in Table 1, each segment is referred to either by its position when the segments are listed in order of decreasing size or by a code based on the largest protein encoded by the segment. Additional proteins are made by alternate splicing, alternate reading frames and leaky ribosome scanning.

Table 1. Segments of influenza A virus and influenza B virus

Segment number	Segment code	Largest protein encoded by segment
1	PB2	polymerase basic 2
2	PB1	polymerase basic 1
3	PA	polymerase acidic
4	HA	haemagglutinin
5	NP	nucleoprotein
6	NA	neuraminidase
7	M	matrix
8	NS1	non-structural protein

42. The RNA segments do not exist as naked RNA but are always associated with multiple copies of viral nucleoprotein (NP) that protect it from host ribonucleases. Each genomic segment exists as a ribonucleoprotein (RNP) with the viral RNA wrapped around the outside of the nucleoprotein (NP) oligomer and attached to the polymerase complex (Figure 4).

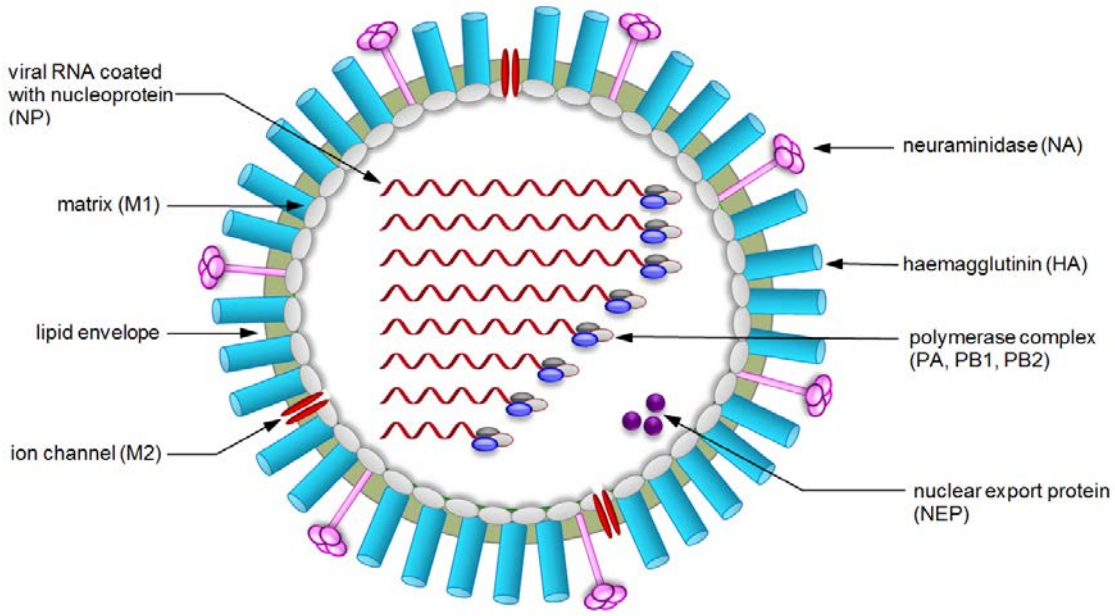


Figure 4. Schematic representation of the influenza virus particle

43. The heterotrimeric polymerase complex comprises polymerase basic 1 (PB1), polymerase basic 2 (PB2) and polymerase acidic (PA). PB1 is a RNA-dependent RNA polymerase that elongates the viral RNA during replication and transcription in infected cell nuclei. Influenza viruses cannot synthesize their own 5′-capped RNA primers. PB2 and PA are involved in cap-snatching which is the stealing of 5’ caps from host mRNAs. PB2 binds the host mRNA while PA, which is an endonuclease, removes the host message by cleaving the mRNA. PB1 then uses the host 5’ cap to prime viral mRNA synthesis.

44. The eight RNPs are enclosed in a layer of matrix protein (M1). M1 is the most abundant protein in the virus; it drives virus budding and controls the intracellular trafficking of RNPs. The viral envelope, which is a lipid bilayer derived from the host cell membrane with viral proteins inserted, lies just outside the M1 layer.

45. In influenza A viruses, the lipid envelope has three integral membrane proteins, namely haemagglutinin, neuraminidase and proton-selective ion channel (M2). Influenza B viruses have the equivalent three proteins (HA, NA and BM2) as well as a fourth protein (NB). The reading frame of NB overlaps with that of NA and its function is not known. M2 and BM2 are proton channels essential for uncoating of viral particles.

46. Influenza viruses encode virulence determinants such as NS1 and PB1-F2 (second reading frame of PB1) in infected cells. NS1 limits host interferon production and regulates apoptosis (Engel 2013). Influenza viruses suppress cell-mediated immunity and block apoptosis to prevent infected cells being cleared by macrophages (Koyama et al. 2000). This allows time for the virus to multiply.

4.4 Haemagglutinin and its role in cellular entry of influenza viruses

47. Haemagglutinin has two main functions: receptor binding and facilitating the fusion of viral and host membranes. This homotrimeric, transmembrane protein is the most abundant protein on the virion surface. Human influenza A viruses have one of three types of haemagglutinin, H1, H2, or H3.

48. The major target cells for human influenza viruses are epithelial cells lining the respiratory tract. The exposed surfaces of these cells are glycosylated and the glycans have sialic acid which is the receptor for influenza viruses. Sialic acids only occur as the terminal sugar in glycans.

49. Newly budded viruses are not infectious as their intact haemagglutinin (HA0) must first be activated by proteolytic cleavage into two peptides (HA1 and HA2). Cleavage generates a short hydrophobic sequence at the N-terminus of HA2 called the fusion peptide and this peptide is required to initiate fusion of viral and host membranes. HA1 forms the receptor binding site (see Figure 5 in Section 4.7, below). HA1 and HA2 remain intertwined after cleavage. Since infection cannot proceed in the absence of the fusion peptide, the pathogenicity of any viral subtype is determined, in part, by the ease of HA0 cleavage.

50. Glycosylation of haemagglutinin affects both antigenicity (Tate et al. 2014) and virulence (Sun et al. 2013). Glycans can hide or modify antigenic sites on haemagglutinin. Mutations that increase glycosylation are associated with antigenic escape. Glycans can also modulate virulence because they interfere with viral attachment to the host cell. The receptor-binding site on haemagglutinin is flanked by glycans, and the sialic acid receptor on the host cell is itself part of a glycan. Steric hindrance occurs between these two sets of glycans and consequently, mutations that decrease the number of glycosylation sites on haemagglutinin can increase receptor affinity and virulence (Wagner et al. 2000). Mutations that increase glycosylation must balance increased antigenic escape with decreased receptor affinity.

51. After receptor binding, influenza viruses enter the host cell either by receptor mediated endocytosis or macropinocytosis, depending on their morphology (Rossman et al. 2012). These entry mechanisms are triggered when a virus attaches to the cell surface. The internalised virus is encapsulated in an endosome and at this point, the viral RNPs are separated from the host cytoplasm by the endosomal membrane, the viral envelope and the capsid.

52. Release into the cytoplasm requires the fusion of the viral and host (endosomal) membranes but membrane fusion is an energetically unfavourable process. Mediation of membrane fusion is the second function of haemagglutinin. The cell acidifies the contents of the endosome to enable destruction by acid hydrolases. The drop in pH triggers a conformational change in haemagglutinin. This exposes the previously buried fusion peptide, which then inserts into the endosomal membrane, resulting in haemagglutinin being attached to

both membranes. Several haemagglutinin trimers, acting in concert, distort the membranes and pores form.

53. The virus must be uncoated concurrently with membrane fusion. When the pH drops in the endosome, protons enter the interior of the virus particle through the proton-selective ion channel (M2 in influenza A virus, BM2 in influenza B virus). Acidification of the virus particle interior disrupts protein-protein interactions and the M1 protein dissociates from the RNPs.

54. The net result of the two events triggered by low pH is the release of the viral RNPs into the cytoplasm of the infected cell. Their subsequent import into the nucleus allows viral replication to begin. The uncoating process takes about 10 minutes (Yoshimura & Ohnishi 1984).

4.5 Viral replication

55. Viral replication does not involve a DNA intermediate. The negative sense genomic RNA (vRNA) serves as the template for the synthesis of both mRNA and the complementary genomic strand of RNA (cRNA). cRNA is the full length transcript of vRNA while mRNA is a truncated transcript. The mechanism that regulates the quantities of either transcript is unknown. Transcription and replication of the influenza virus occur in the nucleus.

56. Influenza viruses replicate very quickly. The half time from attachment to cellular entry is 25 minutes, and entry into the nucleus takes a further 10 minutes (Knipe & Howley 2013). The time from cellular entry to shedding from infected cells is estimated at 6 hours (WHO 2015).

57. Up to 90% of virus-infected cells fail to release infectious progeny. Analysis of viral progeny shows that propagation-competent virions containing one each of the eight RNPs are outnumbered by semi-infectious virions with an incomplete set of RNPs (Brooke et al. 2014). Semi-infectious virions cannot support multiple rounds of viral replication themselves but if they and a complete virion co-infect a host cell, the presence of a second copy of any segment increases the likelihood of reassortment. Some of the reassortants may have a permutation of segments that increase viral fitness. The propagation-competent fraction of virions varies widely between different strains of influenza virus.

4.6 Neuraminidase

58. Neuraminidase hydrolyses the glycosidic bond between sialic acid (N-acetyl neuraminic acid) and galactose.

59. Haemagglutinin and neuraminidase are glycoproteins, and sialic acid is present as a terminal sugar in their glycans. Without neuraminidase, large aggregates of viruses form at the surface of the infected cell, due to binding between haemagglutinin on the newly budded viral particles and sialic acid on the cell surface, as well as between haemagglutinin and sialic acid on adjacent particles. Aggregation of viral particles is the main reason flu viruses do not spread in the absence of neuraminidase (Liu et al. 1995; Palese et al. 1974).

60. In the absence of neuraminidase, viral particles are released slowly from the receptor through natural dissociation but the presence of neuraminidase facilitates the release.

61. The respiratory epithelium, which is the target tissue of influenza viruses, is protected by a layer of mucus up to 50 µm thick. The main protein in mucus is mucin, a highly glycosylated protein with sialic acids decoys that mimic the true receptors on epithelial cells. Influenza viruses that bind these unproductive receptors are trapped in the mucus and removed during mucus clearing, which is part of the innate defence system. Neuraminidase frees the viruses from unproductive binding of the decoy receptor, thereby enabling them to penetrate the protective mucus layer during infection.

62. Neuraminidase also increases virulence of flu viruses by compromising the immune defences at the mucosal surface. It removes sialic acid from T-cells in the mucosa and from immunoglobulin A (IgA)-producing B cells, adversely affecting their function. It also desialidates IgA, resulting in its being cleared more quickly by the hepatic system (Bhatia & Kast 2007).

63. Neuraminidase is not as abundant as haemagglutinin on the viral surface but its catalytic site has to reach all receptor bound-haemagglutinin molecules. Movement of neuraminidase and haemagglutinin is restricted as they are both attached to the viral matrix. Haemagglutinin may also be attached to its receptor, which is itself part of a glycoprotein or glycolipid. These steric restrictions are overcome by the structure of neuraminidase. The catalytic domain is at the end of a long flexible stalk and neuraminidase extends marginally further than haemagglutinin from the viral envelope. This configuration increases the flexibility of the molecule and the likelihood of neuraminidase reaching its substrate.

64. Each neuraminidase monomer has two receptor binding sites. One of these is the catalytic site. The function of the second site is not known.

65. In human influenza viruses, two neuraminidase subtypes are described for influenza A viruses and one neuraminidase subtype has been identified for the influenza B viruses.

4.7 Antigenic determinants

66. The antigenic determinants on the surface of the influenza virus are haemagglutinin and neuraminidase. Haemagglutinin is considered the main antigenic determinant as it outnumbers neuraminidase by between 4:1 to 10:1 on the virion surface (Knipe & Howley 2013; Mitnaul et al. 2000).

67. Different antibodies are produced against the globular head and the stalk of haemagglutinin (Figure 5). The head is distal to the viral envelope, highly exposed to the external environment and the main target of antibodies. It corresponds approximately to HA1, the domain that mediates receptor binding. Antibodies against this region interfere with receptor binding and hence they are neutralising and strain specific. Due to constant immune pressure, the globular head is a highly plastic domain which tolerates mutations and insertions better than other influenza proteins (Heaton et al. 2013).

68. The stalk is proximal to the viral membrane and less accessible to antibodies as it is partially shielded from the exterior by the head. It approximates HA2, the domain that facilitates fusion of the viral envelope with the host endosomal membrane. Antibodies against this region are less potent but cross-reactive (Palese & Wang 2011; Kaminski & Lee 2011) since the haemagglutinin stalk is among the most highly conserved regions in the influenza virus (Westgeest et al. 2014).

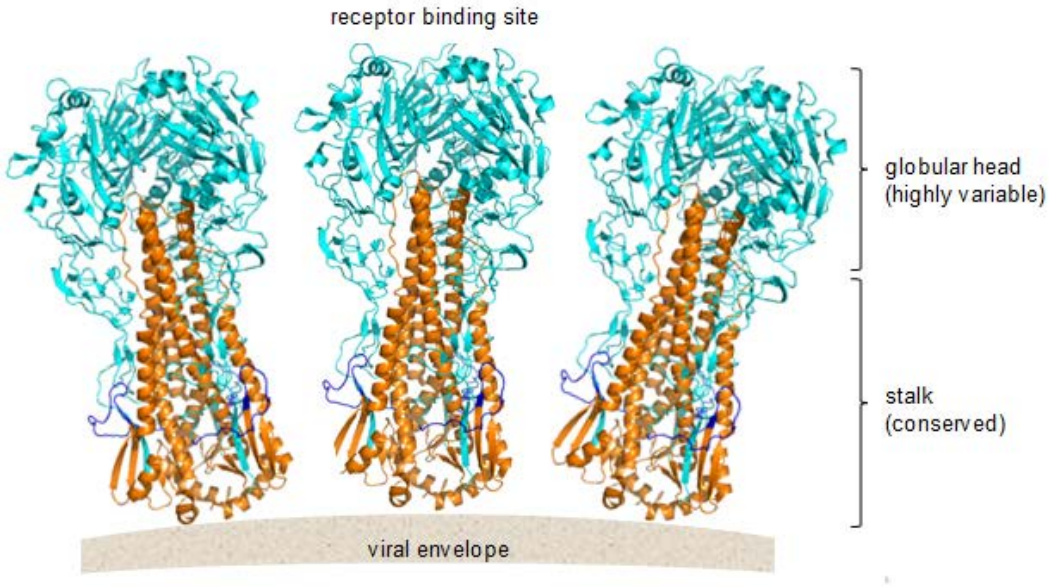


Figure 5. Influenza haemagglutinin domains

HA1 is shown in cyan, HA2 in gold, fusion peptide in blue.

69. Neuraminidase-specific antibodies are not neutralising in that they do not block attachment of the virus to the target cell. However, they can markedly reduce virus replication (Kilbourne et al. 1968) and in doing so, offer partial protection against influenza. This would shorten the severity and duration of illness caused by strain with a novel haemagglutinin but the same neuraminidase (Sandbulte et al. 2007; Kilbourne et al. 2004). Antibodies are raised against both the catalytic domain and the stalk of neuraminidase, as described above for haemagglutinin.

70. The proton channel (M2 in influenza A viruses and BM2 in influenza B viruses), which is partially exposed to the exterior of the virion, is the target of cross-reactive cytotoxic T-cells (Lamb et al. 1985). These proteins are less accessible to antibodies than are haemagglutinin and neuraminidase as they are integral membrane proteins and are shielded by the much larger haemagglutinin and neuraminidase molecules. There are an estimated 16 to 20 molecules of M2/BM2 per virion (Samji 2009).

71. Exposure to influenza viruses can induce heterosubtypic immunity (HSI). This is broad immune cross-protection when challenged with an influenza A virus that differs from the one used for primary infection e.g. immunity to an H1N1 strain of influenza can protect against infection with an H3N2 strain (Nguyen et al. 2007; Garcia et al. 2009). HSI may arise from antibodies against the internal viral proteins.

4.8 Mutation and reassortment

72. Point mutations in the main antigenic determinants, haemagglutinin and neuraminidase, result in antigenically-novel viruses that can cause epidemics in previously resistant or immune hosts. This effect is called antigenic drift.

73. Point mutation rates are very high in single stranded RNA viruses for the following reasons. Firstly, cytosine can spontaneously deaminate to uracil, a natural component of RNA. Uracil, being the smallest of the bases, can mispair with any one of the RNA bases including another uracil. Secondly, viral polymerases lack an exonuclease to excise misincorporated bases and consequently, have no proofreading function. Thirdly, in single stranded viruses, the absence of a complementary strand means that there are no secondary structure changes to

signal the presence of an error. The mutation rate from nucleotide substitutions in RNA viruses is estimated at one error per replicated genome (Sanjuan et al. 2010; Drake 1993).

74. Point mutations occur in influenza A viruses approximately three times faster than in influenza B viruses in the absence of immune or drug selection pressure in laboratory experiments (Nobusawa & Sato 2006).

75. Homologous recombination is rare or absent in influenza viruses as the genomic RNA is single stranded and constantly protected by nucleoprotein from the ubiquitous RNAses (Boni et al. 2008).

76. When a cell is co-infected with two influenza viruses of the same type (i.e. two influenza A viruses or two influenza B viruses), each of the eight RNPs in the progeny virus can originate from either infecting virus because the genome is segmented. Such viral progeny are called reassortants. Theoretically, a single co-infection event, in the absence of any point mutations, could produce 254 novel reassortants. Segment reassortment does not occur between influenza A viruses and influenza B viruses.

77. A cell could be co-infected with influenza A viruses from two different host organisms e.g. a human influenza virus and a swine influenza virus (see Section 4.9). These two influenza A viruses could reassort as they are of the same type.

78. Reassortment can lead to antigenic shift, which is a sudden change in the antigenic determinants. While some novel combinations of haemagglutinin and neuraminidase arising from antigenic shift can lead to pandemic strains, some reassortants are not viable.

79. Due to the significant genetic diversity that exists within the influenza virus population, influenza viruses are considered a quasispecies. Selection pressure from the host immune system or drugs influences the diversity of the quasispecies within the host.

4.9 Host range and zoonotic events

80. Influenza viruses are generally host specific. The principal reservoir of human influenza A viruses is humans but new human subtypes can arise from avian reservoirs. Wild waterfowl are the primary reservoir for most subtypes of influenza A viruses and the most likely progenitor of influenza A viruses that infect all other animals, including swine, horses, dogs and mustelids (eg mink, ferrets).

81. Avian influenza viruses can be of low or high pathogenicity. Low pathogenicity avian influenza infections are often asymptomatic or mild in birds. One factor that influences the pathogenicity of influenza viruses is the ease with which haemagglutinin is cleaved to generate the fusion peptide (see paragraph 49). In low pathogenicity viral subtypes, haemagglutinin has a single basic residue at the cleavage site and can only be activated in a limited number of organs; their replication is restricted to these organs. By contrast, the haemagglutinin of highly pathogenic viruses has acquired a cluster of basic residues (the polybasic cleavage site) that can be cleaved by furin, a ubiquitous protease. This results in systemic infection (Horimoto & Kawaoka 2005; Stieneke-Grober et al. 1992).

82. Direct bird to human transmission is not common and it has not resulted in a sustainable pathogen as avian subtypes transmit poorly between humans. To cross the species barrier, the avian virus must acquire changes in the receptor specificity of haemagglutinin and neuraminidase, and replicate efficiently at the lower human body temperature. Avian influenza virus replication is somewhat restricted by the 32°C ambient temperature of the human nose. The temperature of the avian gut, where the receptors are present and replication would occur, is estimated at 41°C.

83. Human infections with avian influenza viruses generally occur via domesticated intermediates. These infections have not resulted in sustained transmission due to the differences in sialic acid receptors as discussed in Section 4.12 above. The greatest risk occurs during the handling and slaughtering of live infected poultry. Proposed routes of infection include the inhalation of infectious aerosols or aerosolised faeces, and contact with contaminated surfaces.

84. Companion animals such as dogs and cats can be susceptible to human influenza. A human-like H3N2 influenza virus has been isolated from dogs. These dogs shed the virus, had fever, sneezed and coughed (Chen et al. 2015). Cats can be infected with H3N2 canine influenza (Song et al. 2015). Infected cats shed the virus, had elevated temperatures and severe pulmonary lesions.

85. Outbreaks of influenza occur sporadically among farmed animals including swine and mink (Gagnon et al. 2009). Influenza viruses do transmit from swine to individuals involved with pig farming but subsequent person-to-person transmission is very limited (Olsen et al. 2002). The viruses also transmit from humans to swine. Analysis of the H3N1 influenza virus found in swine shows that the H3 originates from a human-like influenza virus (Shin et al. 2006).

86. In Australia, the outbreak of equine influenza in 2007 and of avian influenza (H7N2) in 2013 did not result in human infections. Horses have a sialic acid receptor that is similar to that of avian species (Suzuki et al. 2000).

87. Influenza B viruses have a limited host range with humans and seals being the most common hosts (Osterhaus et al. 2000). There are reports on the seroconversion of dogs inoculated with influenza B viruses but these are contradictory (Rimmelzwaan et al. 2006; Kawano et al. 1978).

4.10 Pandemics

88. Reassortment between zoonotic and human strains of influenza A viruses periodically generates novel influenza viruses and the emergence of subtypes in humans has historically been coupled with worldwide epidemics or pandemics. Pandemics of influenza A viruses occurred in 1918 (H1N1), 1957 (H2N2), 1968 (H3N2) and 2009 (H1N1). These are also referred to as the Spanish flu, Asian flu, Hong Kong flu and swine flu pandemic, respectively. The 1918 pandemic claimed an estimated 50-100 million lives over two years (Taubenberger et al. 2012).

89. Influenza viruses can reassort and evolve in species such as swine, which have the receptors for both human and avian influenza viruses (Garten et al. 2009; Suzuki 1998; Zhou et al. 1999). In 1918, the pandemic virus passed from humans to swine (Dunham et al. 2009), while in 2009 the pandemic virus passed from swine to humans. The 2009 pandemic strain (H1N1pdm09) is a reassortant of four influenza A viruses. It presented an antigenically divergent H1 rather than a novel haemagglutinin. Pig populations currently harbour reassortants that have pandemic potential but do not yet replicate efficiently in humans.

90. Influenza B viruses do not cause pandemics as the limited host range restricts the emergence of new subtypes from reassortment.

91. When a pandemic strain emerges, the contemporary seasonal strain is often observed to disappear (Palese & Wang 2011). In 2009, when pandemic H1N1 influenza virus (“swine flu” strain) appeared, the H1N1 human influenza strain that was in circulation at that point was eradicated within a few months. The 1968 H3N2 human influenza virus had a similar effect on the then-circulating H2N2 virus while in 1957 the emergence of the H2N2 virus stopped the circulation of the H1N1 virus in humans.

4.11 The haemagglutinin-neuraminidase balance

92. The levels of haemagglutinin and neuraminidase activity have to be very finely balanced for productive viral infection. Haemagglutinin and neuraminidase both bind sialic acid but have opposing functions: haemagglutinin binds the receptor for cellular entry while neuraminidase cleaves the receptor to free the virus. If the level of neuraminidase activity is too high, the receptor will be cleaved before the virus can be endocytosed and the host cell will not be infected. Conversely, if the level of neuraminidase activity is too low, the receptor will be cleaved too slowly during budding, viral progeny will aggregate and be prevented from infecting other cells. Some haemagglutinin-neuraminidase combinations recur while others are rarely observed in both natural and laboratory-derived reassortants (see Section 4.8 for discussion of reassortment). The replication fitness of these reassortants could be explained by a mismatch in receptor binding and release (Wagner et al. 2002).

93. Reassortment can result in a haemagglutinin-neuraminidase combination that is structurally mismatched and hampers productive infection. For example, the neuraminidase stalk shortens when H5N1 influenza viruses adapt to chickens. Human infection with this chicken-adapted virus is not common because the short chicken-adapted N1 does not easily reach the slightly longer human haemagglutinin. As a result the virus aggregates and this limits transmission (Blumenkrantz et al. 2013).

94. Haemagglutinin often mutates when adapting to a new host and this is accompanied by mutations in neuraminidase that result in a commensurate level of activity (Matrosovich et al. 1999). A functional balance of haemagglutinin and neuraminidase activities was absent in swine progenitors of the 2009 H1N1 pandemic virus. However, the emergence of influenza strains with weak haemagglutinin receptor binding that was functionally matched by low neuraminidase activity coincided with the human 2009 H1N1 pandemic (Xu et al. 2012).

95. When an inhibitor of neuraminidase or haemagglutinin is used in treatment of influenza infection, a mismatch in receptor binding and release results. Escape mutants re-introduce the balance and can result from mutations in either protein. In viruses that are resistant to neuraminidase inhibitors, many mutations map to haemagglutinin. In such mutants, the affinity of haemagglutinin for the receptor can be reduced to the point where natural dissociation suffices for receptor release and neuraminidase is no longer required (McKimm-Breschkin et al. 1996).

4.12 Sialic acid receptors and viral transmissibility

96. The key reason why avian influenza viruses do not transmit easily to humans is that the receptor differs in humans and waterfowl. However, avian influenza strains are a progenitor of many novel human influenza viruses. In humans, sialic acids commonly occur with an α-2,6 linkage to galactose (SAα-2,6), and human influenza viruses preferentially bind SAα-2,6 receptors (Figure 6). In waterfowl, sialic acids usually have an α-2,3 linkage to galactose (SAα-2,3), and avian influenza viruses favour SAα 2,3 receptors. Exposure to an influenza virus will only result in productive infection if the viral haemagglutinin can bind the host sialic acid receptor and the neuraminidase can cleave it. Influenza neuraminidases can cleave both SAα-2,3 and SAα-2,6 but regardless of the host, all influenza neuraminidases are more efficient at cleaving the non-human SAα-2,3 (Gulati et al. 2005). Since avian haemagglutinin and neuraminidase do not bind and cleave the human receptors (SAα-2,6) well, avian influenza viruses do not transmit easily to humans.

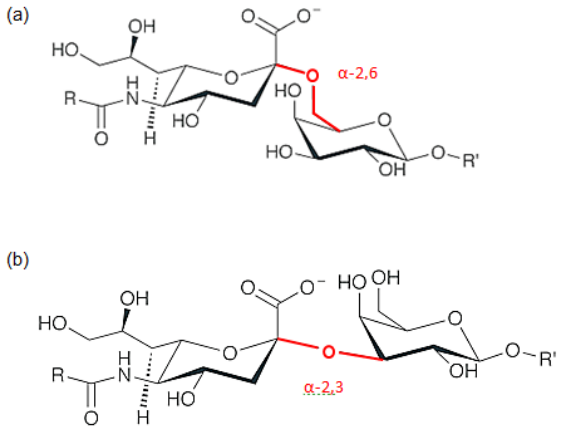


Figure 6. Sialic acid receptors
The SAα-2,6 receptor (a) is more common in humans than is the SAα-2,3 receptor (b).

97. Sialic acid is a generic term for O- or N-substituted derivatives of neuraminic acid, which is a nine carbon sugar. Sialic acid only occurs as a terminal sugar in glycans. In addition to a difference in the linkage to the adjoining sugar, the sialic acid itself can differ. Most species synthesize two types of sialic acid: N-glycolyl neuraminic acid and N-acetyl neuraminic acid. Humans and ferrets only synthesize the latter.

98. Interactions between viral proteins and the host cell receptor are restricted by steric hindrance between the sugars on haemagglutinin, neuraminidase and the cellular glycans. Combinations that decrease steric hindrance are favoured by the virus. Consequently, receptor preference is not only affected by the glycosidic linkage to the adjacent sugar but also other properties of the glycan such as its length, branching pattern and any modifications to the glycoprotein to which it is attached (Knipe & Howley 2013).

99. The tissue localisation of the preferred receptor determines the pathology of the infection and viral transmissibility:

- In humans, SAα-2,6 receptors are found primarily in cells of the upper respiratory tract. Consequently, human influenza is characterised by upper respiratory tract infection, sneezing and airborne viral transmission.
- In geese and ducks, SAα-2,3 are the major sialic acid throughout the gut (Kimble et al. 2010). The virus is generally transmitted through a faecal to oral route, and diarrhoea is a common symptom of infection.

100. Ferrets serve as a model organism for the study of human influenza because, like humans, they have SAα-2,6 receptors and their receptors are similarly distributed through the respiratory tract (de Graaf & Fouchier 2014). As a result, they are highly susceptible to human influenza, transmit the virus between ferrets, undergo the same pathology of infection and exhibit the same symptoms. The ferret body temperature of 38°C is close to that of humans.

101. Many animals such as swine and poultry have both SAα-2,3 and SAα-2,6, providing a suitable transitionary environment in which avian influenza viruses can evolve to productively infect humans.

102. Due to the restrictions of the catalytic site, avian influenza neuraminidase lacks the latitude to evolve specificity for SAα-2,6 to the same degree as human haemagglutinin. Avian influenza neuraminidase has a 50:1 preference for SAα-2,3 over SAα-2,6 while in swine, the ratio is 20:1 (Mochalova et al. 2007). In humans, the preference is between 5:1 and 3:1.

103. Humans have some SAα-2,3 receptors but the binding of these receptors by avian influenza viruses does not always lead to productive infection. Glycans can be short and O-linked or long and N-linked. Due to steric restrictions on neuraminidase (see Section 4.5), short O-linked glycans are a poorer substrate for neuraminidase than the longer N-linked glycans (Air 2012). Human SAα-2,3 receptors found in the nasopharynx and deep in the lungs are generally on O-linked glycans. These are poorly cleaved by avian influenza neuraminidase (Air 2012) and repeated cycles of infection do not always eventuate.

104. Mucin in the mucous layer that protects epithelial cells is effective in its role as a decoy receptor as it contains SAα-2,3 receptors on short O-linked glycans. This combination is bound preferentially by avian haemagglutinin but not cleaved well by avian neuraminidase.

105. Young children express a higher proportion of SAα-2,3 receptors as compared to adults (Nicholls et al. 2007).

106. For an avian influenza virus to adapt to humans, it must acquire changes in both haemagglutinin and neuraminidase that favour the binding and cleavage of SAα-2,6 receptors.

4.13 Disinfectants and antivirals

107. Influenza viruses remain viable on non-porous surfaces such as stainless steel and plastic for up to 24 hours, and on semi porous surfaces such as cloth, paper and tissues for eight to twelve hours (Bean et al. 1982).

108. Contact transmission can be prevented by disrupting the viral lipid envelope through washing hands with soap. Surfaces can be chemically decontaminated with standard disinfectants such as bleach, 2% alkaline glutaraldehyde and 5 to 8% formalin. Physical decontamination includes moist heat at 121ºC for 20 minutes or dry heat at 170ºC for 1 hour (Pathogen Regulation Directorate 2011a; Pathogen Regulation Directorate 2011b).

109. Antiviral agents are 70-90% effective as short term prophylactics (Monto 2003). Neuraminidase inhibitors such as oseltamivir (Tamiflu) and zanamivir (Relenza) may shorten the period of influenza infection.

110. M2 inhibitors such as amantadine and rimantadine, block the M2 proton channel and in doing so, prevent uncoating of the virus and progression of the infection. The spread of a single mutation has resulted in widespread resistance to this class of drugs.

4.14 Flu vaccines

111. Annual vaccination against circulating flu strains is strongly recommended for high risk groups such as the elderly and the immunocompromised (Centers for Disease Control and Prevention 2008).

112. The most common flu vaccines are inactivated (killed) vaccines, which can be divided into whole virus vaccines, split virus vaccines and subunit vaccines. In whole virus vaccines, an immune response is elicited by intramuscular injection of the intact but killed virus. Split virus vaccines use whole virus that has been disrupted by a detergent. By comparison, subunit vaccines only use partially purified haemagglutinin and neuraminidase.

113. Antigenic selection drives mutations in haemagglutinin and neuraminidase. Because of high mutation rates, the WHO recommends flu strains for targeting with vaccines (targeted strains) twice annually. Recommendations are made in February for the Northern Hemisphere flu season and in September for the Southern Hemisphere flu season. The WHO's recommendations are evaluated by the Australian Influenza Vaccine Committee (AIVC) which provides advice to the TGA on the composition of the seasonal flu vaccine to be supplied each year in Australia.

114. For human influenza A viruses, the main subtypes in circulation are A/H1N1 and A/H3N2. For human influenza B viruses, B/Yamagata was the only lineage to circulate until 1983 when both B/Yamagata and B/Victoria, started to co-circulate.

115. Prior to 2012, trivalent influenza vaccines were produced as the vaccine target strains included one strain of A/H1N1 lineage, one of A/H3N2 lineage and only one with a B lineage. However, the dominant B lineage was sometimes wrongly predicted and consequently, the influenza B strain in the vaccine and the contemporary B strain were mismatched in 6 out of 12 influenza seasons in the United States between 2001 and 2012 (Dolin 2013).

116. From 2012, the WHO's recommendations included viruses from both the B/Yamagata and B/Victoria lineages. These together with the two A subtypes (H3N2 and H1N1) resulted in quadrivalent influenza vaccines.

117. A vaccine mismatch occurs when the seasonal flu vaccine contains strains that are antigenically distinct from the strain causing the seasonal epidemic. Mismatches result when the epidemic strain emerges late in the season, or if the targeted strains mutates after vaccine production. Mismatches occur every year due to the propensity of the flu virus to mutate. The extent of the mismatch influences the effectiveness of flu vaccines and the severity of flu for a given season. Even when the vaccine and circulating viruses are not optimally matched, a vaccine may still offer some protection during the seasonal flu epidemic.

118. Over the past 15 years, neuraminidase accumulated more mutations than haemagglutinin (Air 2012). Point mutations warranted eight updates of the H1 component of the flu vaccine between 1977 and 2009 (Hay et al. 2001).

119. In vaccine production facilities and laboratories, influenza viruses are often grown in embryonated chicken eggs because allantoic fluid has a protease which cleaves haemagglutinin, facilitating repeated cycles of infection (Ewasyshyn & Sabina 1986).

120. Strains used for vaccines are generally 6:2 reassortants, with 6 genomic segments derived from attenuated strains that are often high yielding when grown in eggs, and 2 segments encoding the antigenic determinants derived from the seasonal epidemic strain. Reassortant viruses have been used in vaccine production since 1971 (Kilbourne 2006).

121. Cross-protective vaccines targeting conserved antigens on protein other than haemagglutinin and neuraminidase are being tested in laboratories (Price et al. 2014) but are not commercially available. Such vaccines could reduce morbidity in the vaccinated individual but, as they do not prevent infection, they might allow transmission to others.

122. The following is a list of some precautions for influenza vaccines. It should be emphasized that these apply to influenza vaccines in general and are not specially connected with the genetic modifications that are being assessed.

- To be avoided by persons with allergies to the excipients, which are additives to increase the stability of the active components, increase homogeneity or add bulk to the finished product.
- To be avoided by persons aged 2-17 years who are receiving aspirin-containing therapy because Reye's syndrome has been associated with aspirin use in patients with natural influenza infections.
- Vaccination may aggravate symptoms in asthmatics.
- Guillain-Barré syndrome (GBS) is an autoimmune disorder of the nervous system. There was a very small increase in GBS associated with the 1976 inactivated swine flu vaccine.

123. The Product Information document (PI) that would accompany the vaccine provides health professionals with a summary of the scientific information relevant to the safe and effective use of a prescription medicine and would be approved by the TGA. A complete list of individuals for whom influenza vaccines are contraindicated would be detailed in the PI.

Section 5 GM vaccine viruses – nature and effect of genetic modifications

5.1 Introduction

124. The underlying premise of nasal vaccination with live attenuated flu vaccines is that exposure to influenza viruses through the natural route of infection would induce immunity at the most probable site of infection. To achieve this, the GM flu vaccines are sprayed into the nose. The GM viruses are deposited in the upper respiratory tract because the large droplet size retards its movement through the airways. The GM attenuated viruses replicate in the mucosa of the upper respiratory tract, and induce both localised and systemic immunity.

125. The proposed seasonal GM flu vaccine is a tetravalent live attenuated vaccine. It contains four GM flu vaccine strains to induce immunity against four targeted seasonal strains. The proposed pandemic GM flu vaccine, which is also a live attenuated vaccine, would contain one GM flu vaccine strain to target a pandemic influenza virus. The seasonal GM flu vaccine would be manufactured annually but the pandemic GM flu vaccine would be produced only in the event of a pandemic.

126. In the GM vaccine strains, the haemagglutinin and neuraminidase segments in the cold-adapted parent virus are replaced by the equivalent segments from a strain to be targeted by the vaccine, as shown in Figure 7.

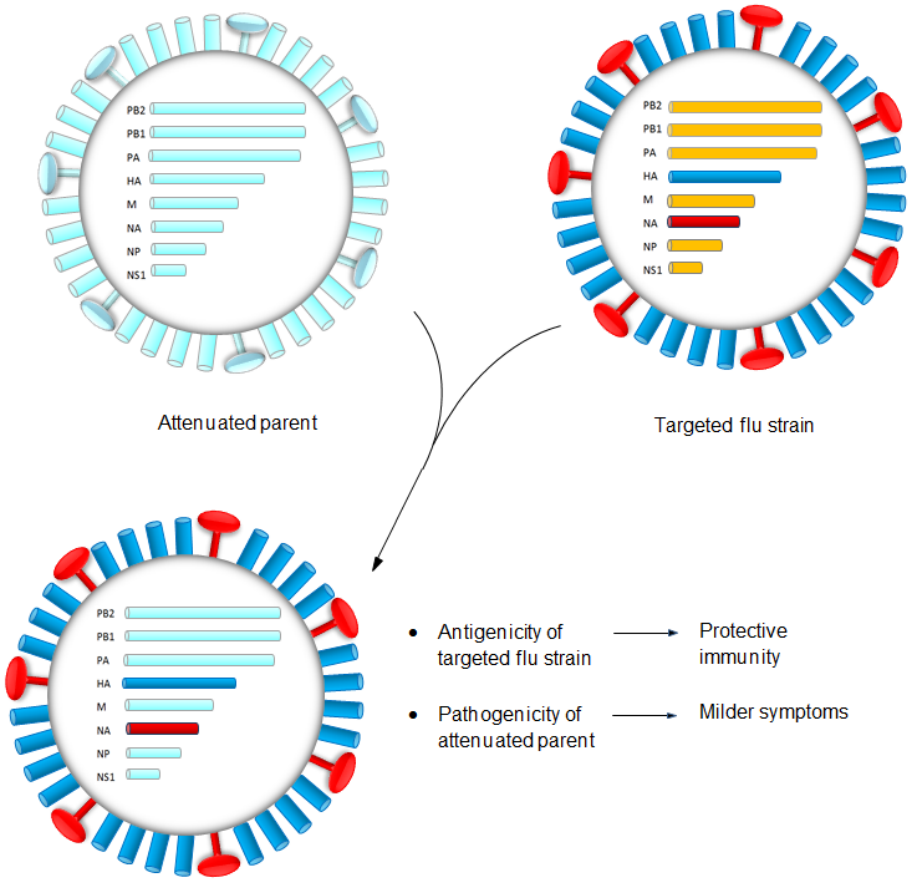
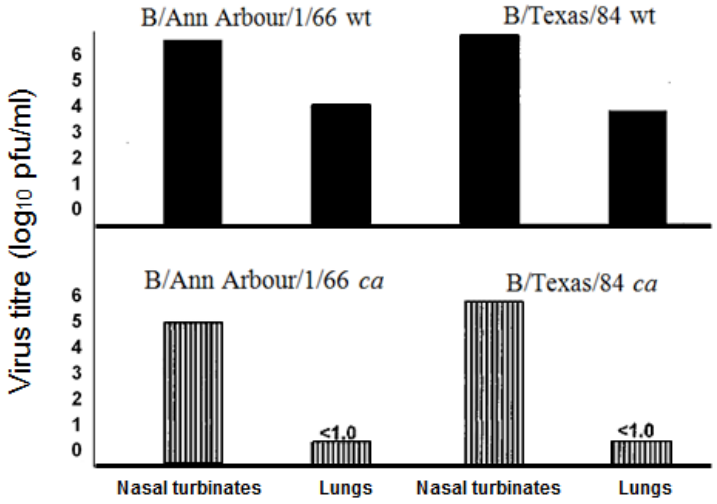


Figure 7. Derivation of the GM vaccine strains

127. The resulting GMOs carry the introduced antigenic determinants to confer protective immunity against the targeted circulating viruses, and the replicative traits of the cold-adapted parent which restrict replication in the lower respiratory tract. The attenuation caused by cold-adaptation decreases the replication level of the GMO by at least 100 fold in the lungs as compared to the wild-type A and B strains. (Figure 8 provides example replication data for two wild-type and corresponding cold-adapted strains of influenza B virus.)



Adapted from Murphy & Coelingh 2002

Figure 8. A comparison of the replication levels of wild-type and cold-adapted viruses in the upper and lower respiratory tract of ferrets.

Wild type B/Ann Arbor/1/66 (top left), the cold-adapted influenza B parent strain (bottom left), wild-type B/Texas/84 as an example of a wild-type circulating strain (top right) and the 6:2 reassortant of the cold-adapted parent carrying antigens from B/Texas/84 (bottom right).

5.2 Annual changes in the seasonal GM flu vaccine

128. Due to the propensity of influenza viruses to mutate, immunity gained during one influenza season will not reliably prevent infection by a strain in a subsequent season. The WHO changes its recommendation on targeted strains twice a year, once each for the Northern and Southern hemispheres. The WHO's recommendations are evaluated by the Australian Influenza Vaccine Committee (AIVC) which provides advice to the TGA on the composition of the seasonal flu vaccine to be supplied each year in Australia.

129. In the seasonal GM flu vaccine, the choice of strain to be targeted is based on the WHO's recommendation of four seasonal influenza viruses to be targeted by the Southern hemisphere influenza vaccine (see Chapter 1 Section 4.14). These vaccine-targeted strains are predicted to be the most widely circulating strains during the upcoming influenza season. Consequently, the haemagglutinin and neuraminidase segments in the GM virus strain in the seasonal GM flu vaccine would change at least once a year.

130. However, the basic construct of GM vaccine viruses would remain constant since the remaining six segments would always be derived from the cold-adapted parent (Figure 7). Therefore, the resultant GM vaccine viruses would always be attenuated.

5.3 Method of genetic modification

131. The reassortant strains can arise naturally but for efficiency, gene technology will be used to construct the GM influenza virus with the desired combination of genomic segments that maintain attenuation and introduce current antigens. The GM viruses are 6:2 reassortants

since six segments are derived from one parent and two segments are derived from a second parent.

132. Influenza viruses do not go through a natural DNA intermediate (see paragraph 55). Manipulation of RNA is technically challenging as RNases are robust enzymes that are stable to heat and detergents, and present on human skin. Single stranded RNA generally lacks secondary structure and is particularly susceptible to RNAses. The difficulty in working with RNA can be circumvented with reverse genetics.

133. The GM viruses will be constructed using reverse genetics, a process in which RNA viruses are generated entirely from the cloned cDNA of vRNA. Reverse genetics enables the desired 6:2 reassortant strain to be produced efficiently and without having to extensively screen numerous reassortants resulting from co-infection and natural reassortment.

134. cDNA from the 6 genomic segments of the attenuated parent virus, as well as the 2 genomic segments encoding haemagglutinin and neuraminidase from each of the target viruses, are cloned into bidirectional plasmids. This allows both mRNA and vRNA to be synthesised.

135. For the targeted virus strains, isolates are obtained from the WHO's Global Influenza Surveillance and Response System (GISRS). No changes are made to the haemagglutinin and neuraminidase segments from the targeted seasonal strains when constructing the four GM viruses that comprise the seasonal vaccine.

136. For the pandemic vaccine, no changes are made to the neuraminidase segment. The haemagglutinin segment will be modified only if a polybasic site is present (see paragraph 49). In this case, the number of basic residues would be reduced to decrease the infectivity of the GM virus.

137. During construction of the GM viruses, the entire segment and not just the gene is replaced in the cold-adapted parent. The eight plasmids corresponding to a desired GM reassortant strain are transfected into a cell line, leading to expression of viral proteins and replication of the GM virus. The resulting virus is then amplified to make a Master Virus Seed which is the starting material for the manufacture of the monovalent bulk for each vaccine virus strain. Each GM reassortant vaccine virus strain is grown up separately in SPF eggs. Four such monovalent bulks are blended to produce the final quadrivalent vaccine.

5.4 Effect of the genetic modification

138. In each of the GM vaccine strains, the haemagglutinin and neuraminidase segments in the cold-adapted parent are replaced by the equivalent segments from a strain to be targeted by the vaccine.

- Haemagglutinin and neuraminidase are the primary antigens that elicit a protective antibody response. Their replacement changes the immune response mounted by the vaccine recipient.
- The six retained segments from the cold-adapted parent ensure that the GM virus also has the *ca*, *ts*, and *att* phenotypes. This would restrict their replication to the upper respiratory tract.
- The net result is an attenuated vaccine virus that can induce protective immunity but replicates poorly at body temperature, resulting in diminished symptoms.

139. No major adverse effects to the seasonal GM influenza vaccines were noted by overseas authorities that analysed the clinical studies conducted to support patient safety, quality and efficacy (PHAC 2011; EMA 2013; FDA 2015). These authorities include the Public Health Agency of Canada (PHAC), Food and Drug Administration (FDA) and European Medicines Agency (EMA). The TGA conduct similar analysis prior to any approval in Australia.

140. In the USA where the seasonal GM flu vaccine has been available since 2003, the most common vaccine side effects are runny nose, nasal congestion and headache in both children and adults. Children may also have wheezing, vomiting, muscle aches and fever. Adults may have a sore throat and cough (CDC 2014). These side effects are similar to those arising from vaccination with inactivated flu vaccines.

5.5 Characterisation of the GMOs

5.5.1 Genotype stability

141. The stability of the GM flu vaccine strains is checked several times during vaccine manufacture. After construction of the GM flu vaccine viruses, they are grown up to form the master virus seed (MVS). This is passaged five times on Specific Pathogen Free (SPF) eggs and then sequenced for assurance of genetic stability. The *ca* and *ts* phenotypes are tested by assaying viral titres at defined temperatures using primary chicken kidney (PCK) cells. The *att* phenotype is tested by infecting ferrets.

142. Reversion to a non-attenuated phenotype would be highly unlikely as the *ca*, *ts* and *att* phenotypes result from several mutations on several segments.

143. Analysis of nine such GM flu viruses showed that they preserved their genotype and phenotype during manufacture (Buonagurio et al. 2006a). The stability of the GM flu viruses through several passages in eggs may be attributed to the cold-adapted parent, which was selected for by passaging in eggs.

144. Studies have also demonstrated that the GM viruses in the seasonal GM flu vaccine retain the attenuated phenotype after replication in humans (Cha et al. 2000; Buonagurio et al. 2006b). 20% of flu virus isolates from vaccinated children were the same as the vaccine strains and the remaining isolates carried one to seven changes per genome. In all isolates, the cold-adaption and attenuation of the virus was retained.

5.5.2 Shedding

145. Shedding and transmissibility of the GM viruses to other persons are matters that would be considered in detail during the TGA's evaluation of patient safety.

146. The GM viruses in seasonal GM flu vaccine are shed from patients i.e. they can be recovered by nasal swabs in newly vaccinated individuals. Shedding is observed on days 1 to 11 after vaccination with the highest titres observed on days 2 to 3. The level of shedding drops by day 10 in children under the age of 8 and by day 6 in older individuals (Block et al. 2008).

147. The findings of several non-clinical studies on shedding after the administration of live attenuated influenza vaccines indicated that shed viral titres were low (Block et al. 2008; Mallory et al. 2011). Shedding decreased with time since vaccination and the individual's age. In adults, shedding may be less likely if an individual has had prior influenza vaccination (Talbot et al. 2005).

5.5.3 Transmissibility

148. For any vaccine virus to transmit from a vaccinated individual to another individual, the amount of virus shed has to be greater than or equal to the infectious dose. AstraZeneca has advised that the human infectious dose (HID_{50}) of the cold-adapted virus for seronegative adults is $10^{5.5}$ $TCID_{50}$, while the infectious dose for children is 10^{3} $TCID_{50}$ (FDA 2002). The administered dose is 10^{7} $TCID_{50}$ per strain. The peak titre shed is 10^{4} $TCID_{50}$ in children and 10^{2} $TCID_{50}$ in adults (FDA 2002). Based on the titres of virus that are shed, human-to human transmission is possible but would be highly unlikely.

149. In a study designed to detect secondary transmission among young children (8 to 36 months of age) in a child care setting with prolonged close contact, a single incidence of secondary transmission of vaccine virus was observed (Vesikari et al. 2006a; Vesikari et al. 2006b; Mallory et al. 2011).

150. Influenza viruses are largely species specific and since the GM flu vaccine viruses are derived from human influenza viruses, they would not be likely to develop a novel tropism for non-human species. The applicant assessed to ability the vaccine viruses to infect a range of avian and mammalian species by inoculating 21 species with the GM viruses. Productive infection was only established in ferrets, hamsters, and guinea pigs. In these species replication only occurs in the upper respiratory tract. This group of animals are particularly susceptible to wild-type human influenza viruses and are used as model hosts for its study. Replication of the GM flu viruses was not observed in swine, horses or other domesticated mammals, or in any tested avian species.

151. Productive infection of other species in the environment depends on several parameters. The ability of influenza viruses to bind and enter cells varies with the viral haemagglutinin and the cellular sialic acid receptor. When the haemagglutinin changes, the affinity for the receptor may change. While there are recorded instances of anthroponotic influenza virus transmission, there is only a very remote possibility that the GM viruses would transmit to other species since:

- the haemagglutinin in the GM viruses is derived from human influenza viruses and would not favour the SAα-2,3 receptors found in species such as birds (see Chapter 1 Section 4.12)
- shedding titres are low and secondary transmission is rare in humans, and secondary transmission to other species would be expected to be even rarer
- the GM viruses are attenuated and exposure would be unlikely to result in productive infection.

5.6 Decontamination and antivirals

5.6.1 Chemical decontamination

152. The applicant has stated the following regarding the susceptibility of seasonal GM flu vaccine to decontaminating agents:

- Detergent solution followed by a hypochlorite solution is recommended for spills of the GM flu vaccines.
- Most hospital disinfectants with the exception of alcohols and phenolic disinfectants will be effective against influenza viruses. However, 0.4% LpHSE (phenolic disinfectant), 0.8% Vesphene (phenolic disinfectant with detergents and builders) and 70% isopropanol did not consistently reduce residual virus load below detectable levels.
- Quaternary ammonium/isopropyl alcohol and bleach detergent wipes eliminated H1N1 influenza virus on reusable elastomeric respirators, whereas 70% isopropyl alcohol alone was ineffective (Subhash et al. 2014).
- 1% Spor-Klenz (peracetic acid, hydrogen peroxide and acetic acid) or 1000 parts per million (ppm) PreSept (sodium dichloroisocyanurate) reduced seasonal GM flu vaccine viral components by > 3.8 $\log_{10}$ fluorescent focus-forming units (limit of detection) on stainless steel.

5.6.2 Other methods

153. GM flu vaccine aerosol droplets produced by the sprayer evaporate in 3 to 30 seconds under ambient conditions, and the vaccine undergoes a 100-fold loss in infectious titre

following desiccation (based on data obtained in spray-drying experiments conducted with the frozen formulation of the vaccine).

154. UV light is highly effective in inactivating viruses in small-particle aerosols (Tellier 2006) such as those that would be produced with the seasonal GM flu vaccine sprayer.

5.6.3 Antivirals

155. The pandemic strains have been tested and found to be susceptible to Tamiflu. It is highly probable that the attenuated GM vaccine strains would also be susceptible to Tamiflu.

Section 6 Receiving environment

156. The receiving environment forms part of the context for assessing risks associated with dealings with GMOs (OGTR 2013). It determines the ease and likelihood of the GMOs surviving outside the site of release.

6.1 Site of release

157. The intended primary receiving environment would be the nose, nasal turbinates and nasopharynx of vaccine recipients. Each dose will be 0.2 mL (0.1 mL per nostril) delivered as a high speed aerosol.

158. The secondary receiving environment would be the room where the vaccine is administered.

159. The principal route by which the GM vaccine strains may enter the wider environment is release following vaccination e.g. the recipient sneezing or shedding.

6.2 Related viral species in the receiving environment

160. The presence of related viral species may offer an opportunity for the horizontal transfer of any introduced genetic material from the GM flu vaccine strains to other organisms in receiving environment.

161. Influenza A and influenza B viruses are endemic in Australia but their levels follow an annual pattern. Infections increase noticeably in May, peak between mid-July and mid-August, and subside in October or November.

162. The *Orthomyxoviridae* family is characterised by viruses with a segmented, negative-sense, single-stranded RNA genome. As there is no DNA intermediate, this family of viruses cannot integrate into the DNA genome of the host. The single-stranded RNA cannot undergo homologous recombination. The segmented genome allows horizontal gene transfer through reassortment.

163. Reassortment only occurs with influenza viruses of the same type. Therefore influenza A virus and influenza B virus do not reassort with each other, with influenza C virus or with other *Orthomyxoviridae*.

164. The most closely related species to *Influenzavirus A* and *Influenzavirus B* is *Influenzavirus C*. Other members of the *Orthomyxoviridae* family include *Isavirus* (infects salmon), *Thogotovirus*, *Quaranfilvirus* and *Lake Chad virus* (arbovirus). The transfer of genetic material between members of the *Orthomyxoviridae* has not been reported.

6.3 Similar genetic material in the environment

165. The balance of a system could be perturbed by the introduction of new genetic material through horizontal gene transfer or through the release of GM flu vaccine strains into the environment. However, the effect of this perturbation would be relatively small if the genetic

material was previously present in the system and did not confer any selective advantage to an organism that gained this genetic material.

166. The haemagglutinin and neuraminidase segments introduced into the GM flu vaccine strains would already be present in the environment, as it is derived from isolates of the targeted strains that are supplied by the WHO's Global Influenza Surveillance and Response System.

167. These haemagglutinin and neuraminidase segments are derived from contemporary influenza strains predicted to be the most common in the upcoming influenza season.

168. All of the genes and genomic segments in the GM vaccine strains would be functionally similar to ones present in other influenza viruses.

6.4 Alternate hosts

169. Influenza viruses are obligate parasites, which cannot replicate outside a host as they depend on the host's proteins for many replicative processes. Their membrane envelope renders them labile in the outside environment.

170. Guinea pigs and ferrets could be actively infected (but would not be infected through shedding) with the GM flu vaccine viruses. These species are the most susceptible to wild-type human influenza. They are kept as pets but are neither farmed nor present in large numbers in Australia. Native birds and seals are less susceptible to wild-type human influenza viruses (see Section 4.9) and therefore, unlikely to be infected through shedding of the GM flu vaccine viruses.

Section 7 Previous authorisations for live attenuated influenza vaccines

7.1 Australian authorisations

171. No GM live attenuated influenza vaccines have been approved for use by the Regulator or the TGA.

7.2 International authorisations and experience

172. AstraZeneca's GM flu vaccines are available commercially in several other jurisdictions as shown in Table 2. The trivalent version of the seasonal vaccine was first released in the 2003/2004 northern hemisphere influenza season. The release of the quadrivalent vaccine occurred after the WHO recommended a second influenza B virus strain for targeting by vaccines.

173. As shown in Table 2, in each of the three jurisdictions where they are commercially available, the seasonal GM flu vaccines have been assessed twice, once for the release of the trivalent vaccine and once for the release of the quadrivalent vaccine. There was a 10-year gap between the releases of these two vaccine versions in the USA and shorter intervals in the EU and Canada.

Table 2: Overseas marketing approvals for AstraZeneca's GM flu vaccines

Released in*	Jurisdiction	Vaccine type	Trade Name
2003/2004	USA	trivalent	FluMist
2012/2013	EU	trivalent	Fluenz
2010/2011	Canada	trivalent	FluMist
2013/2014	USA	quadrivalent	FluMist Quadrivalent
2014/2015	Canada	quadrivalent	FluMist Quadrivalent
2014/2015	EU	quadrivalent	Fluenz Tetra

*Northern hemisphere influenza season

Chapter 2 Risk Assessment

Section 1 Introduction

174. Risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs (Figure 9). Risks are identified within the established risk context (see Chapter 1) and take into account current scientific and technical knowledge. Uncertainty and in particular, knowledge gaps, is considered throughout the risk assessment process.

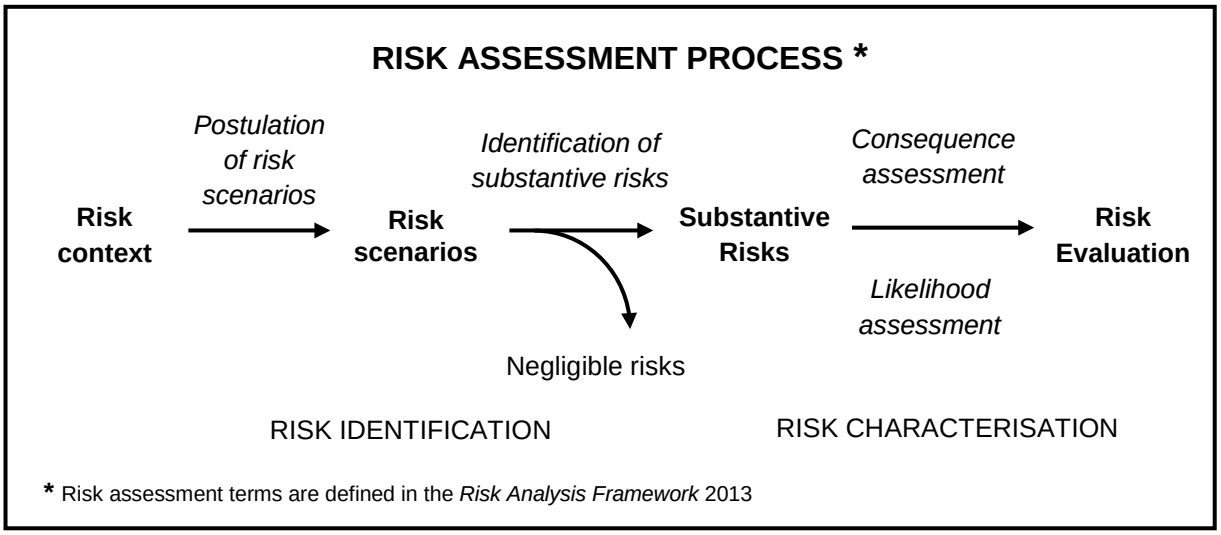


Figure 9. The risk assessment process

175. Initially, risk identification considers a wide range of circumstances whereby the GMO, or the introduced genetic material, could come into contact with people or the environment. Consideration of these circumstances leads to postulating plausible causal or exposure pathways that may give rise to harm for people or the environment from dealings with a GMO (risk scenarios) in the short and long term.

176. Postulated risk scenarios are screened to identify substantive risks that warrant detailed characterisation. A substantive risk is only identified for further assessment when a risk scenario is considered to have some reasonable chance of causing harm. Pathways that do not lead to harm, or could not plausibly occur, do not advance in the risk assessment process.

177. A number of risk identification techniques are used by the Regulator and staff of the OGTR, including checklists, brainstorming, reported international experience and consultation (OGTR 2013). In conjunction with these techniques, risk scenarios postulated in previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

178. Substantive risks (i.e. those identified for further assessment) are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). The level of risk is then estimated from a combination of the Consequence and Likelihood assessments. Risk evaluation then combines the Consequence and Likelihood assessments to determine level of risk and whether risk treatment measures are required. The potential for interactions between risks is also considered.

Section 2 Risk Identification

179. Postulated risk scenarios are comprised of three components:

i. Source of potential harm (risk source)

ii. Plausible causal linkage to potential harm (causal pathway) and

iii. Potential harm to an object of value (people or the environment).

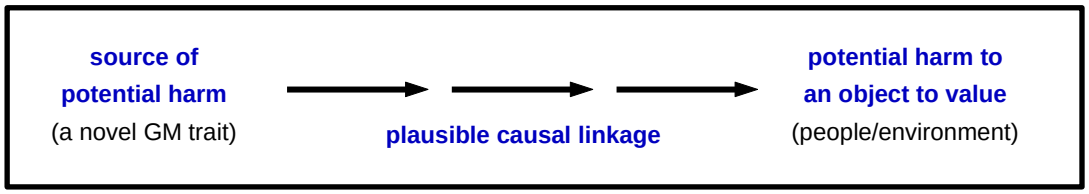


Figure 10. Components of a risk scenario

180. In addition, the following factors are taken into account when postulating relevant risk scenarios for this licence application:

- the proposed dealings, which are import, transport or disposal of the GM flu vaccines and possession (including storage) in the course of any of these dealings
- the proposed controls
- restrictions placed on the import, transport or disposal of the GM flu vaccines by other regulatory agencies
- characteristics of the parent organisms
- routes of exposure to the GM flu vaccine strains, the introduced genes and gene products
- potential effects of the introduced genes and gene products expressed by the GM flu vaccine strains
- potential exposure to the same genes and gene products from environmental sources
- the release environment and
- clinical practices during administration of the GM flu vaccines.

181. As discussed in Chapter 1 Section 2.1, the TGA would regulate quality, safety and efficacy of the GM flu vaccines for use as a therapeutic under the *Therapeutic Goods Act 1989*. This would include:

- assessment of patient safety, vaccine quality and efficacy prior to inclusion on ARTG
- recommended practices for the transport, storage and disposal of the GM vaccines under the *Australian code of good wholesaling practice for medicines in schedules 2, 3, 4 & 8*, and
- requirements for the scheduling, labelling and packaging under the Scheduling Policy Framework for Medicines and Chemicals (AHMAC 2015).

182. Therefore, the current assessment focuses on risks posed to people or the environment, including long term persistence of the GM flu vaccine strains, which might arise from the import, transport, storage or disposal of the GM flu vaccines.

183. Four risk scenarios were postulated as summarised in Table 3. Circumstances sharing common features have been grouped together in broader risk categories. These risk scenarios were evaluated considering both short and long term effects, and in the context of the control measures proposed by the applicant. None of the risk scenarios were identified as a risk that

could be greater than negligible and warranting further scrutiny. Detailed evaluations of these scenarios are provided later in this section.

Table 3. Summary of risk scenarios from dealings with GM flu vaccine

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
Section 2.1: Inadvertent exposure to the GM flu vaccines during import, transport, storage & disposal					
1	GM flu vaccines	Person exposed to the GMOs during waste disposal ⬇ Person infected with GMOs ⬇ Influenza develops	Ill health	No	• Used, unused or expired sprayers would be disposed as medical waste by clinical staff and distributors. • Exposure to unintended recipients would be minimised as the vaccines would be administered by healthcare professionals following well-established procedures. • GM flu vaccine strains desiccate rapidly under ambient conditions. • GM flu vaccine strains are attenuated and growth is restricted at human body temperature.
2	GM flu vaccines	Unintentional release of GMOs during transport or storage ⬇ Person or animals exposed to GMOs ⬇ Person or animals infected with GMOs ⬇ Influenza develops	Ill health	No	• Transport will be according to appropriate standards for medical products. • Storage will be at secure storage or healthcare facilities. • GM virus desiccates rapidly under ambient conditions. • GM virus is attenuated and growth is restricted at human body temperature.
Section 2.2.: Unintended changes in viral characteristics					
3	GM flu vaccines	Person infected with the GMO is infected with contemporary influenza virus carrying a different haemagglutinin and/or neuraminidase from the vaccine strains ⬇ Both viruses co-infect the same host cell ⬇ Viruses reassort ⬇ Reassortant infects host and propagates ⬇ Influenza develops	Ill health	No	• The GM vaccines strains would not contain any novel genetic material. • Co-infection of a host cell by the GMOs and another strain would be uncommon. • The introduced haemagglutinin and neuraminidase genome segments are derived from strains predicted to be the most common circulating strains for the season, so do not add any genetic novelty. • Reassortants containing other genome segments from the GMOs are expected to be less virulent than circulating strains.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
4	GM flu vaccines	Person infected with the GMO is infected with an influenza virus of swine or avian origin ⬇ Both viruses co-infect the same host cell ⬇ GMO and swine/avian influenza virus reassort ⬇ Haemagglutinin and/or neuraminidase segment in GMO is replaced with equivalent segment from swine or avian influenza strain ⬇ Reassortant infects host and propagates ⬇ Influenza develops	Ill health	No	• The GM vaccines strains would not contain any novel genetic material. • Co-infection of a host cell by the GMOs and another strain would be uncommon. • Reassortants are expected to be less virulent than reassortants between a pig or avian flu strain and a circulating strain.

2.1 Ill health from exposure to the GM flu vaccines

184. The parent organisms of the GM flu vaccine strains, human influenza A virus and human influenza B virus, are respiratory pathogens. Details on their transmissibility and pathogenicity are given in Chapter 1.

185. Infection is generally the result of inhalation of aerosol droplets containing the virus or of mucosal exposure to contaminated surfaces. The replication of the influenza virus in respiratory epithelial cells results in their apoptosis, as manifested in disease symptoms such as a runny nose, cough and sore throat.

186. Infection with influenza viruses does not result in latent infection or integration into the host genome.

187. The GM flu vaccine strains are live attenuated influenza viruses with the restricted replicative traits of a cold-adapted parent and the antigenic determinants of a contemporary circulating strain. They have been constructed by reverse genetics, but could arise from natural reassortment of the parent strains.

188. Vaccinated individuals would be intentionally exposed to the GM flu vaccine viruses. Since the GM flu vaccine is dispensed as a nasal vaccine, clinical staff administering the GM flu vaccine may have low level exposure to the GMO. As noted previously, TGA has regulatory responsibility for use of the GMO as a therapeutic. The Regulator does not duplicate the TGA's assessment but focusses on risks posed to people and to the environment associated with activities other than vaccine administration. Other people and animals may be exposed to the GM flu vaccines through accidental release during transport, storage or disposal.

189. The toxicity and allergenicity of the introduced genes and their products were not directly considered but are taken into account in the context of their contribution to ill health.

190. Pathways that could lead to ill health from the GM flu vaccine include:

- inadvertent occupational exposure of staff handling the GM flu vaccine, leading to infection, and
- unintentional release of the GM flu vaccine strains during transport and storage, leading to exposure and infection of humans/animals.

191. These scenarios that could lead to the development of influenza, resulting in ill health are discussed below.

Risk scenario 1

Risk source	GM flu vaccines
Causal pathway	⬇ Person is exposed to GM flu vaccines during waste disposal ⬇ Person is infected with GM flu vaccine strains ⬇ Influenza develops ⬇
Potential harm	Ill health

192. Individuals who handle vaccines may be inadvertently exposed to the GM flu vaccine strains while disposing of used, expired, or unused sprayers of the GM flu vaccine. The locations where these are most likely to occur are:

- the room where GM flu vaccine would be administered
- the distribution warehouse where stocks of GM flu vaccine are held
- facilities for the disposal of the GM flu vaccine and associated clinical waste.

193. Healthcare staff dispose of medical waste routinely and would have standardised procedures for the safe disposal of both used sprayers with residual GM flu vaccine and expired sprayers of GM flu vaccine. This is required by the State governments, which have issued operational guidance documents for the disposal of biohazardous waste to reduce potential risk to waste handlers.

194. Vaccine at the distribution facility may need to be destroyed if its expiry date has passed or when it is superseded by a new seasonal vaccine. The sealed packs of GM flu vaccine sprayers would be placed in containers which are security sealed, tagged and loaded into secure destruction bins. The waste contractor would incinerate the sprayers. Given the sprayers are triple contained during waste disposal, they are highly unlikely to leak in a manner that would lead to exposure of waste handlers to an infective dose the GM flu vaccine.

195. The *Australian code of good wholesaling practice for medicines in schedules 2, 3, 4 & 8* requires (NCCTG 2011):

- specific training for personnel handling medicines that pose high risk to personnel if package integrity is breached or spillage occurs
- waste medicines be collected and destroyed by a person who is licensed or permitted to do so under relevant State or Territory legislation
- medicines for destruction be enclosed in sealed packaging or in a container.

196. Influenza viruses are highly susceptible to desiccation due to their lipid envelope. The GM viruses would not remain viable for extended periods on open surfaces.

197. The GM flu vaccines must be stored between 2-8°C and requires a cold chain, which is a well-controlled and uninterrupted sequence of transport and storage to maintain the vaccine in

this narrow temperature range. GM flu vaccines slated for destruction would be held at ambient temperature and this would increase their rate of deterioration.

198. For productive infection, individuals must be exposed to an infectious dose. Since each filled sprayer would contain the infectious dose of each GM flu vaccine strain, the residual liquid in used sprayers would not contain a sufficient titre to cause productive infection. The same would apply to secondary waste such as gloves that may be contaminated with the vaccine. Influenza viruses cannot replicate outside a host cell and the residual viruses in the used sprayers could not multiply to reach an infective dose.

199. Even if an individual was exposed to the GM flu vaccines, they would be attenuated and therefore, less pathogenic than circulating flu strains.

200. ***Conclusion***: Scenario 1 is not identified as a substantive risk. The GM vaccines are attenuated and it is highly unlikely that persons involved in waste disposal would be exposed to an infectious dose of the GM flu vaccine viruses. Therefore, this risk would be negligible and does not warrant further detailed assessment.

Risk scenario 2

Risk source	GM flu vaccines
Causal pathway	⬇ Unintentional release of GMOs during transport or storage ⬇ People or animals exposed to GMOs ⬇ People or animals infected with GMOs ⬇ Disease develops ⬇
Potential harm	Increased disease burden

201. The cold chain, which is intended to preserve the potency of the vaccine, requires cold packaging/refrigeration and this adds a level of containment during import, storage and transport.

202. AstraZeneca proposes to supply the vaccine in single dose 0.5 mL sprayers in sealed cartons. These packs would be placed in multipack cartons and the cartons packed in corrugated cardboard shipping cartons for distribution.

203. For import under the appropriate International Air Transport Association (IATA) shipping classification, the minimum packing requirements for GMOs are:

- leak-proof primary containers individually wrapped or separated to prevent contact between them
- leak-proof secondary containers that satisfies a pressure differential of 95kPa, and
- sufficient absorbent material to absorb the contents of all primary receptacles placed between the primary and secondary containers.

204. Transport of GM flu vaccines between the port of entry and the warehouse would continue in the above packaging and would also have to satisfy cold chain requirements.

205. Storage, handling and transport would be in accordance with the *Australian code of good wholesaling practice for medicines in schedules 2, 3, 4 & 8* (NCCTG 2011) and the WHO's Good distribution practices for pharmaceutical products (WHO 2010). These guidelines require that:

- in the event of a spill or leak, the GMO be rendered non-viable as soon as possible
- a supply of decontaminant effective against the GMO (spill kit) be readily available
- access to the GM vaccine be restricted to individuals with the appropriate training to deal with the GM vaccines.

These practices would be suitable for the GM flu vaccines and would lower the probability of unintended GMO release.

206. Vaccines are classified as Schedule 4 medicines. The *Australian code of good wholesaling practice for medicines in schedules 2, 3, 4 & 8* (NCCTG 2011) recommends that:

- upon arrival, packaging should be removed, and stock should be examined for the absence of damage or evidence of tampering. Damaged stock should be quarantined.
- packaging and handling of cold chain medicines should alert the receiver of its contents and that the receiver should place the medicines in appropriate storage facilities as soon as possible, and
- wholesalers ensure that persons supplied with medicines are authorised appropriately under State or Territory legislation to be supplied with those medicines.

207. These practices are required by State and Territory legislation. They are suitable for the GM flu vaccines and would ensure examination of these vaccine stocks for any small leaks that may precede larger spills. Restricting supply to select entities would increase the chances of the GM flu vaccines being handled by individuals who would know how to decontaminate a spill.

208. The GM flu vaccine strains are susceptible to common chemical decontaminants such as detergents and hypochlorite (see Chapter 1 Section 5.6). The Material Safety Data Sheet (MSDS) would provide instruction for the clean-up and decontamination of spills. Therefore, there is little potential for exposure of humans or animals to the GM flu vaccine viruses.

209. Should the GM flu vaccine viruses be unintentionally released, it is highly unlikely that they would infect people or animals as they desiccate easily, cannot replicate outside a host and are readily decontaminated. The GM flu vaccine viruses are restricted in replication like their cold-adapted parental strains and would be less pathogenic than circulating flu strains. Therefore, even if an individual or animal is inadvertently exposed to the GM flu vaccine viruses, these factors combined with the attenuation of the viruses would make productive infection unlikely.

210. ***Conclusion***: Scenario 2 is not identified as a substantive risk. The GM vaccines are attenuated and it is highly unlikely that inadvertent release of the GMO would result in persons or animals being exposed to an infectious dose of GM flu vaccine. Therefore, this risk would be negligible and does not warrant further detailed assessment.

2.2 Unintended changes in viral characteristics

211. Horizontal gene transfer (HGT) is the stable transfer of genetic material from one organism to another without reproduction (Keese 2008). All genes within an organism, including those introduced by gene technology, can be transferred to another organism by HGT. HGT itself is not considered an adverse effect, but an event that may or may not lead to harm. A gene transferred through HGT could confer a novel trait to the recipient organism, through expression of the gene itself or through changes to expression of endogenous genes. The novel trait may result in negative, neutral or positive effects. All the genes and genomic segments in the GM flu vaccines would be derived from existing non-GM flu strains (either the parent attenuated vaccine strains or circulating strains) and therefore the GM vaccine strains do not introduce any novel genetic material for HGT.

212. Reassortment, which may be considered a mechanism of HGT, is a continual source of novel influenza viruses and can lead to the emergence of pandemic strains. In the GM vaccines strains, the haemagglutinin and neuraminidase segments from contemporary circulating strains replace the equivalent segments from the cold-adapted parent strains. Reassortment could lead to the introduced segments being replaced. The same process could also result in the transfer of the introduced haemagglutinin and neuraminidase segments to a circulating strain. Other genomic segments could also reassort between the vaccine strains and circulating strains.

213. The two influenza A vaccine viruses in the GM flu vaccine reassorted at low levels in vaccine recipients (Buonagurio et al. 2006b). The reassortants were attenuated as they always carried six segments from the cold adapted parent.

214. Reassortment between influenza A viruses and influenza B viruses does not occur and will not be considered further. Influenza viruses do not undergo homologous recombination as they have single-stranded genomes, and this will not be considered further.

Risk scenario 3

Risk source	GM flu vaccines
Causal pathway	⬇ Person infected with the GMO is infected with contemporary influenza virus carrying a different haemagglutinin and/or neuraminidase from the vaccine strains ⬇ Both viruses co-infect the same host cell ⬇ Viruses reassort ⬇ Reassortant infects host and propagates ⬇ Influenza develops ⬇
Potential harm	Ill health

215. For reassortment to occur, two different influenza viruses of the same type must co-infect a host cell. If this does occur, reassortment could theoretically result in viral progeny having any permutation of the genomic segments of the parent viruses.

216. The temporal mismatch in the presence of GM flu vaccine strains and contemporary strains reduces the chances of co-infection. The GM flu vaccine strains would be present when people are vaccinated. This generally happens in early autumn before the onset of the influenza season. The likelihood of a person being unintentionally infected with the GM vaccine strains is extremely low (see Risk scenarios 1 and 2), and the GM vaccine strains would generally not be transmitted from vaccinated or otherwise infected people (see Chapter 1 Section 5.5.3). The contemporary strains would be present during the influenza season, which usually begins in late autumn and peaks in winter.

217. A study of GM flu vaccine viruses showed that when an attenuated vaccine virus and a wild-type influenza virus reassort, the likely outcome is viruses that are attenuated or less virulent than wild-type strains (Parks et al. 2007). No reassortant was more virulent than wild-type, and the majority of reassortants replicated less efficiently in infected ferrets. This could be explained by the attenuating mutations of the GM flu vaccine viruses being located on different genomic segments, increasing the likelihood of the presence of attenuating mutations in any reassortant.

218. If GM flu vaccine viruses reassort with a circulating influenza virus, the introduced haemagglutinin and/or neuraminidase segments could transfer from the GM flu vaccine viruses to influenza strains in the environment. However, these segments were derived from strains in circulation, and indeed are chosen as vaccine targets because they are predicted to be the most widely circulating strains for the season. Therefore, such a reassortant would introduce no genetic novelty into the environment and would not alter the epidemiology of the influenza season.

219. Different haemagglutinin and/or neuraminidase segments could also be transferred into the GM flu vaccine viruses by reassortment with a circulating strain other than the target strains. The overwhelming majority of genetically novel reassortants would retain the attenuated phenotype of the vaccine strains, and be less pathogenic than the circulating strain.

220. Theoretically, reassortment can generate any combination of genomic segments from the GM vaccine strains and circulating strains but analysis of reassortants shows that not all reassortants occur with equal probability (Angel et al. 2013; Wendel et al. 2015). This may be due to the preferential packaging of some segments, decreased replicative fitness of some reassortants due to mismatches in the haemagglutinin and neuraminidase (see Chapter 1 Section 4.7 and 4.12), or mismatches in other viral components (Treanor & Murphy 2015). Additionally, as noted in Chapter 2 Section 2.2 above, all of the genes and genomic segments in the GM flu vaccines would be derived from existing flu strains, and therefore reassortment would not introduce any novel genetic material.

221. ***Conclusion***: Scenario 3 is not identified as a substantive risk. The GM vaccines strains would not contain any novel genomic segments, co-infection of a host cell by the GMOs and another strain would be uncommon, and reassortants would be less virulent than circulating strains. Therefore, this risk would be negligible and does not warrant further detailed assessment.

Risk scenario 4

Risk source	GM flu vaccines
Causal pathway	⬇ Person infected with the GMO is infected with an influenza virus of swine or avian origin ⬇ Both viruses co-infect the same host cell ⬇ GMO and swine/avian influenza virus reassort ⬇ Haemagglutinin and/or neuraminidase segment in GMO is replaced with equivalent segment from swine or avian influenza strain ⬇ Reassortant infects host and propagates ⬇ Influenza develops ⬇
Potential harm	Ill health

222. As noted in Risk scenario 3, for reassortment to occur two different influenza viruses of the same type must co-infect a host cell. The likelihood of a person being unintentionally infected with the GM vaccine strains is extremely low (see Risk scenarios 1 and 2), and the GM vaccine strains would generally not be transmitted from vaccinated or otherwise infected people (see Chapter 15.5.3).

223. In addition, exposure of people to influenza viruses of avian or swine origin in Australia is very rare for the following reasons:

- swine influenza viruses are not endemic in Australia and transmission requires close contact with infected swine
- DAWR surveillance indicates the H5N1 avian influenza virus is not present in Australia. No cases of H7N9 avian influenza have been reported in Australia.
- migratory birds infected with and suffering the symptoms of avian influenza are unlikely to complete the flight to Australia
- while there was an outbreak of H7N2 avian influenza in Young in 2013, there have been no reports of avian influenza since then
- the most common mode of transmission for avian influenza viruses is the faecal to oral route and they do not transmit efficiently via the airborne route (de Graaf & Fouchier 2014; Sorrell et al. 2011).

224. Reassortment between the GM vaccine strains and influenza strains of avian/swine origin could result in either or both the haemagglutinin and neuraminidase segments in the GM vaccine strain being replaced by the equivalent segments from the swine/avian strain. This would alter the antigenic characteristics of the influenza virus but it would remain cold-adapted and attenuated.

225. Not all reassortants are viable as a novel haemagglutinin-neuraminidase combination may lack the optimal balance between binding to and release from the receptor that is required for productive infection (see Chapter 1 Section 4.7 and 4.12).

226. If avian flu haemagglutinin replaces the GM flu vaccine strain haemagglutinin, non-optimal cellular tropism could prevent or limit infection. Avian flu haemagglutinin preferentially binds the SAα-2,3 receptor which is present in low quantities in the human nasopharynx and lung (de Graaf & Fouchier 2014). In the nasopharynx, the human SAα-2,3 receptors, being O-linked, would be cleaved poorly by avian flu neuraminidase (see Chapter 1 Section 4.12). Virus aggregation would result and this would impede repeated cycles of infection. In the lung, the temperature would restrict replication of the cold-adapted reassortant.

227. Should the abovementioned reassortants infect swine and avian species, their replication would also be restricted. Some segments in these reassortants would be derived from the cold-adapted parent strain and the proteins encoded by these segments would not be expected to function optimally at the higher body temperatures of these species. The vaccines viruses were unable to productively infect experimentally inoculated pigs or birds (see Chapter 15.5.3).

228. If avian/swine neuraminidase replaces the GM vaccine strain neuraminidase, poor receptor cleavage would restrict replication. The avian/swine neuraminidase preferentially cleaves the SAα-2,3 receptor and it would have to act on a human haemagglutinin which preferentially binds the SAα-2,6 receptor (see paragraph 96). Poor cleavage of this receptor would promote aggregation and restrict replication.

229. The attenuating mutations in the GM flu vaccine strains are located on different genome segments. When a GM vaccine strain reassorts with an avian/swine strain, the reassortants are likely to be less virulent than when a circulating human flu strain reassorts with an avian/swine strain.

230. FluMist was in use during the 2009 swine flu pandemic in the USA. This would have provided an opportunity for the GM vaccine strains to reassort with the swine flu virus. When similar reassortants were constructed in the laboratory, they were found to be attenuated (Zhou

et al. 2012). There was no evidence of any such reassortment with the vaccine leading to new, persistent circulating strains during the pandemic.

231. As noted in Risk scenario 3, although reassortment can generate any combination of segments from the two co-infecting viruses, analysis of reassortants shows that not all reassortants occur with equal probability (Angel et al. 2013; Wendel et al. 2015). This may be due to the preferential packaging of segments, decreased replicative fitness of reassortants due to mismatches in the haemagglutinin and neuraminidase (see Chapter 1 Section 4.7 and 4.12), or mismatches in other viral components. Additionally, as noted in Chapter 2 Section 2.2 above, all of the genes and segments in the GM flu vaccines would be derived from existing flu strains, and therefore reassortment would not introduce any novel genetic material.

232. ***Conclusion***: Scenario 4 is not identified as a substantive risk. The GM vaccines strains would not contain any novel genomic segments, co-infection of a host cell by a GM vaccine strain and an avian or pig flu strain would be highly unlikely and reassortants would be less virulent than reassortants between an avian or pig strain and a circulating flu strain. Therefore, this risk would be negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

233. Uncertainty is an intrinsic part of risk analysis[2]. There can be uncertainty in identifying the risk source, the causal linkage to harm, the type and degree of harm, the likelihood of harm or the level of risk. In relation to risk management, there can be uncertainty about the effectiveness, efficiency and practicality of controls.

234. Risk analysis can be considered as part of a structured, transparent process to analyse and address uncertainty when identifying, characterising and evaluating risk (first tier uncertainty analysis). However, residual uncertainty always remains and if it is critical to decision making, it could be further analysed (second tier uncertainty analysis) through building 'worst case' scenarios, or by combining results from several studies (meta-analysis).

235. There are several types of uncertainty in risk analysis (Bammer & Smithson 2008; Clark 2006). These include:

- uncertainty about facts:
  - knowledge – data gaps, errors, small sample size, use of surrogate data
  - variability – inherent fluctuations or differences over time, space or group, associated with diversity and heterogeneity
- uncertainty about ideas:
  - description – expression of ideas with symbols, language or models can be subject to vagueness, ambiguity, context dependence, indeterminacy or under-specificity
- perception – processing and interpreting risk is shaped by our mental processes and social/cultural circumstances, which vary between individuals and over time.

236. Uncertainty can also arise from a lack of experience with the GMO itself. Australia lacks experience with nasally administered GM flu vaccines. However, with these GM flu vaccines the overall level of uncertainty is low given they have been approved and administered in

[2] A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* available from the OGTR website or via Free call 1800 181 030.

several jurisdictions, one for over a decade. None of these releases have resulted in a serious adverse event for health and safety of people, or the environment.

Section 4 Risk evaluation

237. Risk is evaluated against the objective of protecting the health and safety of people and the environment to determine the level of concern and, subsequently, the need for controls to mitigate or reduce risk. Risk evaluation may also aid consideration of whether the proposed dealings should be authorised, need further assessment, or require collection of additional information.

238. Factors used to determine which risks need treatment may include:

- risk criteria
- level of risk
- uncertainty associated with risk characterisation
- interactions between substantive risks.

239. Four risk scenarios were identified whereby the proposed dealings might give rise to harm to people of the environment. This included consideration of whether the genetic modifications could result in altered characteristics that may impact on the disease burden of the GM virus or produce unintended changes in viral characteristics. The opportunity for gene transfer and its effects, if this occurred, were also considered.

240. A risk is substantive only when the risk scenario may, because of gene technology, have some chance of causing harm. Risk scenarios that do not lead to harm, or could not reasonably occur, do not represent an identified risk and do not advance in the risk assessment process.

241. In the context of the control measures proposed by the applicant and the operating guidelines of the pertinent regulatory agencies, and considering both the short and long term, none of these scenarios were identified as substantive risks requiring further assessment. The principal reasons for this include:

- exposure to the proposed GM flu vaccines would be minimised by well-established clinical, import, transport, storage and disposal procedures
- influenza virus survival outside of a host is limited to short periods, and it is susceptible to common chemical decontaminants
- there would be limited opportunity for reassortment between the GM vaccine strains and circulating flu strains
- the proposed GM flu vaccine strains contain a number of naturally occurring mutations that lead to their attenuation
- all of the genes and genomic segments in the GM flu vaccines would be derived from existing non-GM flu strains
- any reassortment between the GM vaccine strains and other flu strains would not be expected to lead to more virulent strains.

242. Therefore, any risks posed to the health and safety of people, or the environment, from the proposed release of the GM flu vaccines are considered to be negligible. The *Risk Analysis Framework* (OGTR 2013), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation. No controls are required to treat these negligible risks. Hence, the Regulator

considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment[3].

[3] As none of the proposed dealings are considered to pose a significant risk to people or the environment, Section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator has allowed up to 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

Chapter 3 Risk management plan

Section 1 Background

243. Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan addresses risks evaluated as requiring mitigation; it considers limits and controls proposed by the applicant together with general risk management measures. The risk management plan informs the Regulator's decision-making process and is affected through licence conditions.

244. Under Section 56 of the Act, the Regulator must not issue a licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in a way that protects the health and safety of people and the environment.

245. All licences are subject to three conditions prescribed in the Act. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. The other statutory conditions allow the Regulator to maintain oversight of licensed dealings: Section 64 requires the licence holder to provide access to premises to OGTR inspectors and Section 65 requires the licence holder to report any information about risks or unintended effects of the dealing to the Regulator on becoming aware of them. Matters related to the ongoing suitability of the licence holder are also required to be reported to the Regulator.

246. The licence is also subject to any conditions imposed by the Regulator. Examples of the matters to which conditions may relate are listed in Section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under Section 152 of the Act.

Section 2 Risk treatment measures for substantive risks

247. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed dealings with GM flu vaccines. These risk scenarios were considered in the context of the proposed receiving environment and the Australia-wide release. The risk evaluation concluded that no controls are required to treat the negligible risks.

Section 3 General risk management

248. All DIR licences issued by the Regulator contain a number of conditions that relate to general risk management. These include conditions relating to:

- applicant suitability
- identification of the persons or classes of persons covered by the licence
- reporting structures
- a requirement that the applicant allows access to specified sites for purpose of monitoring or auditing.

3.1 Applicant suitability

249. In making a decision whether or not to issue a licence, the Regulator must have regard to the suitability of the applicant to hold a licence. Under Section 58 of the Act, matters that the Regulator must take into account include:

- any relevant convictions of the applicant (both individuals and the body corporate)
- any revocation or suspension of a relevant licence or permit held by the applicant under a law of the Commonwealth, a State or a foreign country

- the capacity of the applicant to meet the conditions of the licence.

250. On the basis of information submitted by the applicant and records held by the OGTR, the Regulator considers AstraZeneca suitable to hold a licence.

251. The draft licence includes a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability.

252. In addition, the applicant organisation must have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

3.2 Testing methodology

253. AstraZeneca must provide the Regulator with a method to reliably detect the presence of GM vaccine strains and introduced genetic material in humans. This instrument is required prior to conducting any dealings authorised by the licence.

3.3 Identification of the persons or classes of persons covered by the licence

254. Any person, including the licence holder, may conduct any dealing with the GM vaccine permitted by the licence.

3.4 Reporting requirements

255. The licence obliges the licence holder to immediately report any of the following to the Regulator:

- any additional information regarding risks to the health and safety of people or the environment associated with the dealings
- any contraventions of the licence by persons covered by the licence
- any unintended effects of the release.

256. The licence holder is also obliged to submit an Annual Report containing any information required by the licence.

257. There are also provisions that enable the Regulator to obtain information from the licence holder relating to the progress of the commercial release (see Section 4, below).

3.5 Monitoring for Compliance

258. The Act stipulates, as a condition of every licence, that a person who is authorised by the licence to deal with a GMO, and who is required to comply with a condition of the licence, must allow inspectors and other persons authorised by the Regulator to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing.

259. In cases of non-compliance with licence conditions, the Regulator may instigate an investigation to determine the nature and extent of non-compliance. The Act provides for criminal sanctions of large fines and/or imprisonment for failing to abide by the legislation, conditions of the licence or directions from the Regulator, especially where significant damage to the health and safety of people or the environment could result.

Section 4 Post release review

260. Regulation 10 requires the Regulator to consider the short and the long term when assessing risks. The Regulator does not fix durations, but takes account of the likelihood and impact of an adverse outcome over the foreseeable future, and does not disregard a risk on the basis that an adverse outcome might only occur in the longer term. However, as with any predictive process, accuracy is often greater in the shorter rather than longer term.

261. For the current DIR licence application, the Regulator has incorporated a requirement in the licence for ongoing oversight to provide feedback on the findings of the RARMP and ensure the outcomes remain valid for future findings or changes in circumstances. This ongoing oversight would be achieved through post release review (PRR) activities. The three components of PRR are:

- adverse effects reporting system (Section 4.1)
- requirement to monitor specific indicators of harm (Section 4.2)
- review of the RARMP (Section 4.3).

262. The outcomes of these PRR activities may result in no change to the licence or could result in the variation, cancellation or suspension of the licence.

4.1 Adverse effects reporting system

263. Any member of the public can report adverse experiences/effects resulting from an intentional release of a GMO to the OGTR through the Free-call number (1800 181 030), fax (02 6271 4202), mail (MDP 54 – GPO Box 9848, Canberra ACT 2601) or via email to the OGTR inbox (ogtr@health.gov.au). Reports can be made at any time on any DIR licence. Credible information would form the basis of further investigation and may be used to inform a review of a RARMP (see Section 4.3 below) as well as the RARMPs of future applications involving similar GMO(s).

4.2 Requirement to monitor specific indicators of harm

264. Additional specific information on an intentional release provides a mechanism for 'closing the loop' in the risk analysis process and for verifying findings of the RARMP, by monitoring the specific indicators of harm that have been identified in the risk assessment.

265. The term 'specific indicators of harm' does not mean that it is expected that harm would necessarily occur if a licence was issued. Instead, it refers to measurement endpoints which are expected to change should the authorised dealings result in harm. Should a licence be issued, the licence holder would be required to monitor these specific indicators of harm as mandated by the licence.

266. The triggers for this component of PRR may include risk estimates greater than negligible or significant uncertainty in the risk assessment.

267. Characterisation of the risk scenarios discussed in Chapter 2 did not identify any risk levels that were greater than negligible. The risks were not considered substantive and warranting further detailed assessment. The uncertainty associated with the proposed dealings is considered low and no specific indicators of harm have been identified in this RARMP for application DIR 137. However, specific indicators of harm may be identified during later stages, e.g. following the consideration of comments received on the consultation version of the RARMP, or if a licence were issued, through either of the other components of PRR.

268. Conditions have been included in the licence to allow the Regulator to request further information from the licence holder about any matter to do with the progress of the release, including research to verify predictions of the risk assessment.

4.3 Review of the RARMP

269. The third component of PRR is the review of RARMPs after a commercial/general release licence is issued. Such a review would take into account any relevant new information, including any changes in the context of the release, to determine if the findings of the RARMP remained current. The timing of the review would be determined on a case-by-case basis and may be triggered by findings from either of the other components of PRR or be undertaken

after the authorised dealings have been conducted for some time. If the review findings justified either an increase or decrease in the initial risk estimate(s), or identified new risks to people or to the environment that needed managing, this could lead to changes to the risk management plan and licence conditions.

Section 5 Conclusions of the RARMP

270. The risk assessment concludes that this proposed commercial release of GM flu vaccines poses negligible risks to the health and safety of people or the environment as a result of gene technology. These negligible risks do not require specific risk treatment measures.

271. General licence conditions are proposed to ensure ongoing oversight of the commercial release.

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Appendix A: Summary of submissions from prescribed experts, agencies and authorities on matters relevant to the preparation of the consultation RARMP[4]

The Regulator received several submissions from prescribed experts, agencies and authorities on matters relevant to preparation of the RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered. These issues and where they were addressed in the consultation RARMP, are summarised below.

Table 4 Summary of submissions on the preparation of the consultation RARMP

Sub #	Summary of issues raised	Consideration in RARMP	Comment
1	A condition should be applied to ensure that waste generated from the use of the GMO is disposed of appropriately.	Chapter 2 Section 2.1	Exposure to the GM flu vaccines and inadvertent release into the environment would be minimised by well-established procedures for disposal of waste by trained staff. Relevant codes of practice required by the States and the regulatory agencies are designed to protect the people and environment.
2	Recommended that this matter be directed to NSW Health for comment and distribution to medical practitioners	–	Consultation on the RARMP will include State and Territory Governments, prescribed Australian Government agencies (including the TGA), relevant local councils, the Gene Technology Technical Advisory Committee (GTTAC) and the public.
3	No comment; receipt of notification acknowledged.	–	None
4	As the parent strains are attenuated, the GMOs are not regulated under security sensitive biological agent regulatory scheme.	–	Noted
5	Has no concerns regarding this application.	–	Noted
6	Have identified some pathways to harm that relate to environmental spread and persistence, gene transfer and host range: • spread may be due to accidental release during transport or shedding by patients [reference provided to relevant studies on shedding]. • influenza viruses and their genetic material may persist on, and be transmitted via various surfaces [reference provided]. • viral host range may be altered by genetic changes. Effects could be severe in a new host species. Potential to infect native and domesticated animals should be addressed in the RARMP. • recombination with flu viruses of avian or swine origin could have serious implications, noting that other attenuated viruses have been shown	Chapter 1 Section 4.9, Chapter 2 Section 2	Details of the environmental host range and transmissibility of the GM vaccine has been included in Chapter 1 Section 5.5 of the RARMP. Potential for adverse effects from exposure to the GM vaccine are addressed in Chapter 2 Section 2, including as a result of spills (Section 2.1) and viral reassortment with avian or swine flu (Section 2.2).

[4] Prescribed agencies include GTTAC, State and Territory Governments, relevant local governments, Australian government agencies and the Minister for the Environment.

Sub #	Summary of issues raised	Consideration in RARMP	Comment
	to recombine to produce more virulent stains. Recommends that the uncertainties linked to transmission, recombination and non-targeted effects be addressed in the RARMP.		
7	Identified the following matters for consideration: • environmental release during waste disposal • experience of companies transporting in GM vaccine • administration in medical facilities to ensure waste is appropriately disposed of • protection of medical staff and patient contacts through disposal or shedding of the GMO • monitoring compliance with licence conditions • appropriate scheduling as prescription only • applicant claims regarding low likelihood of the GMOs establishing in environment should be considered by Dept of the Environment.	Chapter 1 Sections 6.2 & 6.4, Chapter 2 Section 2, Chapter 3 & Chapter 4	Exposure to the GM flu vaccines and inadvertent release into the environment would be minimised by well-established procedures for import, transport, storage and disposal from healthcare facilities. Relevant codes of practice required by the States and the regulatory agencies are designed to protect people and the environment. The Environment Minister and the Department of the Environment have been consulted on the application and will be consulted again on the RARMP. TGA approval will also be required for the proposed use of the GM vaccines. TGA will address quality, patient safety and efficacy of the GMO as a therapeutic good. TGA may impose conditions on use of therapeutic products.
8	Provided background information on flu vaccines currently available in Australia and on the proposed vaccines (with references), and noted the following: • live attenuated influenza vaccines have good safety profile • almost zero risk of person to person transmission of virus. • chances of reversion to wild type are remote • efficacy at least as good as flu vaccine currently available in Australia, and better in children • closely mimics immune response during natural infection • more acceptable method of delivery for children • offers potential for rapid vaccine development in response to a pandemic situation Considers the use in non-pregnant people age 2-49 years, as licenced in USA, is reasonable for Australia	–	Noted. TGA approval will also be required for the proposed use of the GM vaccines. TGA will address quality, patient safety and efficacy of the GMO as a therapeutic good. TGA may impose conditions on use of therapeutic products.
9	Identified a number of deficiencies in the application which should be addressed in the RARMP: • reason for choice of release locations • product packaging should state vaccine contains GMOs • insufficient detail on parent virus • lacks detail on claims human/avian influenza virus reassortant will be attenuated • lacks detail on statement that vaccine has no adverse consequences • will there be monitoring of who is vaccinated to assess compliance with recommendation not to treat pregnant women • will persons administering the vaccine know how to clean up of spills, noting ineffectiveness some	Chapter 1, Sections 4 & 5 Chapter 2, Section 2	Details of the parent organism, including reference to adverse effects, have been included in Chapter 1 Sections 4 and 5 of the RARMP. Potential for adverse effects from exposure to the GM vaccine are addressed in Chapter 2 Section 2, including as a result of spills (Section 2.1) and viral reassortment (Section 2.2). Issued associated with use of the GM vaccines as therapeutic goods will be addressed by TGA. TGA may impose conditions on use of therapeutic products.

Sub #	Summary of issues raised	Consideration in RARMP	Comment
	disinfectants		
10	Provided the following issues for consideration: • Potential for residual vaccine/ build-up of vaccine in room after vaccine nasal spraying • Potential for over-exposure of administrator due to aerosol build-up • Potential for unintended exposure of subsequent room occupants to aerosol • How does administrator ensure delivery of full vaccine dose	–	These issues will be addressed by TGA in their assessment of the GM vaccines as therapeutic goods.
11	Recommends that the Regulator consider: • transport, storage and disposal in the context of intended vaccine-delivery workforce and facilities • the need for labelling of packaging • the need for oversight of each new vaccine strain • risk associated with incidental exposure	Chapter 2 Section 2, Chapter 3	Potential for exposure to the GM flu vaccines would be minimised by well-established procedures for import, transport, storage and disposal of medical products. Relevant codes of practice required by the States and the TGA are designed to protect the workforce. The TGA regulates the labelling of scheduled medicines. The TGA will consider exposure through shedding by vaccinated individuals.

Appendix B: Summary of submissions from prescribed experts, agencies and authorities on the consultation RARMP[5,6]

The Regulator received several submissions from prescribed experts, agencies and authorities on the consultation RARMP. All issues raised in submissions that related to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence and were used in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence. Advice received is summarised below.

Sub #	Summary of issues raised	Consideration in RARMP	Comment
1	Does not envisage any GM-related health concerns that have not been assessed. Notes that the Tasmanian *GMO Control Act 2004* is not meant to extend to medical purposes.	–	Noted.
2	Does not have expertise to provide comment	–	Noted.
3	Human influenza viruses are known to infect Australian birds and are likely to infect Australian animals such as seals. Unaware of any cases of human influenza virus infecting native Australian mammals. Supports conclusion in RARMP that the probability of anthroponotic transmission is low.	Chapter 1 Sections 5.5.3 and 6.4	More detail has been added to the sections on transmissibility and alternate hosts. The attenuation of the GM vaccine viruses has resulted in them being unable to replicate in a number of species susceptible to circulating human influenza strains. Native birds and seals are not likely to be infected through shedding by vaccinated individuals.
4	Should consider recommendation not to treat pregnant women on vaccine packaging.	Chapter 2, Section 2	The TGA has regulatory responsibility for quality, efficacy and patient safety of therapeutic goods for use in Australia, including the labelling of scheduled medicines. This issue will be considered by the TGA in their assessment of GM vaccines.
5	It would seem on the information provided that there is minimal risk for this application.		Noted
	Registration status of GM vaccines in jurisdictions outside Australia should be clarified.	Chapter 1, Section 7	Overseas marketing approvals are listed in the summary and Table 2 of the RARMP.
	Where were the parent strains isolated?	Chapter 1, Section 4	Derivation of the parent strains is described in Ch 1, Section 4.2
	Has the replication capacity of parent strain or GM vaccine strains been tested in other species? Has replication of final vaccine dose been tested?	Chapter 1, Section 5	As described in Ch 1, Section 5.5.3, replication of the GM vaccine strains has been tested in a number of birds and mammals. Productive infection of the GM flu viruses was not observed in pigs, horses or other domestic species, nor in any tested species of bird. The viruses replicated only in the upper respiratory tract of ferrets, hamsters, and guinea pigs.

[5] Prescribed agencies include GTTAC, State and Territory Governments, relevant local governments, Australian government agencies and the Minister for the Environment.
[6] No submissions were received from the public.

Sub #	Summary of issues raised	Consideration in RARMP	Comment
	Is there evidence of reassortment or selective growth of particular vaccine strains from laboratory or animal studies?	Chapter 2, Risk scenarios 3 & 4	Reassortment is considered in detail in Ch 2, Risk scenarios 3 & 4. All of the GM vaccine strains carry attenuating mutations and have reduced replication relative to circulating flu strains.
	Are the parent viruses or the GMOs susceptible to existing flu treatments?	Chapter 1, Section 5	The vaccine strains are expected to remain susceptible to neuraminidase inhibitors such as Tamiflu or Relenza. Text has been added to Ch 1, Section 5.6
6	Notes that vaccines would be manufactured overseas and distributed Australia-wide. Does not object to issue of licence.	–	Noted
7	Supports the Regulator's conclusion that DIR 137 poses negligible risk of harm to human health and the environment.	–	Noted
8	Concerned that pregnant women / immune-compromised persons may receive the vaccine in error. Do not see need for a live virus vaccine given availability of multiple inactivated vaccines.	–	The TGA has regulatory responsibility for quality, efficacy and patient safety of therapeutic goods for use in Australia. This issue will be considered by the TGA in their assessment of the GM vaccines.
9	Supportive of the application as the RARMP indicates that the proposed release poses negligible risks to people or the environment. Notes that GM vaccines are also regulated by the other Australian Government agencies.	–	Noted
10	The Regulator should consider clarifying the description of facilities in which administration of the GM vaccine may occur.	Chapters 1, 2 & 3	RARMP has been modified to clarify the range of facilities where vaccines may be administered.
	Agrees with the overall conclusions of the RARMP	–	Noted