

The five-six month timetable for influenza vaccine production from isolation of vaccine strain to release first vaccine lots

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The production of pandemic influenza vaccines requires careful coordination of complex processes which involves public health laboratories including regulatory authorities, the World Health Organization, and vaccine manufacturers. The international influenza surveillance system provides field isolates from nasopharyngeal swabs to the WHO Collaborating Centers for Influenza Reference and Research (currently located in Atlanta, London, Melbourne, and Tokyo). The process to production is summarized as follows:

Activities at WHO Collaborating Centers:

1. **Reference virus strain preparation:** A reference virus strain is prepared in these laboratories, by a process called genetic re-assortment: a process whereby surface antigens (HA and NA, the protective components of the vaccine) from the field-isolated pandemic strain are combined with core components of a traditional laboratory vaccine strain adapted to optimal growth in hen's eggs, the substrate used to produce the vast majority of influenza vaccines under industrial conditions. Reference virus strains can also be produced by reverse genetics, a novel molecular biological technology.

This process requires 3 to 6 weeks.

2. **Verification/Validation:** After preparation of the reference virus strain by the WHO Collaborating Centers, this vaccine candidate undergoes a series of identity testing including the analysis of the virus specific surface antigens (HA and NA) from the circulating virus.

This process requires 3 weeks

3. **Reference reagents:** In parallel, the Collaborating Centers produce official WHO reference reagents, specific antigens and antisera to the selected vaccine strain, which will serve to permit verification of antigen content (potency) and immunogenicity of the commercial vaccines.

This requires producing the reference virus strain, purifying surface antigens from it, immunizing animals, generating and standardizing sera, and distribution of these reagents to manufacturers. This process starts as soon as the reference virus strain is available. It requires roughly 4 months and is often a bottleneck to the overall timeline for manufacturers to generate the vaccine.

At the vaccine Manufacturer:

1. **Manufacturer process optimization and working seed establishment:** Using the reference virus strain received from the WHO collaborating centers manufacturers optimize the production process to increase growth characteristics and yields to expand the reference virus strain into qualified working seed virus banks which are used to inoculate eggs to initiate the production of bulk vaccine batches.

This process requires roughly 3 weeks

2. Vaccine Bulk Manufacture: For most influenza vaccine production this is performed in embryonated (fertilized) hen's eggs. The working seed virus is injected into eggs, and the eggs are then incubated for two-to-three days. The vaccine virus strain grows in the allantoic fluid (egg-white) of the egg to increase the amount of virus that will be harvested for vaccine production. The vaccine is harvested from the egg and purified to remove excess egg material. The partially pure virus is then chemically inactivated for safety and is often then disrupted or split open by mixing with detergents. The remaining proteins important for the vaccine are further purified.

Producing each batch of antigen takes approximately two weeks and a new batch can be started each week. The size of the batch depends on the egg capacity, and the yield of growth in each egg.

3. Quality Control: This can only begin once the reference reagents are supplied by WHO laboratories.

The bulk vaccine is characterized using the reference reagents to ensure that the concentration of the hemagglutinin antigen is correct. In addition, the bulk antigen is also verified for sterility.

This process takes 2 weeks.

4: Vaccine filling and release:

The bulk vaccine is diluted to the desired concentration, and filled into vials or syringes, and labeled according to manufacturers licensed specifications. A sampling of these filled containers are then tested for sterility, which takes two weeks. In addition, testing is done to confirm the antigen content and general safety by injecting into animals.

This process takes 2 weeks.

5. Clinical studies

Clinical trials demonstrating safety and immunogenicity of each new influenza vaccine formulation are required in Western Europe, but not in the United States.

This requires at least 4 weeks.

At the regulatory agency:

1. Regulatory approval

Before the vaccine can be administered to people, approval from local regulatory authorities is required. If the vaccine is made with the same processes as the licensed conventional seasonal vaccine, in the same manufacturing plant, this review can be rapid (1-2 days).

Activity	Month 1	Month 2	Month 3	Month 4	Month 5
At reference labs					
Reference strain preparation	→				
Verification / distribution to manufacturer	→	→			
Preparation of reference reagents		→	→	→	→
At Manufacturer					
Optimise / Establish working seed bank		→	→	→	→
Inoculation of eggs, incubation			→	→	→
Harvesting, purification			→	→	→
characterization, sterility testing			→	→	→
Filling, safety testing, release				→	→
Clinical Trial (in Europe)				→	→
At regulatory agency					
Review and release				→	→

The above procedure requires five to six months before the first final lot becomes available.