

# A review of production technologies for influenza virus vaccines

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Thus, influenza vaccine development and deployment is a critical part of pandemic influenza preparedness. Most resource-constrained countries do not have the means for accessing seasonal influenza vaccines and may face the same challenge during an influenza pandemic. There is therefore a need for these countries to consider establishing influenza vaccine production capacity. Several methods of production and also types of influenza vaccine exist, each with pros and cons for use, but also vastly different requirements in terms of capital investment, technology transfer, and production response time.

Manufacturers in developing countries who are interested in establishing influenza vaccine production capacity need to consider which type of vaccine, and which type of production technology is appropriate for them, and can be sustained. This document outlines the technologies currently available for the production of vaccines against influenza virus, and considers their suitability for influenza vaccine production in developing countries. The key advantages and disadvantages associated with each approach are identified, as well as the main hurdles that need to be overcome in the adoption of each of the technologies.

Investment and time required for implementing production capacity are also discussed. The production technologies considered in the document are egg- and tissue culture-based propagation of influenza virus, for the preparation of live attenuated or inactivated vaccines. The analysis shows that egg-based production of live attenuated vaccine requires the least capital investment to establish and maintain, and therefore appears attractive to manufacturers in resource-poor settings. Nevertheless, parameters such as the regulatory

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pathway to licensure and Intellectual Property Right (IPR) issues also need to be considered.

Egg-based production of inactivated virus is currently the most widely used technology. , However large scale production of inactivated vaccine requires significant capital investment which may be difficult to justify in areas where there is only limited market for seasonal vaccine. Tissue-culturebased production of live attenuated or inactivated vaccines requires greater financial investment, and access to proprietary technologies, but these technologies may have advantages in terms of logistics of vaccine production in event of a pandemic, and may also have long-term advantages for the manufacturer.

These pros and cons of each option are discussed in detail in the document, and graphically presented to assist manufacturers in selecting which technology is the most appropriate for them and for their country's pandemic preparedness plans.

## **Introduction**

The purpose of this document is to provide an overview of the technologies currently available for the production of vaccines against influenza (flu) virus, and to consider their suitability for use for influenza vaccine production in developing countries. The key advantages and disadvantages associated with each approach are identified, as well as the main hurdles that need to be overcome in the adoption of each of the technologies and the investment and time required for implementing production capacity.

The technologies discussed in this document are egg-based and tissue-culture based virus propagation, and the use of inactivated virus (whole, split or subunit, with and without adjuvant) or live attenuated virus as the vaccine. Upstream technologies, including the production of influenza antigens in plants or insect cells, or vaccines based on pure recombinant antigens, peptides or DNA are only briefly discussed. While these technologies may have significant potential for influenza control in the future, there are still many unknowns regarding their development, manufacturing and regulatory approval pathway.

Because of the emergence and circulation of H5N1 influenza strains and the need to prepare for a possible influenza pandemic, it is important to consider methods for the production of seasonal influenza vaccine in the context of their relevance and impact on production of pandemic influenza vaccine and overall pandemic preparedness. Manufacture of seasonal and pandemic influenza vaccines are closely interrelated and emergency production of a pandemic vaccine may be dependent on manufacturing capacity for seasonal influenza vaccine.

The sustainability of pandemic vaccine production preparedness will be dependent on maintaining a facility with trained operators, and this without incurring financial losses. Therefore, in selecting a technology for influenza vaccine manufacture, a country needs to consider the domestic market for seasonal influenza vaccine and also pandemic preparedness, as well as the financial and human investment required to establish and maintain the production capacity.

Other factors which manufacturers embarking on the process of establishing pandemic influenza manufacturing capacity need to consider include: the regulatory pathway to licensure of the vaccine; whether the facilities required for pandemic influenza vaccine production could serve for production of other vaccines, which could minimize the financial burden of maintaining preparedness; supply of critical components of the production process such as fertilized eggs, tissue culture medium and the fact that these supplies may be jeopardized in emergency situations.

## **Surveillance**

Surveillance is undertaken year-round to monitor influenza strains in the population, and at the start of the influenza season WHO announces which dominant circulating strains should be included in the vaccine (Gerdil, 2003). Seed strains WHO Collaborating Centres generate and analyse the seed strains for vaccine production.

Since the 1970s, this has been done by genetic re-assortment. Embryonated eggs are co-infected with the field strain selected for the vaccine and an A/PR8/34 (or similar) master strain that is known to give good yields on eggs. Highgrowth progeny virus is analysed to confirm the presence of surface glycoproteins from the field strain. (Gerdil, 2003). Vaccine seed strains are distributed to the vaccine producers in order to evaluate their suitability for vaccine production.

Factors such as yield when grown in eggs, antigenic stability, inactivation and purification process are evaluated. The results from all the producers are evaluated in meetings with the EMEA and FDA, typically held one month

after the initial WHO decision on formulation (Gerdil, 2003). It should be noted that 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.

## **Vaccine Manufacture**

At the moment of preparing this document all marketed influenza vaccines are grown in embryonated eggs, however it is anticipated that tissue-culture derived vaccines will shortly receive marketing authorization. The basic procedures for viral propagation and options for downstream processing using both egg-based as well as tissue-culture based propagation are outlined in section 5. The expected number of doses to be manufactured needs to be estimated well in advance so that supply of the required number of eggs can be co-ordinated.

Planning for egg supply typically commences six months before the start of vaccine production. Vaccine characterization Quantification of the haemagglutinin (HA) antigen in the vaccine is achieved by a single radial immuno-diffusion (SRID) assay. The reagents need to be specific for the strains in the vaccine, and take approximately three months to be produced and distributed. This can delay the release of the vaccine. Stability studies are also required for each new formulation and presentation.

## **Clinical Studies**

Clinical trials demonstrating the safety and immunogenicity of each new influenza vaccine formulation are required in Western Europe, but not in the United States or the Southern hemisphere (Gerdil, 2003). Overall, the bulk

production campaign for one season takes six months. Two such campaigns take place each year; one for production of vaccine for the Northern hemisphere, one for production of vaccine for the Southern hemisphere.

Inactivated influenza vaccines; composition Seasonal influenza vaccines are usually trivalent, each dose containing 15 µg of each of two influenza A subtypes (e. g. H1N1 and H3N2) and 15 µg of one influenza B strain. There are currently three predominant types of inactivated influenza vaccine: whole virus, split and subunit vaccines. All are currently produced in embryonated eggs. As discussed later, while egg-based production of virus is technically relatively wellunderstood, the need for a reliable supply of fertilized eggs is one factor that may make tissue-culture based production eventually more attractive.

The fact that allantoic fluid does not need to be removed from the virus harvest can simplify downstream processing of tissue-culture (TC) derived virus. Whole virus For preparation of whole virus vaccine, in most cases only one purification step (gradient sucrose centrifugation) takes place before inactivation. Because there is a single inactivation step, a higher concentration of inactivating agent is required than for split vaccines. This may affect the integrity of the antigen.

Nonetheless, whole-virus influenza vaccines are reported to be more immunogenic in immunologically naive populations (Nicholson, 2004). However, they have been reported to be associated with a higher frequency of adverse events compared with other types of influenza vaccine, particularly in children, and have been little used so far (Stephenson et al. , 2004). It is estimated that less than one third of all influenza vaccine  
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production is whole-virus vaccine. Split The majority of influenza vaccines are ‘ split’ vaccines, which are produced by detergent-treating purified influenza virus.

The splitting process breaks the virus allowing the relevant antigens to be partially purified. Removal of some of the viral components results in a less reactogenic vaccine. The inactivation procedure can be less stringent than for the whole virus vaccine since the splitting procedure also serves to inactivate the virus. Subunit or surface antigen vaccines Sub-unit or surface-antigen vaccines are produced as for split virus, but more rigorous purification is carried out so that the vaccine consists almost exclusively of highly purified HA and NA.

These purified antigens may be reconstituted in synthetic lipid bilayers, forming virosomes. Virosomal formulations of influenza vaccine are licensed in Europe.

## **Live Attenuated Influenza Vaccines**

Live attenuated influenza vaccines (LAIVs) were widely used in some parts of the world such as the Soviet Union, however their use declined as economical problems following the collapse of the USSR reduced demand for influenza vaccines.

Recently they have been re-developed in the USA as an alternative to the standard inactivated influenza vaccine (IIVs). Potential advantages of LAIVs include:

- No down-stream processing required (harvested vaccine is simply packaged)



- High yield (as described later, 15 to 20-fold greater than inactivated vaccines)

Needle-free delivery (administration is via an intra-nasal spray), which may facilitate administration in resource-poor settings. Induction of a broad immune response including mucosal, systemic and cell-mediated responses (Nichol, 2001). In contrast parenterally administered inactivated vaccines do not induce mucosal immunity.

The potential to induce a protective immune response after a single administration in naive individuals (yet to be confirmed). Two doses of vaccine are thought to be required for induction of protective immunity in naive individuals with inactivated vaccine.

Live attenuated influenza vaccines are constructed by re-assorting NA and HA from circulating influenza viruses with six genes from master donor strains of two types:

- Temperature sensitive (ts) donor strains. The viruses contain mutations that limit growth at 37–39°C, restricting growth in the lower respiratory tract. Growth is still possible in the upper respiratory tract, so local and systemic immune responses can be stimulated.
- Cold-adapted (ca) donor strains, which are selected to grow well at a reduced temperature, so that growth is favoured in the upper respiratory tract.

Two live-attenuated, cold-adapted vaccines derived from the A/Leningrad/134/57 (H2N2) and B/USSR/60/69 virus have been licensed in Russia (Rudenko et al. , 2001). A LAIV (CAIV, FluMist®, MedImmune

Vaccines) with ca, ts and attenuated (att) phenotypes based on master donor strains A/Ann Arbor/6/60 and B/Ann Arbor/1/66 was licensed in the USA in 2003. LAIVs are currently produced in embryonated eggs (section 7) however several groups are developing LAIVs in tissue culture.

## **Upstream Influenza Vaccine Approaches**

A number of approaches are being pursued to develop alternative forms of seasonal and pandemic influenza vaccines including:

DNA vaccines expressing HA and NA, which are delivered to the epidermis by needle-free ballistic delivery of DNA-coated gold particles (PowderMed, Oxford, UK); Preliminary clinical data suggest that this approach is promising, and since DNA can be rapidly produced, this is attractive for pandemic influenza preparedness.

The need however for a complex formulation and delivery system does remain a barrier to the widespread use of this technology.

Recombinant HA produced by a baculovirus-expression system (Protein Sciences Corporation, CT, USA); or produced in transiently transfected plants (Microbix, USA). The high and rapid yield of antigen make these approaches attractive, however this must be weighed against the limited breadth of immunity that will be induced to a single antigen (compared to a split or live virus). Recombinant M2e, the extra-cellular domain of the ion-channel protein M2 (Acambis, Cambridge, USA). This is a highly conserved viral antigen and it is suggested that immunity to this antigen could theoretically protect against all Astrains of the virus and thus provide universal protection. Because these programmes are at relatively early stages of clinical- or pre-

clinical development or unlikely to be appropriate or available for use in developing countries in the near future, they are not discussed further in this report.

## **Vaccine Production Capacity**

A vaccine against a pandemic influenza strain would be monovalent i. e. containing antigens from the pandemic strain only. In theory only 15 µg of HA per dose would be required (rather than 45 µg); however, for several reasons, the production capacity for a pandemic strain could be less than for current seasonal vaccines. The pandemic strain will be a new strain for which there is little or no immunological memory in the population.

Therefore it is anticipated that two doses of inactivated vaccine will be required in order to induce protective immunity. If a LAIV is used, one dose may be adequate (still to be demonstrated). In order to avoid any possibility of reassortment of an LAIV strain carrying HA and NA from a potentially pandemic influenza strain and used for immunization with a circulating wild-type strain, which might theoretically generate a virulent pandemic strain, use in the general population of LAIV containing HA and NA from a highly virulent avian influenza strain is not recommended before the onset of pandemic.

Manufacturing Current data suggest that egg-based production yields of HA expressed by H5N1 viruses generated by reverse genetics are only 30–40% of the average HA yield for seasonal influenza viruses (Stephenson et al. , 2006), while virus yields are similar to those obtained usually. This could have serious implications for IIV production in the event of a pandemic.

There are also concerns that growth of human influenza virus in eggs can lead to the selection of antigenic variants which may be less efficacious (Katz and Webster, 1989).

Immunogenicity Data from phase 1 and 2 trials indicate that vaccines based on H5N1 strains may be less immunogenic than current seasonal vaccines. In a phase 1 trial of a nonadjuvanted sub-virion H5N1 vaccine (A/Vietnam/1194/04), two doses of 90 µg were required to generate antibody responses that would be satisfactory for licensing (Treanor et al. , 2006). In a further trial in which the sub-virion vaccine was formulated with aluminium hydroxide, two doses each of 30 µg were found to induce immune responses consistent with EMEA requirements for seasonal influenza vaccines.

In contrast, two doses of 10 µg of an aluminium hydroxide-adjuvanted whole-virion vaccine induced seropositivity in 78% of vaccines (Lin et al. , 2006). Therefore, for split or sub-unit H5N1 vaccines to induce protective levels of immunity, it appears that larger doses of HA (compared to standard influenza vaccines) and/or adjuvants will be required. Thus, using current production technologies the antigen production yield is approximately three-fold lower, and six-fold more antigen is required (per strain), and two doses of vaccine (inactivated are required for protection).

When establishing pandemic vaccine production capacity this significant reduction in functional yield compared to seasonal flu needs to be taken into account.

## **Adjuvants and Delivery Routes**

Use of immunological adjuvants or alternative delivery routes may reduce the dose required to induce protective levels of immunity, thereby increasing overall global capacity. Several adjuvants have demonstrated capacity to enable the induction of antibody levels that are considered to be protective, with lower inactivated antigen doses (generally evaluated with split, but also whole cell vaccines).

Aluminium-based adjuvants Aluminium-based adjuvants including aluminium hydroxide, aluminium phosphate, and a combination of the two 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 antigen from potential pandemic-strains, the results are not consistent and for some groups the effect is at best marginal or absent. A likely reason for this difference is the different antigen nature between the groups: for some the split antigen contains a relatively low ratio of lipid and other components (such as matrix protein M1) to the HA, and there is hence limited Other factors such as viral RNA in the vaccine (whole cell), contaminating endotoxins, and the presence of counter-ions which modify the charge on the gel may also affect the impact of aluminium adjuvants on the vaccine immunogenicity.

This indicates that for any influenza vaccine antigen the potential contribution of alum to the immunogenicity and hence antigen dose needs to be evaluated. The use of alumbased adjuvants for pandemic vaccines appears attractive since there are only minimal intellectual property barriers (exception is D'Hondt et al, European Patent EP1618889), however the <https://assignbuster.com/a-review-of-production-technologies-for-influenza-virus-vaccines/>

variable response, as well as formulation and characterization challenges suggests that other adjuvants should be considered. Oil in water emulsions The limited studies conducted to date, with 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 trioleate 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. AS03, 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 (Hannon and Stephenne patent application WO2006/100110).

Both of these adjuvants are however proprietary and their use in vaccines produced by others would require a license. The original patent on MF59 emulsions (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. While the owners of the technology have publicly indicated that in the event of a pandemic they would permit their adjuvants to be used by others, pre-negotiated licenses and supply agreements would need to be established. This would reduce the independence of a company's pandemic response.

## **Other Sdjuvants**

Several other adjuvants have been shown to be effective when used with influenza antigens in preclinical models. Most of these are proprietary, and as for the adjuvants above add to the complexity of manufacturing, formulation and characterization. Intradermal delivery Intradermal delivery has been shown to permit dose reduction for seasonal vaccine (Treanor 2004, Belshe 2004) however in the only clinical study to date with H5N1 antigen this route was not able to induce what are considered protective levels of immunity with a reduced antigen dose.

This approach is attractive since reformulation of the vaccine is not required, however a number of parameters may affect the immunogenicity including the reliability of the intradermal injection, and further investigation of this approach is required.

## **Review of Influenza Vaccine Production Technologies**

For the purposes of this review, each manufacturing technology is considered at two scales of operation: the laboratory and pilot scale manufacture, and industrial scale manufacture.

This separation has been included to permit interested vaccine manufacturers to determine the investment required to proof-of-concept at the pilot level, and then the challenge and investment of scaling up to full production. As indicated below, some technologies are relatively easy to establish at pilot-plant level, but require massive investment to scale up, whereas others are scaled up relatively easily.

Laboratory and pilot-scale manufacture In this case, the requirements to establish and run a process that would be capable of producing a few thousand doses of vaccine under current Good Manufacturing Practice (cGMP) are considered. The material would be suitable and sufficient for phase I and phase II clinical trials to demonstrate immunogenicity and safety of the product.

## **Industrial-Scale Manufacture**

In this case, the investment and infrastructure needed to establish a process capable of producing millions, or tens of millions, of doses per year are considered.

The capacity should be sufficient for meeting local and/or regional demand for seasonal influenza vaccine. It should also be sufficient, or at least readily scaleable, to meet the local and/or regional demand for a pandemic vaccine. For the purpose of comparing production costs and time to establishment between technologies we have arbitrarily chosen to fix the production at 20 million doses per year.

## **Assumptions**

In estimating the resources required to establish influenza manufacturing, it has been assumed that the process and facility will be established by an existing developing-country manufacturer of human vaccines.

It has also been assumed that influenza-vaccine production will be installed on an existing ' campus' with access to trained staff familiar with GMP requirements, as well as all the necessary Quality Control (QC), Quality Assurance (QA) and other supporting functions. Access to vaccine-filling lines



and associated labelling and packaging infrastructure has also been assumed to be available. Where these are not available, the implementation of pandemic influenza vaccine production will be far more costly and take far longer than indicated in the following paragraphs.

Finally, cost estimated are based on figures that would apply in the USA or Europe; they may be different for developing country or resource poor settings.

Inactivated influenza vaccines (IIV); egg-based manufacture Introduction 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.

## **Manufacturing Process**

At the laboratory scale, it is possible to produce hundreds to thousands of doses of IIV manually, without the need for automation and using only standard laboratory equipment. 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 hole is then sealed with wax. The procedure can be carried out on a laminar flow bench.

The inoculated egg is incubated for two to three days in a humidified atmosphere. At the end of this period, it is transferred to 4°C, which kills the embryo Page 12 of 34 Influenza vaccine production technologies 20 December 2006 and aids clarification of the allantoic fluid. The top of the egg is cut off, the membrane pierced with a pipette and clear allantoic fluid is

removed. This is then clarified by centrifugation to remove cell debris. Harvests from the eggs are pooled and sterility tested for three to four days. The degree and method of purification used depends on the type of vaccine being produced.

**Whole virus.** The pooled harvest is concentrated and 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. **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). **Subunit vaccine.** This is <https://assignbuster.com/a-review-of-production-technologies-for-influenza-virus-vaccines/>

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.

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 (Nicholson, 2004). Use of adjuvants would require well-characterized material to ensure consistent formulation. Furthermore, adsorption of alum adjuvants is influenced by the lipid to protein ratio, which varies between the different types of IIV. It should be noted that the methods described above are suitable for production of vaccine candidates for pre-clinical testing and phase I clinical trials. They can provide a useful guide for the development of industrial process, but the final methods used for industrial production would be dependent on, and a result of the know-how of the company developing the process.

## **Resource Required**

The infrastructure requirements for the process are straightforward. Inoculation and harvesting of eggs can be performed manually. Minimum requirements would be:

- a relatively simple laboratory with one or two laminar flow benches;
- a simple incubator;
- and a standard ultracentrifuge.

Assuming access to a manual or small-scale filling-line is available and full QC and QA functions are in place, a facility could be established with investment in the order of US\$100, 000s dollars. Establishing such a facility, developing the process, producing and releasing a batch of vaccine suitable for use in a phase I clinical trial would take approximately 12-18 months (Figure 1), depending on the specific expertise and prior experience of the manufacturer.

In order to produce sufficient inactivated vaccine for routine seasonal immunization against influenza or pandemic preparedness, the process requires automation and scale-up at all stages. A realistic goal would be a facility capable of producing 20 million doses of trivalent IIV in a four to five month production cycle.

## **Resource Required**

Egg supply. In optimized conditions, each egg can produce approximately between 45 and 90 ug of a classical seasonal antigen, which corresponds to one to two doses when formulated as a trivalent vaccine.

As noted above, the yield for H5N1 strains may be significantly less. Manufacture of 20 million doses of trivalent seasonal influenza vaccine requires 15 million to 20 million fertilized eggs over the production cycle. Supplying eggs in these numbers requires careful planning and a long lead-time (6-12 months) in order to reach the numbers required. Furthermore, the supply has to be carefully controlled and timed because the eggs have to be inoculated at a fixed point after fertilization. The egg supply needs to be secure in terms of quantity and quality of the eggs. Automation.

Automatic inoculators, incubators and harvester are required to speed up the process and increase capacity. Automatic inoculators can operate at approximately 10, 000 eggs per hour, enabling a plant to process up to 100, 000 eggs per day. However, this also means that less care is taken with the process because there is no longer the opportunity to inspect each egg for contamination. Careful cleaning and pooling procedures are needed to prevent the contamination of one egg being spread by a harvester or inoculator to many other eggs, resulting in the loss of a whole bulk harvest. Infrastructure and equipment.

A large plant will be required to house the necessary equipment, including equipment for the initial upstream process steps:

- inoculation machines
- incubators
- automatic harvesters
- collection vessel (> 1000 litres capacity) for the harvest
- cold rooms Additional specialized equipment will be needed for downstream processing. The exact nature of this will be dependent on the specific process employed, but is likely to include:
  - high-volume, low-speed centrifugation
  - ultracentrifugation, and possibly diafiltration, which will require thousands of litres of buffer for counter-flow.

Approximately 80% of the mass of the 100, 000 eggs received per day (equivalent to approximately 4, 000 kg) will be solid, infectious waste, which will need to be inactivated and disposed of. It is estimated that the capital investment required to establish a plant for large-scale production of <https://assignbuster.com/a-review-of-production-technologies-for-influenza-virus-vaccines/>

influenza vaccine in eggs on an existing vaccine manufacturing campus is approximately US\$1 per dose of capacity, i. e. US\$20 million investment for a plant capable of producing 20 million doses of trivalent vaccine per year.

Building and validating the large-scale facility would require at least two years (Figure 1).

## **General Considerations**

Laboratory-scale production of IIV in eggs should be readily achievable. The equipment needed is relatively small, standard and inexpensive and the skills required for the process are straightforward. It is estimated that a plant and process could be established and produce a clinical lot in less than 18 months, for an investment in the order of US\$100, 000s.

Scaling up to the industrial process requires significant investment. In addition to the financial cost, one of the major hurdles will be accessing the necessary expertise. Eggbased production of influenza vaccine using automated procedures is a unique process not used in other vaccine manufacturing plants, and the expertise required are not readily transferable from other vaccine-manufacturing plants, even other egg-based processes such as yellow-fever vaccine production where the volumes used are orders of magnitude less.

Since much of the equipment is specific to production of influenza vaccine, the adoption of the technology will require training of personnel in facilities already equipped and performing automated inoculation and harvesting. Finally, the cycle of seasonal influenza vaccine manufacture is such that manufacture for a single market takes place in a four to five month period.

Therefore a manufacturing plant may remain unused for six months of the year; alternatively additional markets in a different hemisphere need to be identified.

Virus grown on embryonated chicken eggs and then inactivated has been widely used for influenza vaccine production for more than 60 years and is the standard method for flu-vaccine production. Billions of doses of IIV have been produced to date using this technology. Reassortant virus vaccine strains have been developed that are optimized for growth in eggs. The regulatory pathway to licensure, as well as the necessary analytical assays are well established with the result that few unknowns remain in this process.

Establishing manufacture at the industrial scale is expensive, and many of the skills and equipment required are not readily transferable to the production of other vaccines. Egg supply. The supply of eggs from a certified source is critical. A lead-time of several months is required to establish a reliable supply of fertilized eggs of suitable quality to meet the requirements of seasonal flu-vaccine production. In the event of a pandemic, scale-up of egg production would take several months.

If the eggs are not produced locally, in the event of a pandemic the limited supply of eggs may be used by the manufacturer in the country of origin of the eggs. Having its own chicken farm and chicken flocks can be a strategic advantage for a manufacturer, both for seasonal and pandemic vaccine. It is technically possible and necessary to secure the flocks against avian influenza, to ensure that egg-based production is possible, even in the worst-case scenario that the pandemic virus infects both mammals and birds.

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Waste disposal. Up to 80% of the mass of eggs used (potentially 4, 000 kg per day) will be solid infectious waste, which will require inactivation and disposal. Selection of variants Growth of human influenza virus in eggs can lead to the selection of variants that differ antigenically from the original seed virus (Katz and Webster, 1989). It is therefore necessary to adapt the strains to egg growth. Yield in eggs. Many years of experience producing reassortant viruses as vaccine strains have made this technology robust and reproducible.

However, individual virus isolates may exhibit different growth characteristics in eggs, thereby affecting the yield of the manufacturing process. This might particularly be the case with an avian-derived pandemic strain. Experience to date with H5N1 viruses generated by reverse genetics has shown that these reassortant viruses produce only 30-40% of the yield of HA of seasonal vaccine strains, despite growing to normal titres in eggs.