Observations on Vaccine Production Technologies and Factors Potentially Influencing Pandemic Influenza Vaccine Choices in Developing Countries

A discussion paper

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Annexes

Acknowledgments

This paper has been written by Edward Hammond for the WHO Regional Office for South-East Asia. It is intended as a contribution to the debate on the sharing of influenza viruses and access to vaccines and other benefits arising from their commercial exploitation, and to efforts to move forward the issues raised by resolution WHA 60.28.

Acronyms

CBW	chemical and biological weapons
DNA	deoxyribonucleic acid
GAP	global action plan
GISN	global influenza surveillance network
IGM	intergovernmental meeting
IIV	inactivated influenza vaccine
LAIV	live attenuated influenza vaccine
MTA	material transfer agreement
PIP	pandemic influenza preparedness
RNA	ribonucleic acid
TRIPS	(Agreement on) Trade-Related Aspects of Intellectual Property Rights
VLP	virus-like particle
WHO	World Health Organization

Introduction

As a result of concerns raised over the sharing of influenza viruses and the lack of affordable vaccines and medicines, the Pandemic Influenza Preparedness (PIP) Intergovernmental Meeting (IGM) is discussing the possible establishment of a new system for sharing of potentially pandemic influenza viruses, as well as sharing of the benefits resulting from research utilizing them.

Among the possible benefits being discussed is expanded transfer of vaccine-related technology to developing countries, and a sustainable financing mechanism for developing country pandemic preparedness. WHO Member States hope that this financing and technology transfer would help close the gap between pandemic vaccine supply and demand.

But what specific technological approaches are best suited for developing countries? Influenza vaccine technologies need to be categorized and assessed for their cost/benefit implications and respective tradeoffs and risks. Not all technologies are freely available or equally easy to use, so this cost/benefit assessment needs to be made in the light of the constraints imposed by intellectual property claims as well as "hard" technology and know-how requirements.

Other important considerations, including export controls and regulation of biotechnology, remain underexplored, but may influence decisions by developing countries with respect to a possible PIP IGM benefit sharing system.

This paper discusses these issues in five sections. Section I provides a short background. Section II describes briefly the main technologies that are currently available or that are under development, as well as their comparative advantages and potential challenges[1]. Section III discusses a number of cross-cutting issues of practical significance (adjuvants, conditions related to seed strains, public perception of some of the technologies, and export controls) that lie outside the production-related questions, but that nevertheless need to be addressed. Section IV considers various options. Finally, Section V contains some concluding remarks.

[1] A table summarizing key features of the various technologies is attached as Annex 1.

1. Background

Ensuring adequate availability of pandemic influenza vaccines is not an easy task in any country of the world, and no single solution will be universally appropriate. Limited global production capacity for human influenza vaccines is the result of limited demand for seasonal influenza vaccines and technical challenges to influenza vaccine production. Adding to the difficulty is a recent sharp increase in patents and patent applications related to influenza vaccines, which may impede access to vaccine production technologies.

Pandemic preparedness efforts cannot be considered in isolation from other public health concerns and must be weighed in the context of programmes to address other priorities, complementing them when possible. For example, the infrastructure to produce some types of influenza vaccine is useful for making other kinds of vaccines, yet paradoxically, the flu vaccine technologies that are most adaptable may be the most expensive and technologically-challenging to utilize, as well as the most impacted by intellectual property claims.

Some have proposed to expand seasonal influenza vaccination in order to expand pandemic production capacity. This strategy is a key part of the WHO Global Action Plan (GAP), whose overarching goal is to increase pandemic influenza vaccine supply by stimulating demand for seasonal influenza vaccines. Greater seasonal demand, it is reasoned, will stimulate the private sector and others to construct additional influenza vaccine production capacity that can then be used in a pandemic.

But in many countries, and especially developing countries, there is low demand for seasonal flu vaccines and limited prospects of expanding it, particularly among citizens in lower economic strata with competing health-care priorities. The cost of implementing the GAP, even with optimistic economic and antigen assumptions, is estimated to rise to US$ 3.5 billion to US$ 5 billion annually by 2012, with an emphasis on spending in *developed* countries to stimulate demand there, and the questionable assumption that excess pandemic vaccine will quickly be used to vaccinate those in other countries.[2]

[2] WHO IVR. *The Global Action Plan (GAP) to Increase Supply of Pandemic Influenza Vaccines, First Meeting of the Advisory Group*, WHO/IVB/08.10, 19 October 2007, Geneva.

It is unwise not to squarely recognize the limitations on seasonal influenza vaccine demand and the great challenges facing the GAP. Even in developed countries where demand and income are higher, and despite hefty economic stimuli, manufacturers currently are hesitant to expand production capacity. This is in large part due to limited seasonal vaccine demand. For instance, a large European manufacturer recently backed out of an agreement to build an influenza vaccine facility in the United States because it said that a US$ 298 million government subsidy was insufficient.[3]

Others have proposed emergency conversion of animal vaccine plants if a pandemic strikes, particularly of poultry vaccine facilities with egg-based production systems that probably can be adapted to produce human influenza vaccine. With global human influenza vaccine production capacity at least 70% short of providing vaccination for the global population within six months of a pandemic,[4] this suggestion makes obvious sense. Where such capacity exists, this could expand pandemic vaccine supply, but there are significant technical and safety hurdles.

Another strategy that has been proposed is to concentrate vaccine antigen production in a small number of developed countries, on the theory that making vaccine antigen is best done in a few expert facilities and that, if these facilities are collectively made large enough, their surplus production can be exported to developing countries in the event of a pandemic. Yet this strategy, encouraged by the WHO GAP, leaves developing countries in a state of dependency and at the end of the queue to receive vaccine.

All of the above factors, together with mounting pressure on health budgets as a result of the global economic downturn, make ensuring availability of influenza vaccines particularly difficult for most developing countries.

Several studies have recently discussed options for expanding prepandemic and pandemic influenza vaccine production capacity. A number of these reports are listed in the annex to this report. While these are valuable and discuss some technical aspects of influenza vaccines in greater detail, there are key issues related to pandemic vaccination strategies that remain under-contextualized for policy-makers. This paper seeks to fill that gap.

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[3] McKenna M. *Plant cancellation shows problems in flu vaccine business* in *CIDRAP News*, 3 Oct. 2008.

[4] WHO. *Business Plan for the Global Pandemic Influenza Action Plan to Increase Vaccine Supply*, February 2008.

This paper assumes that developing countries will largely not be satisfied with reliance on pandemic vaccines and/or bulk antigen exported from Europe, North America, or Japan, particularly because such supplies currently cannot be made available in a timely fashion. Therefore is difficult to argue that such reliance is an adequate pandemic vaccine supply plan. Rather, here it is presumed that developing countries will continue to seek the development of national or regional vaccine production capacity through technology transfer and sharing of benefits of influenza research.

2. Overview of influenza vaccine production technologies

There are a variety of technologies that are used or have been proposed for production of human influenza vaccines. Often, significant parts of the production process are similar. This is especially true in the later stages of manufacture, such as packaging. The technologies may be categorized in several ways. Below, they have been divided into four basic technological approaches.

Classic influenza vaccine produced in eggs

With few exceptions, currently available seasonal and prepandemic influenza vaccines are manufactured through egg-based production methods. The system is cumbersome and inefficient in comparison to the *theoretical* possibilities of newer cell-based production (see below), leading some to characterize egg-based production as antiquated. Such comparisons, however, are invariably made against technologies that have yet to be fully commercially deployed and proven. Moreover, although it may not be new, this decades-old technology is relatively cheap, very well proven, and largely unencumbered by intellectual property claims.

Egg-based production is employed throughout the world for animal and human vaccines. Apart from influenza, however, the only human vaccines for which the egg-based system is utilized are yellow fever and Japanese encephalitis vaccine. This means that apart from making flu vaccine, egg-based production lines have limited broader utility for human public health.[5]

[5] Egg-based lines are important for animal health, however, as discussed below.

Egg-based production requires a supply of fertile chicken eggs produced under relatively stringent conditions (in comparison to eggs produced for food consumption). This is to ensure that they do not carry pathogens that might taint the vaccine. The eggs are infected with a vaccine strain and the fluid harvested from them yields vaccine after separation and further production steps.

The reluctance of H5 viruses to grow to high titer in eggs (because the virus strains are too efficient at killing chicken embryos) is a problem that has bedeviled H5 vaccine development. While this remains a significant technical challenge, the problems with growing H5 viruses in eggs are being overcome, mainly by attenuating the hemagglutinin (HA) gene of the vaccine strain, typically through reverse genetics (see below). It may be noted that some of these techniques are proprietary however.

Major requirements of the egg-based production system include the process of "candling" the eggs (inspection under bright light); equipment to inoculate the eggs with virus; incubators in which to keep the eggs while the virus is reproducing; and equipment to harvest, separate, and purify the vaccine virus after incubation.

Some of the technology required to produce the vaccine strain is specialized; however, none of it is reported to be particularly expensive, complicated or difficult to operate. In the newest facilities the entire process is automated, while in others some steps in production (for example, candling and harvesting) are conducted by human technicians.

Later steps of egg-based vaccine production, including formulation and packaging, may be similar or identical to the process used with other technologies.

Live attenuated influenza vaccine

Live attenuated influenza vaccine, abbreviated "LAIV", is an influenza vaccine production technology in limited commercial use in the Russian Federation and in the United States. LAIV offers the possibility of producing significantly more vaccine than classic egg-based production using same production line; however there are significant additional scientific and intellectual property hurdles that may reduce LAIV's attraction for developing countries.

The production process for LAIV vaccines is similar to that of classic egg-based vaccines, with some notable exceptions. LAIVs are administered live. This means that when the vaccine strain-containing fluid is harvested from eggs, it is not exposed to a detergent. Thus, if adventitious pathogens are present in the eggs, these may survive the formulation process and eventually infect human vaccine recipients. Therefore, eggs used in LAIV production may require even higher production standards than those used to produce classic killed vaccine. This increased danger of contamination means biosafety practices in production need to be more stringent than those used for classic killed vaccine.

While there are a number of well-characterized backbone strains[6] available for classic vaccines, the "cold-adapted"[7] backbones used in LAIVs are proprietary, such as the "Ann Arbor" strain used in the United States and the "Leningrad" strain used in the Russian Federation. LAIVs thus require a proprietary backbone strain[8] and cannot be produced using the vaccine seeds strains currently distributed by WHO global influenza surveillance network (which do not have "cold-adapted" backbones).

Harvesting is simpler for LAIVs (no detergent wash is needed), but the final product is more delicate because the live vaccine must be kept viable ("alive") until it is used. This means LAIVs require cold storage.

The fact that LAIVs are not killed potentially offers a major advantage over classic vaccine, but at a cost. LAIVs reproduce in the body of immunized persons; thus, they effectively act as their own adjuvants, which means they should require a lower dose of antigen than killed vaccine. This means the same production line may yield considerably more LAIV than classic killed vaccine, although estimates of the increased yield vary widely.[9]

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[6] Influenza vaccines typically are comprised of a(n) HA gene(s) taken from a viral isolate that is inserted into another, laboratory-adapted strain by reassortment or recombinant (reverse genetics) means. While the immunogenic HA gene is the most important part of the vaccine, the lab-adapted strain into which it is placed has typically been selected for useful characteristics for lab and industrial use (high growth rate, tolerance for lab conditions and temperature ranges, etc). This lab-adapted strain is called the "backbone" strain.

[7] "Cold adapted" influenza strains are laboratory-adapted types that are suitable for use in live vaccines, which must be kept cold until use in order to maintain the vaccine's viability.

[8] Discussions to license the Russian "Leningrad" LAIV strains for H5 vaccine production are taking place, however, no detailed information concerning the terms and restrictions of any possible license is available, and no final agreement has been reported to have been reached.

[9] The WHO GAP estimate is 4.5 times, whereas others have estimated a yield as high as 10 times that of the classic trivalent killed vaccine process.

One price of this antigen efficiency is that LAIVs are administered as an intranasal aerosol (i.e. sprayed into the nose), rather than being injected. They thus require a special doser instead of standard syringes. Sufficient supplies of this doser are required in order to use LAIVs.

Another limitation of LAIVs is that they are unsuitable for prepandemic vaccines because of the possibility that the live prepandemic vaccine strain could mutate or recombine with circulating strains, potentially causing or contributing to a new epidemic or even pandemic flu strain. While this concern is not applicable to seasonal vaccines (because of the antigens used), it does seriously limit the ability to test LAIV procedures and formulations prior to an actual pandemic.

Of note, in the future it may become practical for LAIVs to be produced in cell culture (see below), although at present they are produced in eggs.

Influenza vaccines from cell culture

Influenza vaccines produced by cell culture are currently under development in several places but so far are not produced commercially on a large scale. In the cell culture process, animal or other cells are infected with a vaccine virus, which is then harvested and formulated into vaccine. The process takes place in vessels called bioreactors (or fermenters), in basic design not dissimilar from those used in brewing.

Cell culture typically starts by growing cells in a nutrient-rich fluid in small containers, scaling up to larger ones as the cells reproduce. When the desired cell density and scale is reached (hundreds or thousands of litres for commercial production), the cells are infected with vaccine strain virus. After the virus reproduces, the cells are harvested and virus processed into vaccine.

In some cell culture systems, gently agitated cells grow freely in a sort of "soup" mixed with nutrients and (eventually) with vaccine virus. In other cell culture systems, the cells grow affixed to a substrate such as tiny gold-coated beads. They are then released by agitation.

For large-scale commercial production, the process requires large bioreactors, from hundreds to thousands of litres in size. Production of cell culture vaccines also requires equipment to build and maintain a "cell bank" to provide a new supply of fresh identical cells after batches of virus-infected cells are harvested.

Cell culture systems are likely to be more flexible than egg-based systems for production of other human vaccines, potentially increasing a facility's utility. For H5 influenza viruses in particular, there are claims of cell culture systems that grow the virus to a higher titer than is possible in eggs.

Although cell culture vaccines are a major focus of research and development (R&D), as yet they remain in limited commercial use. Scientific limitations for their use in flu vaccines include the inability to be certain ahead of time that a particular cell line will be appropriate to grow the pandemic strain, and the need for substantial bioreactor capacity, of which there is little to no global surplus. Although investment may be recouped through a multi-use facility, cell culture has considerably higher facility construction costs at an industrial scale.

In addition to potential patent claims over the influenza genes (which also impact egg-based vaccines) and backbones used in vaccine strains, there are additional intellectual property issues related to cell culture influenza vaccines. The cell lines that are used are themselves often patented, and the information necessary for their use and for regulatory approval is proprietary.

Table 1: *Examples of proprietary cells lines used in cell culture vaccine production*

Cell type	Company	Additional comment
Avian embryonic stem cells	Vivalis (France)	Licensed to Novartis, GlaxoSmithKline, and others
PER.C6 human cell line	Crucell (Netherlands)	Partnership with Sanofi Aventis
African green monkey kidney cells (Vero)	Baxter (United States)	Vero cells *per se* are not proprietary, but Baxter's process of using them is.

To date, few cell culture-produced vaccines have been approved for human use, and they are likely to prompt more intense regulatory scrutiny than egg-produced vaccines. Cell culture vaccines require approval for the vaccine as well as characterization and safety demonstration of the cells used.

Second generation biotech vaccines

If cell culture vaccines, because of their sophisticated biological manufacturing process, may be considered the first generation of biotechnological flu vaccines, then a basket of different technologies currently under development could constitute the second. While supporters of these technologies believe they may be useful for pandemic influenza vaccines, several of them are at early stages of development and none are proven and ready for commercial use. Therefore, although it is difficult to generalize about these new technologies, they are unlikely to be selected in the short term for pandemic vaccine production in developed or developing countries. These technologies depend on the WHO global influenza surveillance network (GISN) for antigens and WHO selections of the best antigens for use in vaccines, but do not utilize WHO candidate seed strain.

Taking a longer a view, however, it is possible that some of these technologies, for example virus-like particles (VLPs), may become viable for large-scale use. The fact that they are new and mainly privately developed, however, means that in general they are heavily covered by intellectual property claims and may require very new kinds of know-how. For most countries it is too early to tell, however, if national patents will be issued, so the extent of intellectual property impediments for any particular developing country remains unclear.

Briefly, second-generation biotech vaccines include, among other approaches:

- production of recombinant HA protein in other, easily grown, organisms (e.g. transgenic bacteria);
- "naked" and plasmid DNA vaccines in which "codon optimized"[10] flu genes are used directly as vaccine, and
- genetically engineered systems to co-express HA, NA, and M2 genes from flu, manufacturing a "virus like particle" (VLP) that is purified from culture and used as vaccine.

[10] "Codon optimized" genes have nucleic acids that have been altered–typically changed from RNA to DNA–so that the gene can be better expressed in a biotechnological application (e.g. a vaccine).

Summary of the basic technological approaches to influenza vaccine production

1. Egg-based "classic" influenza vaccine: Vaccine virus is injected into fertilized eggs. The eggs are placed in incubators and the virus reproduces in the eggs. Fluid is then harvested from the eggs and washed with detergent. The resulting killed virus material is separated and used for vaccine formulation. This type of vaccine is one kind of inactivated (i.e. killed) influenza vaccine, or "IIV".

2. Live attenuated influenza vaccine ("LAIV"): Vaccine virus is grown in eggs (or in the future, potentially in cell culture) in a process similar to classic flu vaccine. The live virus uses a special type of genetic backbone (currently of limited availability since they are proprietary). Harvesting and formulation is simpler than with killed vaccines. The final product is more delicate and requires a cold chain, but the process potentially is considerably more efficient, producing more flu shots with the same number of eggs.

3. Cell culture influenza vaccines: Mammalian, avian, or other cells are cultured in growth media. This culture is scaled up to the desired density of cells in large bioreactors (fermenters) up to thousands of litres in capacity. The culture is infected with vaccine strain, which multiplies in the cells, producing large quantities of vaccine virus. Harvesting, purification and packaging are essentially the same as with egg-based vaccines. This is another type of IIV, produced by a different method.

4. "Second generation" biotechnological vaccines: Many techniques are under study, including: producing recombinant HA protein in other, easily grown, organisms (e.g. transgenic bacteria); "naked" and plasmid DNA vaccines in which "codon optimized" genes are used as vaccine; and genetically engineered systems to co-express flu genes, making a virus-like particle (VLP) that is used as vaccine.

Candidate seed strains and antigens

The WHO system develops and distributes candidate H5 vaccine seed strains. These seed strains are suitable for producing vaccine in eggs and incorporate antigens that have been selected by WHO. In the event of a pandemic, the WHO system may develop and make available LAIV-suitable seed strains; however, WHO does not presently have rights to the proprietary LAIV backbones.

Although at a technical level, current WHO candidate seed strains can be used to produce H5 vaccine, there are legal restrictions imposed on them in a required Material Transfer Agreement (MTA).[11] This is because

[11] For example, the Material Transfer Agreement for the WHO candidate seed strain NIBRG-23, made from an H5N1 strain isolated in Turkey, can be viewed here: http://www.nibsc.ac.uk/flu_site/Docs/spotlight/H5N1_MTA-NIBRG-23.doc

they are created using proprietary reverse genetics technology. (See also the discussion on conditions imposed on commercial use of reverse genetics in section III below.)

The advantage that the WHO seed strains theoretically offer is that the strain is a known quantity that may be quickly used, reducing the amount of work and time needed for new strains to go into production.

Vaccine makers and other companies, however, may choose not to use WHO candidate seed strains for any of several reasons. These may include a desire to avoid the intellectual property restrictions imposed by the WHO MTA, or they may wish to use a technology type for which the WHO strain is not suitable (e.g. LAIVs), or they may wish to make other alterations particular to their production system (for example, to introduce a mutation intended to make the virus grow to higher titer).

If a vaccine maker does not use the WHO candidate vaccine seed strain in actual production, however, it is still highly likely to use the antigens selected by the WHO system as most immunogenic. In this case, the maker would obtain the HA (and/or NA) gene(s) from the WHO system or synthesize them from sequence data. The maker then incorporates the WHO-selected antigen(s) into its own vaccine strain. Thus, particularly in the future, of arguably even greater importance than the WHO candidate vaccine seed strain are the genes that the WHO system determines to be most suitable for use in vaccines, because these will be used by manufacturers whether or not the manufacturer utilizes the WHO candidate seed strain.

3. Issues and challenges

The question of adjuvants

Adjuvants are substances that are added to a vaccine in order to enhance its immunological effect. Most adjuvants act on the human immune system and are not linked to a particular vaccine strain or even a particular disease. Thus a particular adjuvant may be used not only for influenza vaccine; but also in vaccines against other diseases.

Adjuvants can both reduce the amount of antigen needed per vaccine dose (potentially of great importance in a pandemic) and increase the "take" of vaccines–that is, the rate of successful vaccination.

Many adjuvants that may be used in influenza vaccines today are inorganic chemicals. These are sometimes aluminum-related compounds, such as aluminum hydroxide (or gibbsite, ($Al(OH)_3$)), which is more familiar in medicine for its use as an oral antacid. One adjuvant that has long been used, alum, is patent-free and easily obtained, but it is not generally considered promising for H5 vaccines.

Major influenza vaccine manufacturers are increasingly using newer adjuvants of a type called oil-in-water emulsions. Companies claim these offer substantial improvements over other adjuvants. The proprietary oil-in-water adjuvants used by Novartis and GlaxoSmithKline[12] are based on squalene, an organic compound produced in small quantities by many animals and some plants, and are subject to patents and trade secrets.

A large number of biotechnological adjuvants, such as short pieces of DNA that are active in the body and are designed to make vaccines more immunogenic through specific gene or protein-level effects on the immune system, are undergoing research. These, however, remain experimental.[13]

Not all vaccines contain an adjuvant. LAIVs do not need to be adjuvanted because they are alive and reproduce in the upper respiratory tract. One H5 vaccine, produced in cell culture by Baxter International, is a killed virus vaccine that is unadjuvanted.[14]

One problem with assessing the potential use of adjuvants for pandemic vaccine production in developing countries is that they are often highly proprietary. For instance, detailed information on production of vaccines with oil-in-water emulsion adjuvants is limited, as the adjuvants are often patented and their use is covered by trade secrets.

Table 2 provides an overview of a number of adjuvants.

[12] Sanofi's proprietary formulation is reportedly similar, but its exact composition does not appear to have been made public.

[13] Because these adjuvants are varied in nature and generally in earlier development stages, this paper focuses on adjuvants in current use or advanced development.

[14] The Baxter vaccine has an unusual composition and production method. It uses unaltered H5N1 virus isolates that have not been placed on a lab-adapted backbone or had genetic alterations to reduce pathogenicity. Because the live vaccine virus is virulent for birds and, potentially, humans and other animals, it must be grown under very careful biosafety procedures in P-3 (BSL-3) containment. This method of production requires a cell culture system, with the added challenge of stringent BSL-3 practices and facilities.

***Table 2:** Adjuvants that may be used in pandemic influenza vaccines*

	Alum	Other aluminium salts	MF59	AS03	AF03	Biotech adjuvants
What is it?	An inorganic chemical, potassium aluminum sulfate.	Chemicals related to alum, including aluminum hydroxide and aluminum phosphate.	An oil-in-water emulsion, consisting of squalene, polysorbate 80 (Tween 80), and sorbitane trioleate (Span 85).	An oil-in-water emulsion, consisting of squalene, polysorbate 80, and DL-a-tocopherol.	An oil-in-water emulsion, whose formulation does not appear to have been published.	Biological materials designed to boost immune system response.
Proprietary?	No.	Generally not.	Yes *(Novartis)*	Yes *(GSK)*	Yes *(Sanofi-Aventis)*	Yes. Includes JVRS-100 *(Juventus)*, IC31 *(Intercell)* etc.
Use	Has long been used in various vaccines.	Clinical trials are underway of pandemic flu vaccines utilizing these. Also used in other vaccines.	Used in vaccines licensed in some countries.	Used in vaccines licensed in some countries.	Does not currently appear to be licensed.	Experimental; some have advanced to human trials. Regulatory hurdles likely to be quite substantial.
Efficacy issues	Used in trials and in one US-licensed prepandemic vaccine; but often regarded as inadequate for use with H5 vaccines.	Potentially more effective than alum, but less so than proprietary adjuvants. Mixed results in research to date.	Deemed effective and licensed for use in non-influenza vaccines.	Deemed effective and licensed for use in non-influenza vaccines.	AF03 is thought to be similar to MF59 and AS03.	Unproven.

Based on their reported composition, adjuvants such as MF59 do not appear to utilize unusual or expensive ingredients; however, it cannot be assumed that effectively incorporating them into vaccines is as straightforward as their reported chemical composition because details of their use are proprietary.

In some countries, vaccination has been associated with social controversies due to perceived risks. Some vaccine critics have claimed that certain adjuvants are unsafe, including aluminum hydroxide (alleged to be

linked to Alzheimer disease) and MF59 (which has received scrutiny for its use in a controversial US anthrax vaccine). While the scientific merit of these criticisms is debated–the compounds have passed regulatory review in many countries–where concern exists it would be inappropriate to ignore the potential disruption to vaccination campaigns due to widespread worry over adjuvant safety.

It is clear that in the event of a pandemic, the presently limited global vaccine virus production capacity means that the supply of pandemic vaccine antigen (in any form) will be far outstripped by demand, especially in the early stages. With the exception of unadjuvanted LAIVs, in the dominant planning scenario, widespread use of the most effective adjuvants is highly desirable because it will enable more people to be vaccinated with the limited amount of antigen available, especially at earlier stages of the pandemic. Failure to use the most effective adjuvants would "waste" antigen because each suboptimally adjuvanted dose would "rob" antigen from the global supply.

Conditions imposed on commercial use of reverse genetics

Reverse genetics is a relatively new proprietary technology that is being applied to the development of influenza vaccines as well as other products. At present the technology is used in the creation of WHO GISN H5 vaccine seed strains, although it is not strictly technically obligatory to use it when making pandemic vaccine strains. Because of the advantages it offers, however, the technology will likely be increasingly used in future vaccine strains.

Primarily developed by American and British universities, and covered by a large number of patents, reverse genetics intellectual property has been accumulated by Medimmune, a US-based subsidiary of the United Kingdom's Astra Zeneca, a large flu vaccine maker. Medimmune has thus far allowed use of its reverse genetics intellectual property in pandemic vaccine R&D, however, it has indicated that it will not permit commercial use of the technology without a license.

Material transfer agreements for WHO candidate seed strains of H5 vaccines thus include protections for Medimmune's intellectual property and thereby impose restrictions on those that receive seed strains (through contract law), even in countries where Medimmune's patents have not been issued.

Reverse genetics technology involves creation of loops of DNA called plasmids whose key parts encode for influenza genes. When the plasmids

are introduced into cells, the DNA is transcribed into RNA and influenza virus is produced. The technology enables scientists to "edit" the influenza viral genes by making alterations to the DNA plasmid, for example, deleting bases from the HA gene to make the virus avirulent.

In addition to allowing manipulation of individual genes, reverse genetics allows scientists to relatively easily mix and match genes from different influenza strains, particularly when inserting new genes onto "backbone" strains for which plasmid systems are already constructed. This is useful for research purposes and for creation of vaccine strains, because it can be more straightforward and reliable than the traditional reassortment method, whereby cells are coinfected with different strains and the resulting hybrid viruses identified and selected by scientists.

Reverse genetics is potentially a very useful technology for egg-based, cell culture, and other types of flu vaccines. It is, however, controlled by Medimmune and because it is used in current WHO candidate seed strains, recipients of those strains are already obligated to negotiate with Medimmune should they choose to commercially produce vaccine from those strains. This point has perhaps not received the attention it warrants.

Biotechnology and public perception

An important policy and health consideration underappreciated to date is the potential for problems with social and regulatory acceptance of recombinant pandemic influenza vaccines–that is, those that are the product of biotechnology. Some countries may have additional regulatory requirements for such vaccines. This may influence the decisions that governments take in vaccine supplies. Decisions may be complicated by the fact that influenza vaccines make use of biotechnologies that might or might not be popularly and legally understood as "genetic engineering".

It is logical that in the event of a severe pandemic the vast majority of people would opt for vaccination even if concerned about the safety of a recombinant vaccine, for the simple reason that fear of severe illness or death from the disease is greater than concern about the vaccine. It is also true, however, that genetically engineered products used in humans remain controversial in many parts of the world and some citizens may be reluctant to be vaccinated, particularly in scenarios such as a slow-spreading pandemic or widespread use of a recombinant (pre)pandemic vaccine.

Although not strictly tied to biotechnology, recent cases of problems in polio vaccination campaigns and the rejection of childhood vaccination among some religious communities are evidence of the importance of safety perceptions and belief. In the case of pandemic influenza vaccines, the degree to which the vaccine could be termed "genetically engineered" varies by the technology used. Perceptions may be further influenced by other factors, such as use of animal products in cell culture, and whether the vaccine is live or killed, with killed vaccines presumably engendering less resistance.

A brief breakdown of some pertinent influenza vaccine technologies and how they might be considered is given in Table 3.

Table 3: Brief overview of key influenza vaccine technologies

Technology	What is it?	Is it genetic engineering?
Reverse genetics	Assembly of influenza viruses through the creation of DNA plasmids bearing influenza genes that are transcribed into virus in infected cells. Although not strictly necessary for most influenza vaccines, it may offer time savings and other R&D advantages.	Viruses produced by reverse genetics are recombinant products and are, as it is generally understood (and regulated), genetically engineered. If the virus genes have not been significantly changed, however, then the resulting vaccine virus may not substantially differ from reassortant viruses or natural virus isolates.
HA gene deletions	To facilitate safe handling of H5N1 research viruses and vaccine production, part of the HA gene is deleted to make it nonpathogenic. This altered gene is then used in the vaccine strain.	The manipulation of the HA gene creates a recombinant product. The modified HA gene is not transgenic, however, because it does not incorporate foreign genetic material.
Virus-like particles (VLPs)	Insertion of nucleic acids coding for influenza virus genes into other cells, triggering the production of non-living particles that mimic key parts of influenza viruses, and can trigger an immune reaction.	The VLP vaccine itself is non-living; however, it is the product of an organism that is genetically engineered to express non-native genes.
Recombinant LAIV	While it is possible to create LAIVs without use of recombinant DNA, for technical reasons it is likely that a pandemic LAIV would be produced with reverse genetics and possibly incorporate additional genetic modifications.	A live genetically engineered vaccine is the type most likely to encounter stricter regulatory requirements and safety questions.
Recombinant killed vaccine (IIV)	For many of the same reasons as LAIVs, (pre)pandemic killed flu vaccines, produced in eggs or cell culture, may be recombinant products.	These vaccines will contain a genetically engineered product. Regulatory and social concerns may be fewer, however, because the vaccine virus is killed before administration.

Export controls

Export controls are imposed by national laws. They are designed to regulate and sometimes prevent the transfer of technologies that may be used to create nuclear, chemical, or biological weapons as well as certain other items, such as missile-related technology. They are discussed in particular here because they have generally not been discussed with respect to pandemic vaccine production to date.

Export controls are necessary to consider because research on highly pathogenic influenza viruses and production of vaccines require facilities, know-how and equipment that could be abused in biological weapons programmes. As a result, some of the same technologies that can be used to protect public health by producing vaccines can be difficult to acquire because they may fall under export control laws.

Biological export control laws are controversial and have been a matter of intense debate at the Biological and Toxin Weapons Convention. The countries that impose the most rigorous export controls (mainly developed countries) argue that they are necessary for national security and anti-proliferation reasons. On the other hand, the countries that are most often denied technology (mainly developing countries) counter that export controls are arbitrary and unfair, and that they are often motivated by political or economic considerations not related to weapons proliferation.

Export controls are not governed by any international agreement. Some countries that have biological (and chemical) export control systems attempt to coordinate them through the Australia Group, a collection of countries whose stated aim is "to minimise the risk of assisting chemical and biological weapon (CBW) proliferation".

The majority of the members of the Australia Group are OECD Member States. The Group calls itself an "informal arrangement" that "meets annually to discuss ways of increasing the effectiveness of participating countries' national export licensing measures to prevent would-be proliferators from obtaining materials for CBW programmes".[15]

[15] See http://www.australiagroup.net.

For influenza vaccines, export control laws may limit the transfer of a wide variety of research and vaccine production-related technology, and even shipments of vaccines themselves.[16]

Export controls are applied to equipment, organisms, and ideas. The different types of items that can fall under export controls include:

- Physical items used in research and vaccine production such as bioreactors (fermenters), lyophilizers (freeze dryers), separation and packaging (filling) equipment.
- Know-how such as blueprints, design and engineering services for high-containment laboratories and biological production facilities, as well as certain kinds of scientific procedures and knowledge.
- Biological materials–for example, highly virulent disease strains or, in some cases, vaccines.

Export controls apply in different degrees to different countries and technologies. Items considered by export-controlling countries to be of highest risk[17] may be more difficult to export than items that are considered lower risk (for example, vaccines). Generally, when an export license for a controlled item (or technology) is sought, the item is classified for its intrinsic risk and then cross-referenced against a list of countries that themselves have been categorized according to the degree of weapons proliferation threat they are alleged to impose.

An additional pertinent consideration may be the entity in the importing country that seeks access to the technology. For example, a well-known international pharmaceutical company may be less likely to be denied an export controlled item than a government research institute in the same country, if the exporting county is suspicious of the aims of the importing country's government research programme.

Finally, when export licenses are issued, typically they are contingent upon the recipient of the controlled items agreeing to no further transfers of

[16] A particularly severe export control has recently been highlighted in news articles pointing out that export controls in the United States would apply to H5N1 vaccine exports to several countries. See, for example, URL: http://www.exportlawblog.com/archives/406 (accessed 25 November 2008).

[17] For example, a large, high-quality fermenter, which might be used to produce biological weapons agents instead of vaccine.

the technology. While as practical matter this type of re-export restriction is difficult to enforce, entities that transfer export-controlled technologies place at great risk their future ability to obtain export-controlled technologies.[18]

While the Non-Aligned Movement and others have been critical of the Australia Group's biological export control system,[19] there are no signs that export controls are being relaxed even with the prospect of an influenza pandemic. Countries must therefore take into consideration the issue of export controls when making pandemic preparedness decisions. Many developing countries are subject to Australia Group's export control restrictions, which could impede their access to influenza vaccine production technology.

The impact of export control regimes will vary by country and technology. While export controls will not be a major issue for all countries, particular technologies, such as cell culture systems, may be more prone to export control problems than others. Countries that wish to develop a domestic production capacity that utilizes imported technologies will need to address these issues.

4. Options

Timing and technology choices

It is difficult to reconcile the severity of fears of an imminent pandemic with the slow pace of expansion of global influenza vaccination and vaccine production capacity. Years of meetings and rhetoric have passed since the H5 pandemic scare began, yet most countries in the world–including many wealthy countries–have thus far not ensured pandemic vaccine supplies for their own populations.

[18] Countries that impose export controls maintain lists of commercial, governmental and other entities that have received (or sought to receive) export-controlled items for transfer to others without the approval of the original exporting country.

[19] See, for example, the statement of Cuba (on behalf of NAM) and other statements at the 2007 Meeting of States Parties of the Biological and Toxin Weapons Convention, URL: http://www.opbw.org/new_process/msp2007/msp2007_statements.htm

If the pandemic threat is so dire, why is the practical response so muted? Limited resources are certainly a factor; but clearly, not everyone shares the same views with respect to the imminence and likely severity of an outbreak.

Those who warn that a pandemic may envelop the world within months from its onset, and there are many experts that do, suggest a health emergency that arguably would require strong government action such as nationalization of pertinent production facilities and invoking of TRIPS flexibilities to allow for greater availability of affordable treatments. A pandemic could circle the globe so quickly that initiating such steps after the appearance of a pandemic strain might be pointless.

Despite the dire predictions, steps like compulsory licensing of antivirals have yet to be taken, suggesting that governments may be dubious of the claims made by some scientists of the imminence of a severe H5 human pandemic. Is this foolish, or an efficient use of overstretched resources? It will only become clearer in retrospect.

Nobody argues against improved pandemic preparedness now and in the future, for everyone seems to accept that a new pandemic will occur, sooner or later. Yet, at the same time, it is clearly not possible today to abandon other public health efforts because the argument that a highly lethal pandemic strain is nearly upon us may turn out to be correct.

For those seeking to get ready for a pandemic now, proven technology–mainly egg-based production of classic flu vaccine–offers degrees of certainty that emerging biotechnologies cannot. Methods to grow H5 viruses in eggs are improving, and egg-based production is already available and does not require any potentially expensive and unreliable "bleeding edge" technology. And in theory, the same production facility can also be used for production of pandemic LAIVs.

Although egg-based production is sometimes maligned as "antique", it is telling that major vaccine makers investing in biotechnology remain heavily reliant on egg-based systems for their own flu vaccine production. The major problem, of course, is what–if anything–to do with the production capacity when it is not required for (pre)pandemic vaccines, in view of the fact that there is limited other use for egg-based facilities and, for many developing countries, seasonal flu vaccination is a losing economic proposition. Maintaining an unused production base is expensive. WHO estimates that maintaining an idle capacity to produce 200 million *seasonal* vaccine doses would cost US $100 million per year.

Viewed in a longer timeframe, technology selection may become more complicated. The flexibilities and potential efficiencies of cell culture are attractive because they may offer a faster pandemic response and, especially, a facility with potentially broader public health uses–if the technology is available and markets exist for the other types of human vaccines that may be produced in cell culture.

However, the relatively unproven status and considerably greater cost of hardware for cell culture technologies (estimated at ten or more times the cost of egg-based facilities), both in terms of equipment and intellectual property, at present make them a daunting proposition for most developing countries.

Fill/finish projects and importation of bulk antigen

Indonesian and Mexican vaccine manufacturers, with WHO support, are developing fill/finish capacity for local vaccine sales. In the fill/finish approach, developing country manufacturers import bulk vaccine antigen produced by an overseas company and use it in a locally branded, finished product. In the current WHO-supported projects, the antigen manufacturers are Biken (to Indonesia) and Sanofi-Aventis (to Mexico).

The imported bulk antigen, suitable for a classic killed vaccine, is processed in-country into a finished product. The national manufacturer creates filling and packaging facilities, and some associated technology transfer takes place.

Importation of bulk antigen and filling/finishing in developing countries favours the argument, advanced by some, that it is rational for global influenza antigen production to be concentrated in a few locations with well-developed capacity and expertise.

Local manufacturers importing bulk antigen remain dependent, however, on product supplied from abroad, which is unlikely to be available in the event of a pandemic (particularly in its early stages), so long as global production capacity remains well below that which is necessary.

The option of animal vaccine plant conversion

Current global capacity for human influenza vaccine production falls well short of that needed for pandemic response, even with optimistic assumptions about demand/yield of pandemic antigen. Often unmentioned is the substantial additional manufacturing base that uses egg-based production systems to make animal vaccines. These facilities could lessen the gap between pandemic vaccine supply and demand. They use a very similar production process as that used for human influenza vaccines. Estimates of the global size of the egg-based animal vaccine industry, however, vary wildly.

On the high end, according to one source[20] the annual global egg-based animal influenza vaccine capacity, as of 2006, was approximately 41 billion avian doses (at 100 doses per egg), or about 410 million eggs. In terms of human vaccines, this implies a capacity of approximately 410 million doses of human *trivalent* seasonal vaccine. Using this capacity estimate, output of a monovalent pandemic LAIV could be between 1.8 billion (WHO conversion factor) and up to 4 billion *or more* vaccinations per year (other conversion factor),[21] depending on antigen assumptions. In either case, this would allow vaccination of a substantial proportion of the world's population.

But WHO GAP consultants, also citing industry sources, come up with very different numbers for the potential contribution of animal vaccine facilities. They report that the animal vaccine industry can handle only about 78 million eggs annually. This implies an annual pandemic LAIV output of approximately 340 to 750 million human vaccine courses per year, a much lower but still substantial figure.

It is thus difficult to be precise about (pre)pandemic capacity of animal vaccine facilities because of conflicting and limited data and the

[20] Heldens, J G M. *Production capacity for human and veterinary influenza*, June 2006, at URL: http://www.dutchbio.org/meetings/list/dutch_vaccines_group/files/influenza_dag/DVG%20jacco%20Heldens,%2026.06.06.pdf (Heldens represented Akzo Nobel, which owned Intervet, a major animal vaccine maker, until it was sold to Schering Plough in 2007.)

[21] See: Fedson DS, Dunnill P. *New approaches to confronting an imminent influenza pandemic*. Perm J. 2007;11:63–9, URL: http://xnet.kp.org/permanentejournal/SUM07/influenza-pandemic.html and Fedson DS, Dunnill P. *From Scarcity to Abundance: Pandemic Vaccines and Other Agents for "Have Not" Countries* in Journal of Public Health Policy (2007) 28, 322–340. doi:10.1057/palgrave.jphp.3200147

variety of assumptions that could be made about antigen production and vaccine type. But even a low-end estimate would represent a large addition to human production capacity. Notably, a large proportion of global animal vaccine production capacity is located in Asia, and additional capacity exists in Latin America.

Converting an animal influenza vaccine facility to human vaccine production is not, however, as simple as switching vaccine seed strains. There can be significant hurdles, the severity of which will vary with the specific equipment and process used at each manufacturing plant.

Major issues to be addressed in such a conversion are regulatory certification of the manufacturing process to human vaccine standards, ensuring appropriate biosafety practices, adequate egg supply, improved virus purification processes, and adoption of adjuvants approved for human use.

Regulatory hurdles will be country-specific. In some places, animal vaccine plants are already held to manufacturing standards near or equal to those for human vaccines; however, this is not always the case. A related issue is biosafety practices which, in some animal vaccine plants, would need improvement–both in operating procedures and, potentially, to equipment.

Conversion of animal facilities to human vaccine production may also strain egg supplies, especially in countries or regions where H5 vaccination of poultry currently occurs, because eggs laid by hens vaccinated against H5 cannot be used to produce vaccine.

Human flu vaccines produced in eggs go through an extensive filtration process to remove egg proteins and other contaminants that can cause an adverse reaction. Animal vaccines are generally not subjected to the same level of filtration, and improvement of filtration in converted animal vaccine plants would be necessary.

The adjuvants used in animal vaccines are not typically approved for use in humans, and animal vaccine plants would have to switch to appropriate adjuvants, unless they are producing a human pandemic LAIV (which is unadjuvanted).

Human vaccine producers and pharmaceutical companies own a significant proportion of global animal vaccine production capacity. For example, Merial, the world's largest animal vaccine maker, is a joint venture of Sanofi Aventis and Merck. Ft. Dodge, another large animal vaccine company, is a division of Wyeth. Intervet, a third large animal vaccine maker, is owned by Schering Plough. Other drug companies, such as Pfizer and Novartis, also have animal vaccine businesses. Thus, human and animal vaccine makers should not be thought of as wholly separate industries.

5. Concluding discussion

Which technologies should developing countries seek multilaterally to improve pandemic preparedness? The answer, of course, depends on many factors.

Reliance on a small number of developed country sources for pandemic vaccine and/or antigen is unlikely to remain an acceptable solution for most developing countries, particularly in view of the fact that the developed country industry is not currently in a position to offer sufficient quantities of antigen in a timely manner after the appearance of a pandemic strain.

Practically, the present situation of dependency, which is effectively unaltered by the WHO Pandemic Action Plan, means that the vast majority of developing countries will only receive significant quantities of vaccine after the needs of developed countries are met, which will likely be many months after the onset of a pandemic–months during which pandemic mortality may be severe.

As a result of the inequity, in the event of a pandemic, developing countries will suffer a disproportionate burden of serious disease and death, a problem that could be ameliorated by increased and equitably distributed global vaccine supplies, particularly in the developing world. These vaccine supply problems may be further exacerbated by non-health factors, in the form of export controls that may inhibit the ability of some countries to prepare for a pandemic because some kinds of technology transfer are unavailable to them.

Developing country leaders are likely to face question from their citizens if they remain vulnerable while the citizens of wealthy countries are vaccinated; this situation could become especially tense if a pandemic is severe enough to cause serious socioeconomic disruption.

Vaccination for the population at the earliest point possible following the onset of a pandemic isn't the entirety of pandemic preparedness; but it is a high priority. But at present, there is little consensus among experts about how best to achieve that.

It is also clear that no single technological approach will be appropriate for all countries or regions and that greater funding and improved access to proprietary technologies will be necessary for developing countries to improve protection of their citizens from pandemic flu. Regional cooperation in production and technology to take advantage of economies of scale will likely be far more fruitful than trying to go it alone for most countries.

Several options for financing and technology transfer have been mentioned in the context of the Pandemic Influenza Preparedness Intergovernmental Meeting (IGM). These include increasing vaccine production in developing countries, possibly supported by royalty-free licensing of vaccine production technology. Contributions to a global fund, and contributions of vaccines to a WHO stockpile by entities that use pandemic preparedness biological materials in research and development of vaccines and other biomedical items have also been proposed.

While a WHO stockpile may be useful to help stamp out or slow down the emergence of a pandemic influenza strain, it is not designed–nor will it serve–to ensure any country's vaccine supply. A WHO vaccine stockpile is also mandated by WHO Member States outside the WHO PIP IGM discussions, and is thus not a central objective of the benefit-sharing discussion.

Because increasing national or regional vaccine production capacity in developing countries requires flexibility in technological approaches, no single technology transfer and cooperative arrangement is likely to be effective. There is strong evidence that proprietary and emerging technologies, such as reverse genetics, adjuvants, and in the future cell culture, could serve to greatly increase the efficiency of preparedness efforts. Specific technology selections, however, must be made in the regional and national contexts.

In principle, developing countries may seek to formalize a system of equitable reciprocity wherein those developed country companies and other entities that utilize Global Influenza Surveillance Network (GISN)

materials to develop vaccines commit to transfer their vaccine technologies so that they may be used by developing countries.

Therefore, in the PIP IGM negotiations, developing countries have explored the possibility of creating a mechanism for transfer of influenza vaccine technology, through mandatory royalty-free licensing and other low or no-cost means, including for both formal patents and related know-how and trade secrets. The technologies prioritized by any such pandemic preparedness technology transfer program should be those that are used by industry to manufacture products that include WHO GISN materials (e.g. H5 vaccines) or are developed utilizing WHO GISN materials.

Reducing proprietary barriers to the technology needed to produce pandemic vaccines would represent a significant step forward; however, making technology available does not guarantee that it will be effectively used. Ways to optimize the use of technologies include, for example, the creation of a financing mechanism by which the real-world transfer of these technologies can be effected (for instance, to pay for the necessary equipment and training to utilize them). A pandemic preparedness cooperation fund could also be established, with contributions from manufacturers that utilize WHO GISN materials in commercial products (to be defined in a WHO material transfer agreement), and possibly contributions from governments. A cooperation fund could also help enable the use of nonproprietary technologies, such as egg-based production lines and fill/finish capacity, which will be important elements of any national or regional effort to increase vaccine production capacity.

Pandemic preparedness is a problem of daunting complexity, and solutions will only come with time and contributions from many quarters. The PIP IGM is an important process but not one that by itself can solve all problems. Developing countries may wish to focus on important specific benefits that will enhance their preparedness.

With the timing of a pandemic uncertain, but the time needed to construct and validate vaccine facilities typically measured in years, it is urgent that progress be made now to expand developing country vaccine production capacity. Developing countries will have to work together to identify the best technologies for their circumstances. The PIP IGM's decisions may help to make key technologies affordably available to developing countries, and through a cooperation fund, provide means through which to effect their transfer and use.

Annex 1

Overview table of influenza vaccine technologies

	Egg-based "classic" flu vaccine	Cell culture – produced vaccines	Live atenuated ("LAIV")	"Second generation" Biotech (e.g. VLPs, DNA vaccines, etc.)
Description	Inject vaccine virus into fertilized eggs, allow virus to grow. Harvest fluid from eggs, wash with detergent to kill cells, separate out virus material for vaccine formulation. Generally, the vaccine strain consists of the HA and NA genes of a "wild type" of influenza fused onto a lab-adapted "backbone" strain with the other viral genes.	Animal, insect, or other cells are cultured in growth media, scaling up the quantity to the desired density of cells in industrial bioreactors (fermenters) of hundreds to thousands of litres capacity. The culture is infected with vaccine strain, producing large quantities of vaccine virus. Harvesting, purification and packaging is essentially the same as with egg-based methods.	A vaccine seed strain using a special type of backbone (from a lab-adapted flu strain) is grown in eggs (or potentially cell culture). The live virus is harvested, purified and formulated for use. Harvesting and formulation is simpler than with killed vaccines, but the live final product is more delicate.	Many techniques are under study, including: – Producing recombinant HA in other, more easily grown organisms (e.g. transgenic bacteria). – "Naked" and plasmid DNA vaccines in which "codon optimized" flu genes are used directly as vaccine. – Genetically engineered systems to co-express HA, NA, and other flu genes, making a "virus-like particle" (VLP) which is used as vaccine.
"Hard" technology requirements	Large BSL-2 space, incubators, inoculation and harvesting equipment. Centrifuges (separation and purification) and packaging equipment.	Large BSL-2 bioreactors for cell culture, equipment to maintain cell bank and scale-up. In some cases growth substrates. After harvesting of virus, purification and formulation requirements are the same as for eggs.	Large BSL-2+. May be grown in eggs or bioreactors (see respective requirements at left), but currently the process is done in eggs. Purification and formulation differs from that for killed egg and cell culture vaccines.	Will vary with specific technology; however, all are likely to require BSL-2 GMP space and microbial fermentation / cell culture capacity.

	Egg-based "classic" flu vaccine	Cell culture – produced vaccines	Live atenuated ("LAIV")	"Second generation" Biotech (e.g. VLPs, DNA vaccines, etc.)
Major advantages	The technology is well known and has been successfully utilized for decades. Essentially the same technology is used for some poultry vaccines, making conversion of animal vaccine plants and personnel a possibility in an emergency.	Theoretically higher antigen output than eggs, theoretically more scalable. May grow some H5 vaccines viruses to higher titer. Cell culture vaccine plants likely to prove more flexible for producing other kinds of human vaccines than egg-based plants.	Live vaccine is likely to be effective in much lower doses than killed vaccines. Assuming production capacity can be harnessed, more LAIV vaccine may be made available in a shorter time period than with other types.	Dependent upon specific technology. Nearly all purport to be able to provide more vaccine faster, but these claims are as yet unproven. May offer more dependable vaccine yield per production run.
Major limitations	Not as efficient as cell culture *theoretically* is. Requires many eggs, the availability of which may be limited in a pandemic (eggs may be available from the broiler industry). Egg-based plants are unlikely to be used for other *human* vaccines (except for Japanese encephalitis vaccine).	Cell culture vaccines are a major focus of R&D; but as yet they are in limited commercial use. Inability to be certain that a particular cell line will be appropriate to grow the pandemic strain. Requires substantial bioreactor capacity of which there is little to no global surplus. Much higher cost facility at industrial scale.	Not suitable for prepandemic vaccines due to recombination risks. Difficult to test with prepandemic strains. Requires more stringent conditions on egg supplies than killed vaccine production. Higher biosafety requirements for production. More difficult to store vaccine. Intranasal administration requires special delivery device.	Dependent upon specific technology.
Regulatory/ safety approvals	Fewer impediments as these vaccines will be produced in the same manner and with the same facilities as seasonal vaccines, although converted animal vaccine plants likely will not already possess approvals to produce human vaccine.	Few cell-culture produced vaccines have been approved for human use and they are likely to prompt more intense regulatory scrutiny. Require approval for the vaccine as well as characterization and safety demonstration of the cells used.	*Seasonal* LAIVs have limited use in the US ("FluMist") and in Russia; however, most regulatory authorities would be encountering a live (and likely genetically engineered) influenza vaccine for the first time.	These products, if successful, will be new to regulatory systems and are highly likely to require substantial safety review.

	Egg-based "classic" flu vaccine	"Cell culture produced vaccines"	"Live attenuated flu vaccine" ("LAIV")	"Second generation" Biotech (e.g. VLPs, DNA vaccines, etc.)
Administration	Syringe	Syringe	Intranasal (requires appropriate delivery device)	Method of administration (injected, oral, intranasal, etc.) will depend on the specific product
Recombinant (genetically-engineered?)	Probably. Seed strain may be produced with reverse genetics and, for example, may contain a modified HA gene (deletions) to make the virus less pathogenic. Virus is killed before use.	Same as egg-based production. Other genetic modifications of the vaccine strain may occur to optimize growth in cell culture.	Probably, with the notable difference that the vaccine is administered live.	Vaccine will be genetically engineered or be the product of a genetically-engineered organism.
Adjuvanted (pandemic vaccine?)	Almost certainly, to make more efficient use of bulk antigen and potentially to reduce the number and size of required doses.	Same as egg-based. One exception is unadjuvanted killed "wild-type" virus (being tested by Baxter); however, producing such a vaccine is a biosafety challenge, requiring cell culture in large scale BSL-3 containment.	Probably not. The vaccine virus replicates in the upper respiratory tract, stimulating immune response. Nevertheless, some research has focused on increasing immune response to LAIVs with adjuvants.	Depends on specific technology.
Intellectual property	Few IPR problems for egg-based process, except for adjuvants where IPR and supply problems may exist. Potential additional problem if seed strain is produced using reverse genetics.	Many IPR impediments. These include patents on cell lines and production systems, as well as trade secrets on safety profile of cells. Cell characterization is only reportedly publicly available for Vero (monkey) cells.	Only a small number of backbone strains are suitable for use. Intellectual property impediments exist on the use of strains; patents and trade secrets cover the formulations. Seed may need reverse genetics.	Impediments will depend on specific technology; however, it may be anticipated that these technologies will have robust IPR coverage as they are mainly being developed by biotech companies and/or universities seeking to sell this technology.

	[illegible]	Cell culture produced vaccines	Live attenuated (LAIV)	"Second generation" Biotech (e.g. VLPs, DNA vaccines, etc.)
Other issues	Some (generally minor) side effects from egg proteins and other possible impurities.	Requires supply of growth media and other relatively exotic supplies. In addition to technical challenges, cell culture production may be especially prone to export control issues for a number of countries.	Safe production will require more stringent biosafety procedures than killed vaccines to prevent contamination of live final product. Societal resistance may be significant, particularly for seasonal use.	The vast majority of R&D in these lines of research appears to be conducted by companies in a handful of developed countries.

Annex 2

Relevant reports available online

Friede M, Serdobova I, Palkonyay L, Kieny MP. Technology transfer hub for pandemic influenza vaccine. *Vaccine*. 2008 Nov 18. (http://dx.doi.org/10.1016/j.vaccine.2008.10.080 - accessed 9 January 2009).

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This paper presents an overview of technologies currently available for the production of influenza vaccine, as well as others that are under development. It draws attention to pertinent issues and challenges that policy-makers in developing countries may need to consider when reviewing their options for accessing influenza vaccine production technologies. It is intended as a contribution to the debate on the sharing of influenza viruses and access to vaccines and other benefits arising from their commercial exploitation.

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