ANTIGENIC CHARACTERISATION OF SEASONAL INFLUENZA VIRUSES ISOLATED IN VACCINE-QUALIFIED CELL LINES

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Scope and purpose of this document

This document describes the process to be used by WHO Collaborating Centres for Reference and Research on Influenza (CCs) when undertaking the antigenic characterisation of influenza viruses propagated in vaccine-qualified cell lines that may be used in seasonal influenza vaccine production for use in humans. Antigenic characterisation is performed to ensure that the test virus is antigenically similar to the prototype vaccine virus that has been selected for incorporation into a particular influenza vaccine for use in humans. This process is equivalent to the one used for the testing of egg propagated high growth reassortant influenza viruses (1). Antigenic characterization results are generally evaluated by advisors from WHO CCs and WHO Essential Regulatory Laboratories (ERLs) in the course of the bi-annual WHO Consultations on the Composition of Influenza Virus Vaccines (VCMs).

This document does not address issues on safety, adventitious agents or Good Laboratory Practise/Good Manufacturing Practise (GLP/GMP) matters associated with the use of cell propagated influenza viruses in vaccines for humans and other documents should be consulted to address these issues (2, 3).

Background

A number of vaccine manufacturers are either developing or have developed cell lines that are suitable for both the isolation and subsequent propagation of influenza viruses to generate influenza vaccines for use in humans. Influenza Vaccine manufacturers have developed a variety of different qualified cell lines, including Vero cells, MDCK lines and PerC6 cells, to propagate influenza viruses (4).

To date manufacturers have used influenza viruses that were initially isolated in embryonated hens’ eggs to develop seed viruses for manufacture of vaccines in their qualified cell lines. There is now a desire by some manufacturers to expand the number of seed viruses available by also including viruses initially isolated in qualified cell lines. Cell-isolated viruses potentially provide another source of supply of viruses for vaccine production in cell culture. In addition, viruses isolated and propagated exclusively in cell cultures generally lack the haemagglutinin (HA) amino acid substitutions that occur during egg isolation, and might more closely resemble the antigenic characteristics of circulating viruses. (5, 6).

In addition, human influenza viruses isolated directly in qualified cell lines might also be grown subsequently in embryonated eggs to support vaccine manufacture in eggs. The relative importance of this “cell-to-egg” process, and the priority of such activity, may fluctuate depending on the availability and performance of candidate vaccine viruses.
propagated solely in eggs. The antigenicity and other testing requirements for these “first cell- and then egg-” propagated viruses is not addressed in this document.

Cell culture candidate vaccine virus isolation and preliminary characterization

Figure 1 shows a notional work flow that would be involved in the isolation, expansion and the antigenic characterisation of influenza viruses required to establish their suitability as potential cell culture Candidate Vaccine Viruses (pccCVV). Stage 1 involves the initial isolation and characterization of influenza viruses, usually incorporating at least the assessment of the haemagglutination titre, sequencing of the HA and neuraminidase (NA) genes, and a 1-way haemagglutination-inhibition (HI) assay using appropriate reference post-infection ferret antisera which would take place in a WHO CC. Following successful completion of these assays, larger scale propagation of selected viruses might be undertaken and the process would then move to Stage 2, which is most likely to take place at a manufacturer’s facility. If required Stage 2 may take place at a number of different manufacturers using various cell lines, providing there is sufficient material available from the initial isolation to distribute more widely. At the end of Stage 2 it would be expected that there would be a small number of pccCVV that would then be available for further testing/characterisation by a WHO CC (Stage 3). Some elements of Stage 3, for example Stage 3.1 (Figure 1) where the raising of post-infection ferret antisera to the pccCVV is required, may be done outside of a WHO CC’s by contractors/collaborators/manufacturers with suitably equipped facilities.

Antigenic characterization of potential cell culture candidate vaccine viruses

Stage 3.2 (Figure 1) involves the full antigenic and genetic characterisation of a pccCVV. Those pccCVVs that meet the 1-way HI and HA/NA sequence requirements when compared to the requisite prototype virus, are used to infect naïve ferrets and raise convalescent ferret antisera for use in further HI assays (known as a reciprocal or 2-way HI assay). The post-infection ferret antiserum ideally will have a titre of ≥160 with the homologous pccCVV for use in the 2-way HI. Other ferret antisera raised against reference influenza viruses propagated in cells are also used in the 2-way HI assay along with the homologous ferret antiserum. Comparisons with ferret antisera raised against egg derived reference viruses may be performed if relevant. Antigenic analysis of pccCVVs will be carried out at a WHO CC. It would be expected that a ferret antiserum raised against a WHO-VCM designated cell propagated prototype virus should recognise the pccCVV in a HI assay with a titre of <4-fold lower compared to the titre of the antiserum with the homologous virus. Similarly, a convalescent ferret antiserum raised against the pccCVV should recognise the WHO-VCM designated cell propagated prototype virus in HI tests at a
titre <4-fold lower compared to homologous titre of the antiserum with the pccCVV. If this is the case then the pccCVVs shall be considered as antigenically equivalent to the WHO VCM designated cell propagated prototype virus.

**Characterization of data for potential cell culture CVVs**

A full pccCVV characterization report will be prepared by one of the WHO Collaborating Centres with all the available genetic and antigenic data, and submitted to the WHO VCM Advisors for consideration. The data will support evaluation of the following requirements:

- Satisfactory reciprocal HI assay relative to WHO VCM-recommended vaccine virus
- Satisfactory HA and NA gene sequences

**Review and decision on status of pccCVV**

The WHO VCM Advisors will review at their biannual meetings (or as appropriate e.g. by email) the pccCVV data and make a determination as to whether it meets all of the above requirements. Once approved the test virus can then be considered a cell culture candidate vaccine virus (ccCVV).

Following the review and agreement by the VCM Advisors that the virus is a suitable ccCVV, WHO will list this virus on its website. Reassortants and other viruses that are to be used for egg-based vaccines will be described in a separate document and listed on the WHO website. ccCVVs will be free of intellectual property right claims but could be subject to liability waivers. Distribution of the ccCVV, preferably at the same passage level as was tested by the WHO CCs/ERLs, to requestors will be free of charge (excluding shipping charges which may be recovered from the consignee). The distribution will be the responsibility of either the manufacturers which developed ccCVV or the WHO CCs/ERLs.

**Note that the final acceptance of the ccCVV as a suitable seed virus for influenza vaccine production for use in humans is generally the responsibility of the country’s/regions regulator (e.g. US Food and Drug Administration (FDA), the European Medicines Agency (EMA), the Australian Therapeutic Goods Association (TGA)) and significantly more information will be required in addition to the antigenic characterisation of the virus as described in this document.**
References


Figure 1: Development of cell culture candidate vaccine viruses (ccCVVs)

Aliquots of respiratory secretion samples that were previously deemed to contain antigenically variant influenza viruses (or possibly replacement ccCVVs for poorly growing/yielding ccCVVs), that could potentially be included in vaccines, will be selected for further characterization by genetic and antigenic analyses at WHO CCs. To this end, promising viruses isolated in vaccine-qualified cell lines will be evaluated to determine if the viruses meet the appropriate criteria for antigenic properties and also provide suitable yields for use in vaccine manufacturing. See article text for details.