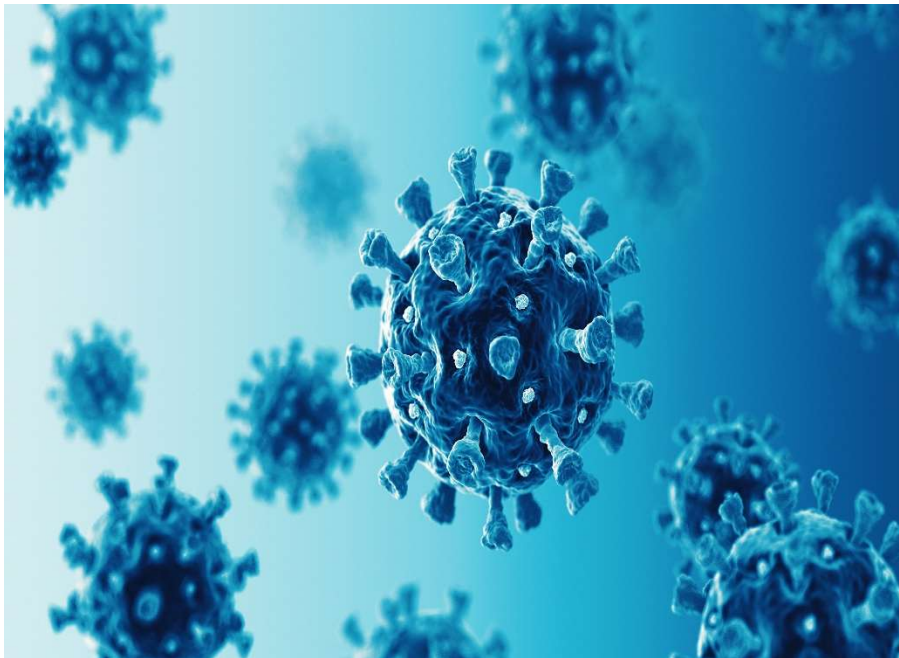


Technical Description of the Influenza Vaccination

As we enter the Fall season, not only do we notice the push for the upcoming Holidays, but we also see an increase in advertisements for the cold and flu season. This includes “reminders” to get your vaccines for the Flu (Influenza), Pneumococcal, and Covid 19 boosters. Once these are acquired, does anyone wonder how they are made? According to the National Institute of Allergy and Infectious Diseases, the flu vaccine production process is labor-intensive and time-consuming, taking months, even years, to complete. The process for the Influenza Vaccine is completed using a very peculiar way, but the actual process might shock you.



When scheduling your “flu shot,” you are given a questionnaire. On this questionnaire, you will always be asked, “Do you have an allergy to eggs?” Seems like a strange question when scheduling a flu vaccine, but it is a very important question for your Medical Provider or a pharmacist.

Commercially available vaccines (especially those offered and given in the United States) are made and produced by private sector manufacturers. What this means is that any vaccines that are FDA-approved and distributed within the United States are required to meet strict safety and effectiveness requirements. These standards, set forth by the CDC and the World Health Organization (WHO), follow very strict standards, and an imperfection in the design that alters the strength will forfeit production.

To begin, the Influenza vaccine is not necessarily grown in a test tube. Currently, there are three different influenza vaccine production technologies available in the United States. These

technologies have been approved by the U.S. Food and Drug Administration (FDA). These vaccines are the egg-based flu vaccine, the cell culture-based flu vaccine, and the recombinant flu vaccine. And yes, the egg-based flu vaccine does begin in a chicken egg. Yes, you read that correctly. The Influenza vaccine begins its life cycle within a chicken egg. This process, as strange



as it sounds, has been used for more than 70 years and is the most common method. Egg-based vaccine manufacturing is used to make both inactivated (killed) vaccines, which are known as the “flu shot,” and live attenuated (weakened virus) virus, which are known as the “nasal spray flu vaccine.” Egg-based vaccine production begins with the Centers for Disease Control (CDC) or another laboratory that is a

permitted partner in the WHO Global Influenza Surveillance and Response System, which will provide a private sector manufacturer with candidate vaccine viruses (CVVs) grown in chicken eggs per the standard FDA regulatory requirements. These CVVs are then injected into fertilized chicken eggs and incubated for several days. This process allows the viruses to replicate. Once replicated, the fluid within the egg containing the virus is then extracted (or harvested) from the eggs. In regard to the inactivated influenza vaccine, which is the flu shot, the vaccine virus is made inactive (killed), and the virus antigen is purified. For the live attenuated influenza vaccine (LAIV), which is known as the nasal spray flu vaccine, the beginning CVVs are used to make live and weakened viruses that are then used in vaccine production.

To begin this process, CVVs are required. As noted previously, the CVVs (candidate vaccine viruses) are used in the production of the influenza vaccine. The production of the CVVs is the first, and most important, step in the production of the flu vaccine. CVV production begins when



Scientists acquire two genes from the bird flu virus. These two genes are the [NA] neuraminidase and [HA] hemagglutinin genes. Upon receiving these two genes, reverse genetics is then performed. The reverse genetics involves the tedious practice of taking these specific two genes and then combining them with six other genes of a commonly used human flu virus. The [HA] and [NA] genes from the bird flu virus can either be prepared synthetically or by using the viral RNA of the wild-

type virus (if this is available). The [HA] is then modified by removing the site that is responsible for making the virus deadly to birds and chicken eggs. This is called a polybasic cleavage site.

Then this prepared DNA is introduced into cells, which are typically “vero” cells within a laboratory, and are then allowed to grow for three days. These are then collected on the third day from the cells and injected into eggs to begin the growth of the virus stocks. The virus stocks undergo further testing to determine if a sufficient amount of the virus is present in the eggs. The CVVs are analyzed further to ensure that they are suitable for vaccine development in terms of compliance with regulatory requirements. The CDC then continues to test the vaccines.

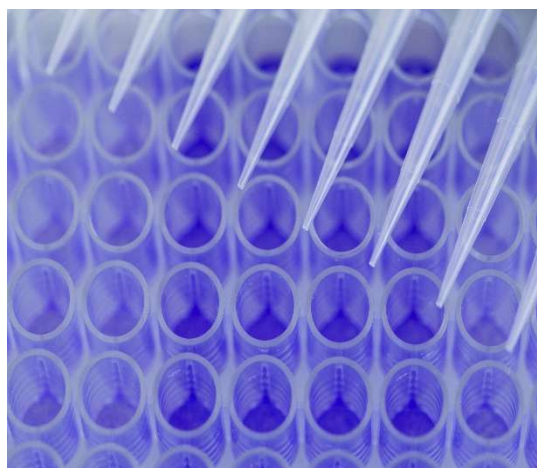
Once the CVVs are created and complete the quality testing that is required, the viruses undergo more testing, which is part of the quality assessment. The 6 steps involved during this part of the process are as follows, and are known as the quality assurance testing.

Gene Sequence Verification. This verification step requires that the flu experts verify that each CVV produced is genetically stable and hasn’t undergone any unintended changes or mutations. PCR and full genome sequencing are used in this process to ensure that the CVVs are as close as possible to the wild-type bird flu virus that they are intended to protect against.

Impurity testing. This verification step makes sure that the CVVs do not contain any bacteria or fungi.

Exclusivity testing. Here, the CVVs are tested to ensure that it only contains the virus intended and is not mixed with any other viruses.

Trypsin-Dependent Plaque Assay Testing. This testing is a laboratory test that ensures that the polybasic cleavage site, the part of the virus that makes the virus deadly in poultry and chicken eggs, has been removed.



Embryo Lethality Testing. This test confirms that the CVV does not kill chicken embryos and is a requirement for the egg-based vaccine production.

Finally, there is the Antigenic Characterization of the virus. This testing portion is completed by the CDC and uses the hemagglutinin inhibition [HI] test to antigenically characterize the CVVs to ensure that the CVVs are antigenically similar to the bird flu virus (as in nature) and that the CVVs will work to protect against the necessary strain. In other words, the virus that is made is the virus that it is intended to control.

Following the quality assurance testing, the CVVs undergo the Determination of Attenuation tests. These two tests. The First is known as the Pathogenicity testing in Chickens. Chickens are inoculated with the newly produced CVVs and watched to ensure they do not get sick or die. This testing verified that the CVV is not highly pathogenic within domestic poultry. It further signifies that the CVV will not harm agricultural interests and can be safely used during the egg-based

production. The Pathogenicity testing in chickens is completed by outside collaborators. One highly regarded one is the United States Department of Agriculture's [USDA] Southeastern Poultry Research Laboratory [SEPRL]. Once this testing is completed, the SEPRL will send the results to the CDC. The other testing is the Pathogenicity testing in Ferrets. The testing procedure is the same as with chickens, but with ferrets, the effects of the disease in ferrets closely resemble the virus process in humans. This is why ferrets are often used as a model to infer flu disease and transmission characteristics.

After all testing has been completed, the CVVs are then shipped to Essential Regulatory Laboratories (ERLs), and vaccine manufacturers begin the process of mass production.

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