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Introduction to Vaccine Development and Production

Influenza vaccines have been used in the U.S. for more than 50 years and are the primary method for preventing influenza and its complications. There are several ways to decrease population vulnerability by improving the ability to immunize with a safe, effective vaccine: make vaccine available earlier; make more vaccine doses; and make a more immunogenic vaccine that is highly effective in all populations.

Annual influenza vaccine development is an efficient and well-coordinated process that includes input from international organizations, advisory committees, Department of Health and Human Services (HHS) agencies and licensed vaccine manufacturers. This time-sensitive, multi-step process typically takes nearly a year and requires year- round work.

The amount of vaccine that can be produced in time to be used in an influenza season is a function of the capacity of the industrial manufacturing base and the growth characteristics of the viruses selected and used to produce the vaccine. In 2004, approximately 100 million doses of trivalent influenza vaccine are projected to be produced for the U.S. market, equivalent to approximately 300 million doses of a monovalent vaccine containing 15 micrograms of antigen against a newly emerging pandemic strain. This may not be sufficient to meet U.S. needs, since the entire population could require vaccination and two doses may be necessary to ensure full immunity in individuals with no prior exposure to similar viruses. Therefore, in the event of a pandemic increased domestic influenza vaccine production capacity would enhance the supply of vaccine using current production techniques.

II. Background and Current Status

Influenza viruses were first isolated in the early 1930s. Subsequently, it was recognized that two types of orthomyxovirus (influenza A and B viruses) produce clinical influenza in humans. Changes occurring by point mutations in the hemagglutinin (HA) or neuraminidase (NA) proteins of influenza viruses result in what is termed antigenic drift and is the reason that influenza vaccines are redesigned every year to match the strains that are predicted to be circulating during the influenza season. In contrast, marked changes that occur as a result of reassortment between influenza viruses of human and animal origin ("antigenic shift") are those that are most likely to result in a pandemic strain. *(See Annex 1: Overview of Influenza Illness and Pandemics for a detailed discussion of shift and drift.)* The exchange of the HA or NA in the reassortment is the minimum needed for detection of antigenic shift, but any of the eight genes may be reassorted, which has possible implications for viral replication, virulence and transmissibility. Influenza B viruses are not different enough to be divided into subtypes and have no known reservoir other than humans, but significant antigenic drift occurs in influenza B, and reassortment with exchange of HA or NA has been found. Fifteen HA subtypes and nine NA subtypes exist within influenza A viruses, all of which are found in avian species. Of these, only three HA and two NA subtypes are known to have regularly infected humans during the 20th century. H1N1 influenza A viruses caused infection in human populations from the early 1900s until 1957 when an antigenic shift occurred and H2N2 viruses emerged. A second antigenic shift occurred in 1968 when H3N2 influenza A viruses appeared. In 1978, H1N1

viruses reappeared and have co-circulated with H3N2 viruses since that time. The antigenic shifts to H2N2 and H3N2 viruses appear to have been the result of natural reassortment; however, the reappearance of H1N1 viruses may represent an accidental reintroduction of laboratory virus.

The successful propagation of influenza virus in embryonated chicken eggs in 1937 paved the way for the first influenza vaccines. In 1943, the U.S. military sponsored extensive clinical trials with egg grown, formalin inactivated, whole virus vaccine. These vaccines were immunogenic and demonstrated 70 percent protection during a severe H1N1 influenza A epidemic. In 1945, licenses were issued to several companies in the U.S. for inactivated vaccines that were purified by adsorption and elution from red cells.

A. Use of Chicken Eggs

The vast majority of inactivated influenza vaccines are still grown in eggs, but inactivated influenza vaccines produced in tissue culture have been developed by several companies during the 1990s and are approved but not yet produced for commercial use in Europe. There also have been substantial improvements in purification techniques, most notably zonal centrifugation and column chromatography, to reduce the presence of egg or tissue culture proteins and improve safety/reactogenicity profiles of the vaccines. Most of the vaccines produced today are chemically disrupted, split, or subunit vaccines. Although they have been available in Russia since the 1980s, live attenuated influenza vaccines produced in eggs were first approved for use in the United States in 2003.

B. Composition of Annual Influenza Vaccine

The composition of the inactivated influenza virus vaccine is reviewed and revised yearly. In most years, one or two of the three strains are replaced by more recent ones because of virological and epidemiological surveillance data that detects antigenic drift in influenza A and B viruses. Each January, information collected by the World Health Organization (WHO), Centers for Disease Control and Prevention (CDC), Food and Drug Administration (FDA), National Institutes of Health (NIH), Department of Defense (DoD), and others is presented to the FDA's Vaccine and Related Biological Products Advisory Committee (VRBPAC). The information includes data on surveillance of new influenza strains in the U.S. and the rest of the world, on epidemiology of influenza virus infection and illness, on influenza virus antigenicity and molecular changes, on an assessment of the probability that current influenza vaccines will produce antibodies that react with the vaccine strains and the newly circulating strains, and on the availability of strains suitable for vaccine production.

Collection of surveillance and epidemiology data are done continuously throughout the year. Data from immunization studies are usually collected in the fall when the most recent vaccine is available. The current organization and timing of selection of vaccine strains resulted largely from experience gained during the preparation for the possible spread of a swine influenza strain in human populations during 1976-1977. The valuable lessons learned during that campaign have been incorporated in the yearly preparations for contemporary antigenic drift in all influenza strains, and are partly responsible for the continued and increasing efforts in surveillance for the early identification of new (shifted) influenza strains to provide as much time as possible to choose strains for vaccine production. Minor modifications and improvements

have been incorporated throughout the years, but the basic strategy for recommending new strains for inactivated influenza virus vaccines has continued with the format established in the early 1980s.

C. Vaccine doses

Current vaccines are trivalent (H1N1, H3N2, and B), and contain 15 micrograms of each antigen. Vaccine production for the U.S. has increased since 1985. About 100 million doses of trivalent inactivated vaccines are anticipated to be produced for the U.S. market in 2004-2005.

Studies done in anticipation of the return of the H1N1 strains in human populations provided the basis for the assumption that two doses of vaccine would be needed in immunologically naïve hosts. Studies done in the late 1970s instituted the system of potency assignment using single radial immunodiffusion (SRID) through the use of a standard antiserum and a positive control antigen to determine the quantity of HA in individual vaccine preparations. Initially, the dose of HA for vaccines was set at 7 micrograms per antigen, since higher doses did not appear to increase immunogenicity. In subsequent years, the dose was increased to 15 micrograms per antigen to maximize the production of heterologous antibodies without the increasing reactogenicity of vaccines.

D. Production Process

Vaccine production occurs over a several month period and requires careful coordination among the HHS agencies and influenza vaccine manufacturers. Each year, manufacturers project the vaccine demand and negotiate contracts with farmers to provide fertile eggs for vaccine production. Usually, the flocks of chickens are hatched in summer to reach maturity and begin laying eggs by December or January when the annual production begins. Egg size and eggshell characteristics are critical to large scale, automated inoculation and harvesting operations. Eggs from the new laying hens are more likely to meet requirements for shell thickness, egg size, quantity, bioburden, and viability of the embryos. Therefore, aged flocks are sacrificed at the end of the production season in the following summer. In addition, egg quality may be less desirable during hot months because of increased bioburden. However, experiences in several years suggest that it is possible to extend the life span of the flock if additional vaccine production is needed. In 1998, WHO began publishing recommendations for the composition of influenza virus vaccines for use in the southern hemisphere. The recommendations for the southern hemisphere have prompted many manufacturers to make the necessary arrangements (including development of flocks to provide the required number of eggs) for nearly year round production of influenza vaccine.

At full capacity, current manufacturing facilities are capable of inoculating, incubating, and harvesting up to several hundred thousand eggs daily. Each monovalent component is produced separately and is dependent on how well the specific viral strain replicates. Therefore, yield of a specific influenza virus is perhaps the largest limiting factor to the amount of vaccine that can be produced in a given period of time. Yield in eggs is increased by use of reassortant strains produced using an egg-adapted donor to provide all viral genes except HA and NA, which are derived from the wild type strain. However, there remains substantial unpredictability and differences in growth properties among reassortant strains.

Recently, it has been possible to selectively introduce specific genes of influenza viruses into reassortants using the molecular biologic technique of reverse genetics. The technique consists of copying influenza virus genes, transfecting a cell line to introduce all of the genes to make the desired virus, and selecting reassortant clones with the antigenic and genetic properties of interest. The method permits recovery of reassortants in a manner that may avoid some of the unpredictability associated with the classical reassorting techniques. However, at this time, reverse genetics techniques are not incorporated in the production of the annual influenza vaccine.

Figure 1: Influenza Vaccine Manufacturing Process

Eggs are held in incubators until the proper age (9-11 day embryos). Eggs are candled to permit nonviable eggs to be discarded.

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After inoculation of the specific influenza virus, incubation for optimal time (usually 48-96 hours) at optimal temperature (33-36 C) is carried out before the eggs are candled again to discard additional nonviable eggs.

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Eggs are pre-chilled in refrigerators to increase yield at harvest of the infected allantoic fluids. The allantoic or tissue culture fluids are further processed to remove egg or cell proteins and cell debris, chemically inactivated, and stored as bulk vaccines until formulation can proceed.

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Potency assignment is done on each monovalent vaccine pool using SRID, which requires a standard antigen of known HA quantity, and a HA specific antiserum

The manufacturing operation consists of several steps for which inoculum, time, and temperatures are predetermined by small-scale studies to maximize viral yield (see Figure). In contrast to egg-based production, when vaccines are produced in tissue cultures, a similar process is used except that fermentors containing cells grown over several days to the proper density are inoculated with virus and after incubation the virus containing fluid is harvested

To assure that the antigen content of the vaccine is standardized, antiserum is produced by inoculating large animals (sheep) with a highly purified preparation of HA and may be a rate-limiting step in the formulation of final vaccine. To prepare the antiserum to match the vaccine strain, sheep need primary and booster doses to produce antibodies of sufficient titer to permit SRID to be done – a process that may requires six weeks or longer. Since the immunization procedure is time consuming, it begins as soon as vaccine composition is determined. However, if final vaccine composition is not determined until March, the antiserum is usually available in May, but can require additional time if the response of individual animals is not optimal. *(See Table: Approximate Timetable for Production of Inactivated Monovalent Vaccine).*

Overall, primary vaccine production (inoculation and harvest of influenza viruses from eggs or tissue cultures) for the United States typically begins in January and proceeds through the summer (and sometimes later) depending on anticipated vaccine demand, viral replication, and availability of standardization reagents. Although vaccine production is time and labor intensive, it is likely that in a pandemic situation, the capability to produce 300 million doses of a monovalent vaccine consisting of 15 micrograms per dose currently exists in licensed manufacturing facilities. The ability to increase further (surge capacity) depends on the number of manufacturers actively making influenza vaccines and their total production capacity, how many lines are available for filling product into final containers, and the growth characteristics of the virus in production facilities.

E. Vaccine Production Considerations for Highly Pathogenic Influenza Viruses

As with other highly pathogenic infectious agents, additional requirements to ensure laboratory worker and public safety will need to be considered. For example, transport of strains between laboratories may require a permit from USDA for each strain being sent. For strains such as the highly pathogenic 1997 and 2003 strains from Hong Kong, biological containment at the Biosafety Containment Level 3 (BSL3) or higher is a requirement.

F. Clinical Trials *(See Annex 8: Pandemic Influenza Research)*

Ideally, clinical evaluation of vaccines against new HA (antigenic shift) will determine immunogenicity and reactogenicity in target groups. A model for these studies would be the studies conducted in the 1970s with the reappearance of H1N1 influenza A. At a minimum, the groups evaluated would cover the age spectrum (6 months to 3 years; 3 years to 10 years; 10 years to 20 years; 20 years to 65 years; and over 65 years). Certain populations might also be targeted in each age group (such as healthy elderly and institutionalized elderly). For each group, studies would be done to determine preexisting antibodies to the pandemic strain, the immunogenicity of a range of doses of vaccine (such as 7.5 and 15 micrograms) and the need for one, two, or more doses of vaccine to achieve a specified level of antibody (for example, greater than 1:40 for 90 percent of the population). Each group studied would need to be of a size to provide statistical confidence and, if possible, done at more than one center to highlight potential geographic differences in populations.

III. Actions to Improve Vaccine Development and Production

The time required to develop, produce, evaluate and deploy an adequate number of doses of a safe, effective vaccine against the pandemic strain is an important obstacle to an optimal pandemic response. Currently, it takes up to nine months to produce egg-based influenza vaccines. Several actions can be implemented that can reduce the time required to complete the complex series of steps in the vaccine development process and decrease the time during which the population is vulnerable.

The first critical step is early identification of the strain of the influenza virus causing the pandemic. Influenza virus strains are identified from global surveillance of human cases and, in the case of strains with pandemic potential, from animal sources as well. Intensifying

surveillance of novel animal and human influenza viruses can lead to earlier development of reference strains that can be used for vaccine production.

Once strains are identified FDA can produce reference influenza viruses that are adapted to high-growth in eggs. Similar work on the reference strains by vaccine manufacturers improves the efficiency of the process and enables manufacturers to make more vaccine in a given time. Coupled with the expansion of manufacturing facilities, these activities have helped to support the growth of influenza vaccine production for the United States from about 20 million doses of trivalent influenza vaccine in 1990 to nearly 90 million doses for the 2003-2004 season. This process is important since it provides information about optimal production methods and, if the HA of the emerging pandemic viruses closely resembles one of the reassortants in the library, could provide the opportunity to quickly scale-up to industrial levels of production of vaccine. However, even if the strain that causes a pandemic is not similar to one in this library, the experience gained in developing, producing, and evaluating candidate vaccines will improve the efficiency and speed of the process when a pandemic occurs; and the information gained assessing the immune responses to novel influenza viruses, the optimum amount of antigen to include, and the number of doses needed for immunity can guide preparation, formulation, and use of a pandemic vaccine.

Once sufficient doses are produced, the candidate vaccine will need to undergo thorough evaluation, including studies to determine the optimal immunization dose and schedule.

A. Vaccine Production

Most influenza vaccines available in the U.S. today are inactivated vaccines made by chemical inactivation and disruption of the virus. Although a live attenuated influenza vaccine was approved for use in the U.S. in 2003, the number of doses available in the early years of production likely will be small relative to inactivated vaccine production. All licensed influenza vaccines in the U.S. are manufactured in embryonated chicken eggs, but influenza vaccines produced in tissue culture are being developed for commercial use. The ability to expand capacity for a pandemic is limited by the available supply of eggs that are required for production.

New approaches are examining whether vaccines could be efficiently produced in tissue cultures instead of eggs. This system will still rely on growing the viruses in cells and will be subject to many of the same time issues in terms of preparing the cells for infection with influenza virus; growing the viruses; purifying the viruses; formulating and filling the vaccines; and performing all quality-control testing. Preparation of vaccines by recombinant DNA technology can also be done, but this too requires cell culture for individual virus products and must go through purification steps, formulation, and quality control. All of the technologies require substantial careful preparation of the facilities before the work can commence. However, the advantage of tissue and cell cultures is the technology is not dependent on variable egg production by chickens.

B. Production Capacity

As noted earlier, the current U.S. capacity for a pandemic vaccine is estimated to be approximately 300 monovalent doses. Steps to encourage expansion of capacity are currently underway and will continue to be driven by increasing vaccine demand during the inter-pandemic period. *(See Pandemic Influenza Preparedness and Response Plan: Core Document for discussion of influenza pandemic phases.)* Because the growth of vaccine strains may differ among manufacturers, as was observed during the 2000-2001 vaccine shortage, having more licensed manufacturers working in parallel increases the probability of successfully producing sufficient vaccine.

Increasing capacity through the use of incentives to encourage the diversification of vaccine manufacturing approaches and/or attract additional manufacturers into the U.S. market will take several years to accomplish. Discovery of methods to increase the yield of vaccine per unit of production, however, offers opportunities to expand the number of vaccine doses produced more quickly. Reference strains from which influenza vaccines are produced (reassortants) include the hemagglutinin (HA) and neuraminidase (NA) proteins of the wild type strain as well as other viral proteins from a strain known to grow well in eggs. Development of new molecular techniques, a better understanding of the genes that regulate growth of influenza virus, and methods to more rapidly identify and select high-growth reassortants all may increase the speed and the yield of vaccine per unit of production. Methods to increase virus yield also can be identified through systematic exploration of viral factors that affect growth in various culture system and through systematic assessment of various cell lines that are certified for production of vaccine.

To advance pandemic vaccine preparedness specifically, HHS recently announced two Requests For Proposals (RFPs) designed to encourage U.S.-based influenza vaccine manufacturers to have adequate surge capacity so that they can make large quantities of vaccine in the setting of a pandemic. In addition to ensuring that the manufacturers who make vaccines in eggs have the raw materials they need at any time of the year, these RFPs ($50 million in fiscal year FY '04 and $100 million in the President's FY '05 budget request) are also seeking to accelerate the development of domestically-produced U.S.-licensed cell-culture based vaccines. Not only will this potentially shorten the timelines to the production of large numbers of doses of vaccines, but also will decrease the potential vulnerability to egg-based production should an avian influenza epidemic threaten the egg supply.

C. Research *(see Annex 8:Pandemic Influenza Research)*

The experience with current and past influenza vaccines suggests two doses may be required to induce adequate levels of immunity to a pandemic strain of influenza. Enhancing the immunogenicity of a new vaccine so that only one dose is needed to provide adequate levels of immunity could stretch available vaccine supply to protect more people. Enhancing the immunogenicity may require inclusion of an adjuvant – a substance included in vaccines to increase the strength of the immune response. Considerable work has been done to explore adjuvants combined with influenza vaccines. Further investigation needs to be done to understand whether adjuvants will be useful in a pandemic situation.

D. Vaccine Preparedness

The ideal pandemic influenza vaccine is one that can be produced in the shortest amount of time, protect the largest number of individuals, and is efficient, safe, and easy to deliver. Actions to achieve this goal are listed below.

Inter-pandemic phase preparedness actions

- Expand global surveillance for the earliest possible detection of the emergence of a pandemic strain.
- Prepare a library of high growth reassortant viruses against influenza strains with greatest pandemic potential. This collection will have the greatest value if the pandemic strain is identical or similar to one of the strains included. Even if the strain is different, however, the experience gained by the development of high growth reassortant viruses to novel influenza strains will increase the speed with which a high growth vaccine seed virus can be developed to a pandemic strain.
- Prepare and clinically evaluate investigational lots of vaccine made against novel influenza subtypes. Going through the process of vaccine development and evaluation will help identify parts of the system that can be made more efficient and provide experience that can be applied when a pandemic occurs.
- Develop standardization reagents that will be required to assess the potency of investigational vaccines for potential pandemic viruses.
- Improve the yields of high-growth reassortant viruses to increase the number of available vaccine doses through systematic evaluation of growth in various tissue culture systems available for vaccine production. This effort will be complemented by ongoing fundamental research into the factors that affect growth characteristics of various influenza strains in a variety of tissue culture systems.
- Increase demand for yearly vaccine thereby increasing the size of the vaccine market and providing the financial incentive to expand capacity. Increasing demand and vaccine coverage has the additional benefit of preventing disease and death during the annual influenza epidemics.
- Provide incentives for new vaccine manufacturers to enter the U.S. market to increase production capacity and, through diversification, enhance the probability that vaccine will be produced rapidly and made available early.
- Prepare a clinical protocol that can be immediately implemented to rapidly evaluate the safety and the optimal dose and schedule of a pandemic vaccine in various populations. If vaccine containing less than 15 micrograms per dose is effective in inducing immunity, available production capacity could lead to a greater total number of doses. In addition, two doses may not be needed by all segments of the population, and will be dependent on whether a strain similar to the pandemic strain has ever circulated previously.
- Encourage development, evaluation, and licensure of an influenza vaccine that contains an adjuvant. An influenza vaccine that contains an adjuvant may produce a good immune response with a lower dose of antigen, allowing existing production capacity to be divided into more vaccine doses. Also, a vaccine that contains an adjuvant may produce protective immunity to a novel influenza strain after a single

dose, reducing the total number of doses needed to protect the population. Development and licensure of any new influenza vaccine needs to be done in the inter-pandemic period because studies needed to demonstrate safety and efficacy cannot be done quickly enough at the time of a pandemic to be of value.

- Support the clinical development of promising alternate vaccine products. New technologies under study may decrease the time needed to produce influenza vaccine or to increase the yield or efficiency of production.

Table: Approximate Timetable for Production of Inactivated Monovalent Vaccine