Mapping of Intellectual Property Related to the Production of Pandemic Influenza Vaccines

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This document is prepared as a technical-level input to support discussion on influenza vaccine production capacity building, does not seek to advance, advocate or endorse any policy position, and does not present any official view attributable to WHO, its Member States or its Secretariat.

1. Introduction

Because of the emergence and circulation of H5N1 influenza strains and the need to prepare for a possible influenza pandemic, numerous approaches are being evaluated to improve the production capacity and efficacy of pandemic influenza vaccines. The approaches include *inter alia*: increasing the number of vaccine production sites and particularly establishing influenza vaccine production in developing countries; using production processes which give higher yields, produce vaccine more rapidly, or are less subject to potential supply issues which could arise in the event of a pandemic; adding adjuvants to the antigen to enable immunization with less antigen; using live attenuated vaccines; using vectors to deliver antigens; and using conserved antigens to enhance the breadth of immune protection. For all of the approaches, it is essential to consider the intellectual property relating to that approach.

In this document we have attempted to review the intellectual property issues relating to the manufacture and use of pandemic influenza vaccines. Rather than conducting a large patent map of all intellectual property relating to influenza vaccines, we have instead identified the approaches that are most likely to yield increased global capacity within the next 10 years, and evaluated the intellectual property issues related to each of the approaches. This bottom-up approach is aimed primarily at new manufacturers interested in entering the field of influenza manufacture using technologies that have already been described in the literature.

This document attempts to identify patents which manufacturers should consider, and also tries where possible to identify the patent holder. This document does not claim to cover the entire field, and does not claim to be exhaustive. Researchers and manufacturers considering any approach should be aware that there may be intellectual property covering that approach which has not been considered in this document.

For manufacturers interested in establishing pandemic influenza vaccine production, patents are only one aspect of intellectual property that can affect the feasibility and speed at which local production and regulatory approval of the vaccine can take place.

Indeed for the majority of existing vaccines, and in particular for influenza vaccines, patents do not represent significant barriers, protecting primarily improvements to processes. The other barriers include:

- Technical know-how: Production even of conventional egg-derived influenza vaccines requires technical know-how and skills that are not readily found outside of existing influenza vaccine production plants. While there are a few consultants assisting developing-country manufacturers to install appropriate machinery and establish production processes, the process is likely to be faster when technology-transfer is provided from existing manufacturers. Thus, even for procedures for which there are no patents, partnerships with technology-holders may be necessary.
- Access to regulatory dossiers: as described below for several strains of live attenuated influenza vaccines and also some adjuvant compositions for inactivated vaccines, there is no IP coverage in developing countries. Making 'generic' versions of pandemic vaccines based on these technologies would hence be possible without invoking any compulsory license under TRIPS, however the generation of a regulatory dossier *ab initio*, including extensive preclinical and clinical safety data, genetic stability etc may take years. Licensing such products from manufacturers who have already generated the data would avoid the need to repeat many of these studies, and result in a rapid regulatory submission.
- Market, sustainability, and capital investment: in the absence of a market for seasonal influenza vaccines, the maintenance of a production plant and skilled manpower ready to produce influenza vaccines in the event of a pandemic may represent a significant capital sink.

Scope of patent search: Publicly available databases exist on patents filed with the PCT, and filed in Europe or in the USA. These databases do not provide reliable information on whether a patent has been filed or granted in other countries, particularly developing countries. Hence in this document we have identified patents that have been either filed with the PCT, or have been granted in either USA or Europe, and have not considered whether the patent has been filed in any other country. Manufacturers need to be aware that if the patent in question has not been applied for in the countries in which they plan to manufacture or sell the vaccine then the patent in the USA or EU may not be of relevance to them. One exception, discussed later in this document, refers to the use of reference strains that have been produced in countries where the methods used to produce such strains are patented, even if the final vaccine production is not in those countries.

Technical readers not familiar with patent procedures and the PCT system are directed to the WIPO website (http://www.wipo.int) for further clarification. Below we provide a brief explanation of the relevance of a PCT application:

> A PCT application has the effect of a national application in each of the PCT contracting states at the date of filing of the PCT application (list of PCT Contracting States under: http://www.wipo.int/pct/guide/en/gdvol1/annexes/annexa/ax_a.pdf). However, the

publication of the international application does not mean that a patent is already granted. Also, it does not mean that a patent application will be pursued in all the PCT countries. The patent applicant would have to decide separately to initiate a corresponding application under national law, using the PCT application as a basis.

Whether an actual patent should be granted is decided under the jurisdiction of the national law and the national competent authority and depends on a specific procedural step, known as the entry into the national phase. The applicant has to decide which of the PCT Contracting States it is most interested in obtaining patents, and has to take specific steps to progress the patent application in those countries. This includes furnishing translations of the application into the various national languages, as necessary, paying national fees, and complying with certain national requirements. Unless the applicants request early entry into the national phase, no PCT Contracting State may process the application before the expiration of 30 months from the earliest priority date (PCT Article 23).

In practice, most international applications are pursued on the national level only in a relatively small number of PCT Contracting States. In all other PCT Contracting States, the application loses effect when the national phase is not entered timely so that the claimed invention enters into the public domain in these States.

2. Pandemic influenza vaccine production technologies and processes.

The various methods that are currently being used to produce candidate pandemic influenza vaccines, and the IP associated with each approach, are summarized below. WHO has produced a guidance document which describes the steps required to undertake egg-based or tissue-culture-based production of inactivated or live attenuated influenza vaccines in more detail
http://www.who.int/vaccine_research/diseases/influenza/Flu_vacc_manuf_tech_report.pdf.

2.1 Egg-derived inactivated vaccines.

Producing influenza vaccine in embryonated hens' eggs has been carried out for more than 60 years, and has not yet been replaced as the standard industrial process, despite the recent development of alternative methods. Given the age of the process, it is clear that there can be no patent which create an absolute barrier to the production of vaccines via this process, however several more recent improvements to the process and to final composition have been patented and do need to be considered.

The process: Embryonated eggs from a certified source are obtained and used 9–12 days after fertilization. The eggs are candled to locate the air sac. The egg is pierced under aseptic conditions, and the seed-virus is inoculated into the air-space with a syringe. The procedure can be carried out manually on a laminar flow bench or automatically by machines. The inoculated egg is incubated for two to three days in a humidified atmosphere. At the end of this period, it is maintained at 4°C, which

kills the embryo and aids clarification of the allantoic fluid. The top of the egg is cut off, the membrane pierced, and clear allantoic fluid is removed. Again his can be done manually, or by automated machinery. The allantoic fluid is then clarified, normally by centrifugation to remove cell debris, and then subject to inactivation and further purification according to the type of vaccine being produced.

The process is well described in old patents, now expired. Recent improvements to the process, particularly aiming to improve yields, are covered by IP including **WO 04/113518** which describes the use of high-salt to enhance the yield of virus extraction, and **WO 2005/113756** which describes a method of enhancing yields by introducing reagents such as amino acids into the egg. **WO02/067983** which describes a purification process for making antigens suitable for intradermal delivery:

WO 2004/113518	Process to increase recovery of virus from allantoic fluid by use of high-salt	Microbix
WO 2002/067983	process of purifiying antigen for intradermal vaccine	GSK
WO 2005/113756	enhancing influenza production in eggs by introducing amino acids, serine proteases,	GSK

Whole virus.
The pooled harvest is concentrated and typically purified by ultracentrifugation on a sucrose gradient. After harvesting from the gradient, the virus is diluted, and inactivated either by formaldehyde or betapropiolactone (BPL). The concentration of the inactivating agent needs to be determined and validated for each strain. A filtration step may be included to remove egg debris. Because relatively little purification is involved, the yield of whole-virus vaccine per egg is higher (roughly 2-fold) than with the other types of IIV. In addition whole viruses may be more immunogenic than their split or subunit counterparts since they contain viral RNA which may serve as an adjuvant. On the other hand inactivation needs to be thorough since there is no splitting step. The existing IP on this process is primarily aimed at the inactivation procedure:

EP 865296	Whole virus heat inactivated with thimerosal no formalin	Yissum
US 5698432	Process for producing inactivated whole virus: use of nucleic acid inactivating agent	Retroscreen

Split virus.
The procedure above is followed to the point of harvesting from the sucrose gradient. At that point, the virus is diluted and then ‘split’ by the addition of detergent such as Triton X-100, sodium lauryl sulphate or Tween 80 to extract

proteins from the lipid membrane. The optimal conditions for each strain need to be determined but typically this step can take two to three days. The preparation is then sucrose-gradient purified once more and the HA-rich fraction is harvested. Alternatively, the detergent may be removed by diafiltration. The product is inactivated and sterile filtered.

Compared to the whole-virus preparation, split vaccines are better characterized, contain less ovalbumin and are claimed to be less reactogenic. However the yield of HA can vary significantly from strain to strain and year to year, and from one manufacturer to the next (between 0.7 to 3 eggs required per dose of trivalent vaccine). Numerous processes for splitting are well described in expired patents. Some new improvements are described in:

US 6048537	Process of purifying viral antigens	Sanofi
US 2005/201946	process for preparing split vaccine	GSK

Subunit vaccine.
This is prepared in a similar manner to split virus; however, different, more extensive purification steps are used in place of the second sucrose-gradient purification. This results in the isolation of relatively pure HA with minimal contaminating N, matrix protein, nucleoprotein and lipid. Numerous patents, now expired, describe this process including **US 4158054, US 4140762, US 4064232, US 4064232.** Since these are expired, the processes described can be freely used to make subunit vaccines.

Whether whole, split or subunit vaccine is being produced, a final dilution is made in the formulation buffer. Typically, no adjuvant or stabilisers are used, although preservatives such as thiomersal are frequently added. The addition of adjuvants is described later in this document.

2.2 Cell culture derived inactivated vaccines

Growing influenza virus in tissue culture is being developed in order to overcome some of the limitations of egg-based production. The use of cell lines for influenza virus production is analysed in a WHO publication:

http://www.who.int/vaccine_research/diseases/influenza/WHO_Flu_Cell_Substrate_Version3.pdf

The potential advantages of this approach compared with egg-based manufacture include:

- No requirement for a supply of large numbers of eggs
- A rapidly scaleable process (using technologies that are used in other vaccine production plants)
- Improved aseptic handling during manufacture
- Avoidance of the risk of embryonated eggs containing avian retroviruses.

- Elimination of egg components in the vaccine and the resultant risk of potential allergic reactions

Three cell lines are currently in late-stage development as substrates for the growth of influenza virus. Several manufacturers have filed for the approval of their influenza vaccines produced in cell lines.

Vero cells
Vero cells are a well-characterized and widely used African green monkey kidney-cell line. They have been used for the production of polio vaccine for more than 20 years. They are an adherent cell line and some strains can be cultured in serum-free medium in stationary culture or on microcarriers. The Vero cell line can be obtained from WHO, in a form that still requires characterization, but can also be purchased from ATCC in the form of a fully characterized working seed.

MDCK
Madin Darby canine kidney cells were originally isolated in 1958 and have been widely used for the production of veterinary vaccines. They can produce high quantities of influenza virus. Several companies have developed and characterized proprietary Master and Working Cell Banks derived from the original MDCK cell line, and have adapted the cells to grow under serum-free conditions, including at least one in suspension.

PER.C6
PER.C6 is a relatively new, immortalized human retinal-cell line. It can be cultured in serum-free medium, but only grows in suspension cultures.

Others
Several other cell lines that may be particularly suitable for production of influenza virus are under investigation, including a chicken-derived cell line from Vivalis (Nantes, France). These cell lines have not yet been fully characterized and several years of research and characterization will be required before these can be approved for the production of human vaccines. An potential alternative is the use of chicken embryo fibroblasts which are used in the production of measles vaccine.

IP:
Several of these cell lines have been used for many years and hence are not covered by patents and can be obtained from public sources. However, in order to get regulatory approval for vaccines produced in a cell line requires that Master and Working cell banks are established and characterized, a very lengthy and costly process. Manufacturers who have undertaken such processes are unlikely to share their characterized cell line. Hence even in absence of IP on the cell line, there is a significant barrier to the use of these cells. A possible exception to this is the Vero cell line which can be purchased with full characterization.

There is IP on specific variants of these strains (e.g. MDCK-B-702 described in US 6825036, a MDCK line with higher susceptibility to infection described in **WO 2005/113758**), and on the new cell lines including the PerC6 cell lines (e.g. **US7192759**) and avian embryonic lines (**WO 2006/108846**).

WO 2003/048348	Cell over expressing a nucleic acid encoding sialyltransferase for production of virus	Crucell
WO 2005/113758	A MDCK cell with higher susceptibility to viral infection than parenteral line	ID biomedical
US 6825036	process for preparing MDCK cell in serum-free suspension -specifically MDCK-B-702 line.	Kumamoto-ken, JP
US 7192759	human embryonic retinoblast cell encoding E1 gene of adenovirus to produce influenza	Crucell
WO 2006/108846	avian embryonic stem cell. Replicating virus in suspension	Vivalis
EP 1108787	method for producing flu virus or antigen in cell encoding adenovirus E1 gene.	Crucell

In addition, as discussed below, there is IP on using cell lines for producing influenza vaccines and also processes involving these cell lines, including the VERO strains.

Process: The cells need to be expanded to the desired quantity from working cell banks. Commercial production of vaccines on cell lines needs to be done in a chemically defined medium, hence conditions for growth of the cell line and propagation of the virus under serum-free conditions need to be identified. The cells are then infected with influenza virus and incubated for several days. Parameters such as multiplicity of infection, incubation time and temperature need to be optimized for each cell line and each strain of virus. A prerequisite for a successful infection is the addition of proteases to the medium. After the incubation period, the virus is harvested by removing the tissue-culture supernatant.

There is IP on specific processes or use of cell lines to produce influenza vaccines, or adaptation of cell lines to serum-free medium including :

EP 891420 (Chiron) claims a process of growing viruses in cells, including MDCK and Vero etc, where protease is added to the cells before or during infection. **US6951752** (Baxter) claims a method for the production of virus on any cells, where the cells are first grown to confluence on microcarriers, then infected with the virus. Both of these patents claim specific production processes, which could presumably be worked around if required, even if the resulting process was less efficient.

US6146873, US 5753489, US5698433 (Baxter) which describe and claim the use of continuous monkey kidney cell-line, particularly **Vero** cells, growing in protein-free medium for propagation of influenza virus. Since Vero cells are the most readily available to new manufacturers, the claims in this patent family need to be considered in detail by manufacturers intending to embark on tissue-culture based

propagation of influenza virus. There does not appear yet to be any granted equivalent of this in Europe (equivalent at EPO: **EP1213030A1,** submitted as divisional application of EP0791055A1, also not yet granted) . The PCT application **WO9615231** only lists US, EU, Japan, Finland and Canada as designated states, so it is possible that there are no equivalents to this family in developing countries.

Several patents also describe the use of **MDCK** cells and specifically derivatives of the original MDCK cell line, adapted to grow in serum-free medium and optimized for influenza viral growth including **US6455298** (Chiron/Novartis). An application by this company (**US2005/118140**) claims a method for producing virus in MDCK cells wherein the cells are first infected with the virus and then cultured on a commercial scale. **US 2006/188977** (MedImmune) claims a cell culture composition comprising non-tumorigenic MDCK cells which are derivatives of the ATCC CCL34 line.

Primary chicken embryo cell lines, such as chicken embryo fibroblasts (CEF) have also been considered for influenza virus propagation and vaccine production. Currently this has not been achieved on a large scale, however these cells are used for other vaccines (measles, mumps) by numerous manufacturers so their application to influenza vaccine production could facilitate capacity building. **US5698433** (Baxter) describes and claims a method for producing influenza virus on primary chicken embryo cell lines requiring the process to comprise adding a serine protease and then a serine-protease inhibitor to portions of the culture medium. Manufacturers wishing to use CEF for influenza vaccine production should consider the claims of this patent and whether any equivalent has been filed and granted in their country. The PCT application **WO9615231** only lists US, EU, Japan, Finland and Canada as designated states.

The harvested supernatant is concentrated (typically using diafiltration or centrifugation) and further downstream processing is then required to remove contaminating host-cell DNA. Typically, this involves ion-exchange chromatography to reduce the level of DNA, combined with the addition of benzonase (or a similar reagent) to reduce the size of the DNA fragments that are still present.

Several patents claim cell-culture derived influenza vaccines with methods to reduce DNA content of the final vaccine including **EP870508**, which describes the use of DNAse and detergents,

The yield of influenza virus production in cell culture can be enhanced by several methods which are the subject of IP, including modifying the cell genes coding for PKR or 2-5a (**US6673591, US6686190**), or modifying the viral backbone with an alternative NS1 gene eg **WO 2005/024039**. There is also IP on methods for enhancing the viral growth in cell lines by modifying the PKR or 2-5A genes of the cell (**US7132271**) or using a cell line which over-expresses sialyltransferase..

Procedures for splitting and inactivating the virus are similar to those as described for egg-based manufacture (see above). Inactivated whole-virion vaccines are currently not produced by cell culture.

IP on cell culture inactivated vaccine production process

US 7132271	method for enhancing production using cell with targeted deletion in PKR or 2-5A gene	U. California
US 6673591	use of cells with targeted deletion in PKR gene to enhance virus production in cells	U. California
US 6686190	use of cells with targeted deletion in at least one ISG gene to enhance production in cells	U. California
WO 1997/008292	process to enhance virus production in cells by inhibiting PKR 2-5a synthetase	U. California
US 6344354	Vaccine comprising flu virus produced on cells (isolate not been passaged on eggs)	St Judes
US 6656720	MDCK cell adapted to suspension growth for influenza virus production	Novartis
US 6455298	use of MDCK 33016 for replication of influenza virus in serum-free medium.	
EP 870508	process to reduce DNA content in cell derived vaccine by use of DNAse and detergent	Duphar
US 4500513	influenza vaccine production in liquid cell culture	Miles Lab
US 5698433	Producing flu vaccine in Avian embryo cells: Infection prior to growth on monolayer.	Baxter (Immuno)
US 5753489	producing virus in serum free monkey kidney cells	Baxter (Immuno AG
US 6146873	producing virus in serum free monkey kidney cells	Baxter (Immuno AG
US 5756341	producing in serum free monkey kidney cells, with modified cleavage site in HA	Baxter (Immuno AG
US 5840565	Use of PKR antisense polynucleotide to enhance production of influenza virus in cells	U. California
WO 2005/024039	Replacing NS gene of APR/8 with NS from A/England to produce high titer virus in cells	St Judes
WO 2005/028658	two vectors, at least one containing a pol II promotor linked to a ribozyme sequence	Wisconsin alumni
WO 2006/027698	tests to ensure that no pathogens other than influenza virus are growing in cell culture	Chiron Behring
WO 2006/067211	use of bacteriophage polymerase promoter to produce influenza antigen in cells	Solvay
WO 2007/002008	expression vector containing canine RNA polymerase regulatory sequence; MDCK	MedImmune
WO 2007/045674	method for producing flu virus or antigen in cell encoding adenovirus E1 gene.	Crucell
US 7037707	method of making a reassortant growing to high titer by using an alternative NS gene	St Judes

Production of recombinant influenza antigen in non-eukaryotic cells:

The procedures described above involve the propagation of an influenza virus in eggs or eukaryotic cells, and the subsequent treatment of the virus to make a vaccine. An alternative, described further down is the production of one or more recombinant influenza antigens in cells. These can be eukaryotic cells (including plants), insect cells, yeast, or bacteria.

2.2.2 Dose reduction of inactivated vaccines by the addition of adjuvants

Adjuvants are compounds that are added to vaccines to enhance the immune response to the vaccine. Depending on the adjuvant used, the immune enhancement can have several beneficial effects including enabling one to induce protective immunity with a lower antigen dose, thus increasing capacity.

There are many adjuvants under development, and most are the subject of intellectual property. While many of these adjuvants could be combined with inactivated influenza vaccines to permit dose reduction or to improve protective efficacy, only a few have been evaluated in the clinic with positive results. These include aluminium salts and oil-in-water emulsions.

Aluminium-based adjuvants

Aluminium-based adjuvants including aluminium hydroxide, aluminium phosphate, and various combinations of these with inactivated whole-cell, split or subunit antigens have been evaluated by numerous groups. While these adjuvants have been shown by some groups to permit induction of comparable antibody responses with up to five-fold less split antigen from potential pandemic-strains, the results are not consistent and for some groups the effect is at best marginal or absent. For inactivated whole-cell vaccines the effect may be more reliable, and aluminium hydroxide is a component of a licensed whole-cell seasonal vaccine.

EP 1216053	AlOH plus AlPO4 (alum) in vaccine containing pandemic strain low dose	GSK
WO 2001/022992	Whole killed virus with alum	GSK
US 6372223	reduced dose of cell-culture derived antigen with alum	Baxter
WO 2007/052060	extemporaneous addition of alum to influenza antigen; kit containing alum and antigen	Novartis

The use of alum-based adjuvants for pandemic vaccines appears attractive since there are few intellectual property barriers. One granted patent (**EP1216053**) requires the combination of both aluminum hydroxide and phosphate as an adjuvant to achieve dose reduction of a pandemic strain dose. One granted patent (**US6372223**) claims the use of aluminium salts as adjuvant for low-doses of cell-derived influenza vaccine.

There is an application on the use of aluminum salts with whole-cell vaccine (WO **2001/022992**) and an application claiming a process where the aluminium salt is added to the flu vaccine just prior to injection (extemporaneously) and kits

containing both an antigen and an aluminium salt to be combined prior to injection.

Oil in water emulsions
Several studies using oil-in-water emulsions have shown a greater dose-reduction potential than aluminium salts. MF59, an oil-in-water emulsion produced by Novartis (Chiron) comprising squalene, sorbitan rioleate and Tween 80 and which is a component of the company's seasonal influenza vaccine has been shown to permit the use of doses of pandemic-strain (H9N2) antigen as low as 3 µg. AS3, an oil-in-water emulsion produced by GlaxoSmithKline has been shown to permit significant dose sparing of H5N1 antigen, achieving what are considered protective levels of immunity with 3 µg per dose, whereas the unadjuvanted vaccine required 90 µg. Sanofi-Pasteur has also announced that its adjuvant AF03, an oil-in-water emulsion, enables induction of protective immunity with similar doses of antigen.

EP 0399843	Adjuvant composition comprising a submicron oil-in-water emulsion	Novartis
US 7029678	Vaccine comprising antigen and oil-in-water emulsion of metabolisable oil and a-tocopherol	GSK
US 2007/141078	metabolizeable oil-in-water emulsion with alpha-tocopherol and reduced dose antigen	GSK
WO 2006/100109	oil-in-water emulsion to enhance CD4 and B memory repsonse to influenza antigen	GSK
WO 2006/100110	multivalent vaccine comprising metabolizeable o/w emulsion with sterol	GSK
WO 2007/052061	oil-in-water emulsion with free surfactant as adjuvant for influenza vaccine	Novartis
WO 2007/006939	A thermoreversible oil-in-water emulsion	Sanofi

The original patent on submicron oil-in-water emulsions including MF59 (**EP0399843**) has been revoked in the European Patent Office (decision of 17 August 2000), however it may still be valid in other parts of the world. The combination of GSK's AS3 adjuvant with influenza antigen is claimed in recent application **US2007/141078**, however the composition of this oil-in-water emulsion containing alpha tocopherol is already claimed in several granted patents including **US7029678.** The Sanofi-Pasteur adjuvant has not been widely described, however their recent application WO **2007/006939** describes a thermoreversible oil-in-water emulsion.

While the GSK and Novartis have publicly indicated that in the event of a pandemic they would permit their adjuvants to be used by others. However pre-negotiated licenses and supply agreements would need to be established, and regulatory approval would require clinical evaluation of the proprietary adjuvant

with the antigen. This could reduce the independence of a company's pandemic response.

It should be borne in mind that even if IP to either of these adjuvants may not be a barrier in certain countries, the development of such adjuvants, demonstration of efficacy and safety and their regulatory approval is a lengthy process. Hence even in the absence of IP it may be in the manufacturers interest to negotiate with the technology holders.

Other potentially relevant IP:

There are hundreds of patents on novel adjuvants, many of which could potentially be applied to pandemic influenza. These are not considered here since their application to influenza vaccines has not been specifically claimed and not demonstrated. There are also numerous patents and applications that describe influenza vaccines which contain specific adjuvants, including fusion of flu antigens to immunostimulatory molecules such as flagellin, stress proteins, CD40L, tripalmitoy cysteine etc, or combination with immunostimulants such as MPL, GM-CSF, CT, LT, etc.. The majority of these are still at the exploratory stage and it is not yet known whether such products will be efficacious in the clinic. Some identified IP is summarized in the table below.

EP 941315	Fusion of influenza antigen with stress (heat shock) protein	Stressgen
US 5679356	Use of GM-CSF as adjuvant for influenza	Schering
WO 2007/052055	non-virion antigen derived from cell culture with an adjuvant	Novartis
WO 2006/100111	vaccine containing o/w emulsion with MPL	GSK
WO 1994/019013	influenza antigen with MPL	GSK
WO 2005/117958	virosome plus saponin adjuvant for flu vaccine.	GSK
WO 2007/053781	influenza antigen with calcium phosphate	Novartis
WO 2007/052059	Influenza antigen and Th1 adjuvant	Novartis
US 2007/141078	metabolizeable oil-in-water emulsion with alpha-tocopherol and reduced dose antigen	GSK
WO 1993/020846	HA or NA epitope fused to flagellin	Yeda
WO 1994/019013	influenza antigen with MPL	GSK
WO 1998/023735	influenza antigen fused to all or part of a stress protein (DNA or protein)	Stressgen

WO 2001/021151	nasal delivery of non-live antigen with surfactant as adjuvant	GSK
WO 2001/021207	Tween 80 as adjuvant for mucosal delivery	GSK
WO 2003/063899	CD40 ligand conjugated to influenza antigen	Adjuvantix
WO 2004/016281	intradermal influenza vaccine with ADP-ribosylating toxin (e.g. CT, LT).	GSK
WO 2004/030608	nano-emulsion based on oil and solvent. Use for vaccines, including influenza.	U. Michigan
WO 2004/075829	influenza antigen with ADP ribosylating protein and chitosan	Novartis
WO 2005/117958	virosome plus saponin adjuvant for flu vaccine.	GSK
WO 2006/069262	Use of Pam3cys with membrane protein of influenza	Vaxinnate
WO 2006/100111	vaccine containing o/w emulsion with MPL	GSK
WO 2006/124630	use of a probiotic to enhance immune response	Tufts
WO 2007/008918	enveloped virus envelope-bound immunomodulatory protein e.g. IL-1 etc	Wayne State U.
WO 2007/047831	VLP Virus-like particle comprising M1, H5 and N1.	Novavax
WO 2007/052055	non-virion antigen derived from cell culture with an adjuvant	Novartis
WO 2007/052058	influenza vaccine with particulate antigen and immunostimulator	Novartis
WO 2007/052059	Influenza antigen and Th1 adjuvant	Novartis
WO 2007/052061	oil-in-water emulsion with free surfactant as adjuvant for influenza vaccine	Novartis
WO 2007/053781	influenza antigen with calcium phosphate	Novartis
WO 2007/056266	fusion protein of CD40 ligand conjugated to influenza antigen, or DNA coding for	Sidney Kemmel

2.3 Live attenuated influenza vaccines (LAIV)

Live attenuated influenza vaccines (LAIV) have been undergoing development for more than 30 years. Two LAIV vaccines have also been licensed in Russia (Rudenko et al., 2000). In 2003, one cold-adapted LAIV, FluMist® was licensed in the USA (Glezen, W. 2004). They are delivered intranasally and so are expected to elicit a similar immune response to natural infection with influenza. Clinical studies have reported the induction

of specific nasal IgA, serum antibodies, and cell-mediated responses by LAIV (reviewed by Belshe, 2004). All licensed LAIVs are currently produced in embryonated specific pathogen-free (SPF) eggs. A cell culture (MDCK) cold-adapted LAIV is also being developed.

A cell-culture (MDCK cells) live cold-adapted attenuated vaccine based on the A/Leningrad/134/47/57 (H2N2) cold-adapted donor strain has been proposed (Ghendon et al., 2005). Development and pre-clinical evaluation of an H5N1 LAIV produced in MDCK cells is now underway.

IP:
Since LAIV have been used for over 30 years, there is clearly no existing IP on the basic concept of making and using an attenuated influenza virus (although US 5149531 does claim a novel approach for using LAIV as a therapy). Several attenuated strains were developed and patented in the past, including the Brigit strain (an attenuated B/Russia/69 described in **US4278662**, now expired), processes for making attenuated vaccines by reassortment with A/PR/8/34 (**US3953592**, now expired) etc., and several of these attenuated strains are available from sources such as ATCC. The attenuated strains A/Leningrad/134/57 and B/USSR/60/69 which were developed and used in Russia are covered by IP which is geographically limited to Russia (e.g. **RU2248395** which describes the reassortant of the Leningrad backbone with the circulating strain). The absence of IP does not, however, mean that one can readily manufacture vaccines based on the attenuated backbones: even if one could acquire the backbone, without the original development and regulatory dossier documenting characterization and safety, vaccine development will be lengthy and costly.

The MedImmune Flumist® vaccine is based on the use of the A/Ann Arbor/6/60 and B/Ann Arbor/1/66 strains. In addition to owning the proprietary documentation describing the characterization, safety and efficacy of these strains, MedImmune also has IP on specific characteristics of their vaccine which could prevent one acquiring the backbone by simply purchasing a dose of Flumist®. This IP includes IP on mutations within the PB2 protein which confer attenuation (**US 6974686, US 6843996**).

Several other groups have IP on specific attenuated strains (e.g. attenuated A-X31 strains described in Korean application **KR19970064854**), and means of attenuating influenza vaccines by introducing mutations or insertions in the NS1 gene.

WO 2005/116258	LAIV 6:2 reassortant of pandemic strain HA and NA. Not Ann Arbor.	MedImmune
US 6974686	mutation in PB2 protein	MedImmune
US 6843996	PB2 temperature sensitive mutations	MedImmune
US 6800288	A/PR/8 virus containing modified NS gene	Green Hills
EP 1259629	Genetically engineered virus with insertion of epitope in NS1 gene at aa 124 .	Green Hills
WO 2006/083286	attenuated swine influenza virus with mutation in NS1	Mt Sinai

WO 2007/061969	LAIV with specific mutation in NS1	Rutgers
WO 2007/064802	chimeric avian influenza / Newcastle disease or other virus	Sinai
KR19970064854	HTCA A101, B102: (attenuated A-X31)	
WO 2001/083794	Dual promoter system to facilitate production of attenuated reassortant virus	St Judes
WO2006051069	Defective virus particle lacking PB1 or PB2.	Solvay

Production of pandemic LAIV:
The production of a LAIV against a circulating strain is normally done by conventional reassortment. Embryonated eggs are co-infected with the field strain selected for the vaccine and the attenuated backbone strain (Leningrad, Ann Arbor or other). High-growth progeny virus is analysed to confirm the presence of surface glycoproteins from the field strain. When the circulating strain is not highly pathogenic and can grow on eggs or cells, classical reassortment can be achieved in a few weeks and is not dominated by IP. For Pandemic strains however there is some IP that may cover the use of classical reassortment with attenuated strains that are not Ann Arbor derived: **WO 2005/116258** application by Medimmune describes 6:2 reassortments of pandemic HA and NA with strains that are not Ann Arbor.

For pandemic strains, the classical reassortment approach may not work, or the time to isolate the appropriate reassortment may be too long. In such cases the use of reverse genetics is required to construct the reassortment. The IP on reverse genetics is described later in this document.

Other IP on LAIV includes methods of stabilization to permit the vaccine to be stored in a refrigerator rather than freezer (**WO2006/041819**). There are also numerous patents not specific to influenza, which cover methods of stabilizing viruses, proteins and vaccines, generally by the use of cryoprotectants, drying procedures etc. Such IP is out of the scope of this document.

WO 2006/041819	method of making refrigerator-stable LAIV by purification of vaccine.	Medimmune

Delivery:
LAIV are delivered to the nose either through a spray or as drops. The regulatory approval of the vaccine includes the delivery device, hence manufacturers need to identify a device that is suitable for delivery and possibly for storage of the vaccine within the device. There are numerous devices for nasal delivery of pharmaceutical products and vaccines ranging from simple droppers to multi-dose sprays which generate defined droplet sizes and delivery accurate doses. Some of these devices are covered by IP. Analysis of such IP is outside the scope of this document.

2.4 Recombinant and Synthetic antigens

The procedures described above involve the multiplication of an influenza virus in eggs or eukaryotic cells, and either use of an attenuated form of virus or inactivation of the virus to make a vaccine. An alternative is the production of one or more influenza antigens in heterologous cells. These can be eukaryotic cells (including plants), insect cells, yeast, or bacteria.

Numerous approaches are being investigated: the majority involve the production of haemaglutinin, since this is currently the antigen against which immunity is thought to correlate with protection, however many groups are investigating the production and protective immunity conferred by alternative antigens including neuraminidase, and potential broad immunity conferred by conserved antigens such as nucleoprotein, matrix protein 1 and matrix protein 2.

The most advanced of these technologies is the production of HA in insect cells. This is described in **US 6245532** assigned to Protein Sciences who are currently seeking approval for a vaccine based exclusively on this antigen. Insect cells are also being used to produce HA along with NA and Matrix protein assembled to form virus-like particles (VLPs) described in (**WO 2007/047831**).

Numerous other approaches identified in the patent literature include production of HA in microbial systems; expression of fragments of HA or NP; fragments of the Matrix protein M2 expressed as fusion partners with other proteins or as synthetic peptides; and combinatorial antigens containing multiple peptides corresponding to possible HA mutations. While several of these approaches are under evaluation it is not yet known whether they will be able to induce protective immunity in humans.

One approach which has demonstrated some promise in clinical studies is the use of DNA vaccines, where a piece of DNA coding for the HA (in a plasmid under control of a suitable promoter) is injected. There is specific IP on the use of DNA to induce an immune response including but not limited to: **US6214804** (Vical) and the associated family, DNA coding for a protein of influenza **WO 2005/116270** (Vical) , the use of particles to enable the delivery of the DNA **US7207967** (Powdermed) and the use of vaccines containing chimeric promoters **WO 2006/082398** (Powdermed).

US 6245532	multivalent vaccine comprising baculovirus expressed influenza antigens	Protein Sciences
US 4659669	expression of flu antigens in microbial system (under lac operon)	U. California
WO 2007/022425	recombinant soluble HA lacking c-terminal produced in insect cells (drosophila)	Hawaii Biotech
WO 2007/047831	VLP Virus-like particle comprising M1, H5 and N1.	Novavax
EP 176493	HA2 petide fusion protein	GSK
US 4474757	short synthetic peptides conjugated to a carrier inducing	Yeda

	neutralizing antibodies	
US 4981782	peptide of Matrix protein (79-104 region; 64-80; 149-169)	SRI
US 5136019	Synthetic peptide corresponding to 215-235 of Matrix protein	SRI
US 5243030	peptide of Matrix protein attached to macromolecular carrier	SRI
WO 1994/017826	HA2 subunit	GSK
WO 1994/026903	peptide from Matrix protein which binds human MHC-1 complex	Rijks university
WO 2004/009028	mutated M1 protein or DNA coding for mutated M1 protein causing altered budding	Myriad Genetics
WO 2004/076621	polynucleotide coding for viral component	Yeda
WO 2005/107797	antigen from avian influenza as vaccine for human influenza	Novartis
WO 2005/116270	DNA vaccine coding for a protein of influenza	Vical
WO 2006/082398	DNA vaccine comprising chimeric promoter sequence of Hcmv early promoter plus	Powderject
WO 2006/111616	conjugate vaccine with peptides from HA sialoside binding site	Glykos
WO 2006/128294	vaccine containing short peptides corresponding to specific HA sequences	Variation Biotech
WO 2007/011904	recombinant influenza vaccine based on fusion with plant virus capsid VLPs	Dow
WO 2007/016598	DNA vaccine coding for NP and M1 and NS1	Cure Lab
WO 2007/051036	combinatorial antigen of 5-50 defined peptides corresponding to predicted mutations	Protelix
WO 2007/066334	two peptides, one from M and one from HA.	Yeda
US 4752473	Glycosylated peptides from HA produced in yeast	U. California

Vectors:

An alternative approach to replicating the influenza virus or producing and purifying an influenza antigen, is to express the influenza antigen of interest on another organism such as a non-pathogenic virus. This approach has been widely explored for diseases such as TB and HIV, for which vectors including adenovirus, modified Vaccinia virus Ankara (MVA), yellow fever virus and others have been considered.

This approach is still experimental for influenza vaccines. While it appears attractive since the live vaccine can be administered by mucosal routes, and any risk of recombination with wild-type virus is avoided, the interference of pre-existing immunity may significantly hamper this approach.

US 2007/3576	replication deficient adenovirus vector encoding influenza antigen	U. Pittsburgh
WO 2006/113214	adenovirus vector expressing antigen of avian flu	US HHS

3. Reverse Genetics and Engineering of Vaccine Strains

WHO Collaborating Centers, who provide reference strains to industry, currently generate the seed strains for vaccine production by genetic re-assortment. Embryonated eggs are co-infected with the field strain selected for the vaccine and a master strain that is known to give good yields on eggs (such as A/PR8/34 or similar) . High-growth progeny virus is analyzed to confirm the presence of surface glycoproteins from the field strain. Currently the seed strains that are provided are intended for production of inactivated vaccines. The production of seed virus for live attenuated vaccines requires further re-assortment with the attenuated strain.

This process takes time, and for highly pathogenic avian strains can be difficult to achieve since they cannot be readily propagated on eggs. Hence a process of plasmid rescue reverse genetics is used: With this technique, certain amino acids associated with H5N1 virulence are removed from the HA cleavage site, and plasmids containing the genes for the avian virus HA and NA antigens are then cloned and transfected into Vero cells along with plasmids containing six genes from a donor strain (typically A/PR/8). The progeny virus is recovered from cell culture, purified, propagated in embryonated eggs, and tested for stability and pathogenicity.

There are four dominant patent portfolios associated with the reverse genetics technology including: **WO 91/03552**, (Palese, Mt Sinai), **WO 00/60050** (Kawaoka, University of Wisconsin) and **WO 01/83794** (St. Jude) and **US 6544785** (Palese, Mt Sinai). Negotiating access to these used to be complex, however these have now been licensed exclusively to MedImmune Inc (USA).

This patent portfolio is highly relevant to the issue of access to pandemic influenza vaccines since pandemic influenza vaccine reference strains will be produced using this procedure by WHO collaborating centers in countries where the patents have been issued or applied for. Such preparation, and distribution of the resulting reference strains requires a license to the patents.

MedImmune, Inc., has taken steps to ensure that its patent rights do not inhibit the development and commercialization of a pandemic influenza vaccine. Specifically, MedImmune, Inc., proactively notified the World Health Organization in December 2003 that it would grant free access to its intellectual property to government organizations and companies developing pandemic influenza vaccines *gratis* for public health purposes. In addition, MedImmune, Inc., has given similar notification to NIH and NVPO in the United States, and the National Institute for Biological Standards and Control (NIBSC) in the United Kingdom. For corporate manufacturers considering the *commercial* sale of

pandemic influenza vaccines produced by reverse genetics, MedImmune, Inc., has indicated to manufacturers that it is offering licenses to its intellectual property. MedImmune, Inc., has made it clear to its commercial peers that it will waive royalties on its intellectual property for any and all pandemic influenza vaccines that are offered free of charge in the interest of public health.

MedImmune has recently been acquired by Astra Zeneca. It is not yet known what effect, if any, this acquisition will have on the access to the reverse-genetics intellectual property.

4. Conclusion:

Influenza vaccines have been manufactured for decades by numerous techniques, and there is therefore no absolute intellectual-property barrier to influenza vaccine production. Certain optimized vaccines, and in particular certain processes, cell lines and novel adjuvants are the subject of patents or patent applications.

It is essential that manufacturers entering the field of influenza vaccine production pay attention to patent rights in considering which technology to develop. This document analyzes many of the patents but does not claim to be exhaustive and does not cover all granted patents and applications. It is the responsibility of the manufacturers to perform their own patent analysis. In particular many patents relating to influenza vaccine technology have limited territorial scope and may not exist in certain developing- or emerging-economy countries.

While there may be no, or only limited, intellectual property barriers to many of the optimized technologies, it will generally be in the interest of new manufacturers to negotiate access to a technology with a manufacturer who has already achieved registration of the vaccine made by that technology. Such negotiation could provide access to the safety, clinical and regulatory information, which would greatly facilitate regulatory approval of the vaccine made by the new manufacturer.