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Guideline on Quality Aspects on the Isolation of Candidate Influenza Vaccine Viruses in Cell Culture

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Executive summary

This Guideline lays down the quality recommendations for cells used to isolate candidate influenza vaccine viruses, the conditions under which the viruses are isolated and the subsequent passage of the viruses until the manufacturer's seed is prepared under GMP conditions.

1. Introduction (background)

Many influenza vaccine manufacturers are developing cell culture processes for the production of inactivated vaccine using a variety of cell types and several such vaccines have been licensed within the EU. Manufacturers of cell-derived vaccine typically use the recommended egg-derived candidate vaccine virus to derive their seed virus; this may be the wild type egg isolate or a high growth reassortant (hgr), especially for influenza A viruses. There is currently no evidence that the use of an egg-derived hgr provides a growth advantage in cells compared with the wild type egg-derived recommended strain – it is simply the vaccine virus that is available from WHO collaborative laboratories that supply such viruses.

Manufacturers of cell-derived influenza vaccine would prefer to use a cell-only passaged virus and not one that has been egg-adapted. This is because research indicates that when a human influenza virus is adapted to grow in eggs, it undergoes phenotypic changes that might include changes to its antigenicity/immunogenicity. Virus isolated on mammalian cell cultures do not, at least initially, undergo the type of selection that occurs during initial passage in eggs and typically the haemagglutinin (HA) of a cell isolated virus is structurally identical to the virus found in clinical material in contrast to egg-adapted variants in which specific HA amino acid substitutions have been identified [1]. Thus a cell-isolated virus may be more clinically relevant for vaccine than an egg isolate.

For the reasons mentioned above, manufacturers are now keen to use non-egg adapted viruses which are antigenically closer to the wild type virus. However, cells in use by National Influenza Centres and WHO Collaborating Centres for virus isolation are not qualified/validated for use in deriving a candidate vaccine virus and so currently only egg-isolated viruses are taken forward as vaccine candidates.

This document provides essential guidance to influenza vaccine manufacturers and to WHO Collaborating Centres on the quality issues associated with isolating candidate vaccine viruses in cell culture, including the cells used to isolate the virus, the conditions under which viruses are isolated and the subsequent passage of these viruses until the manufacturer's seed is prepared under GMP conditions. The quality aspects of the establishment of a manufacturer's seed lot and subsequent use in a cell vaccine manufacturing process have been described previously in the guideline 'Cell Culture Inactivated Influenza Vaccines, Annex to note for guidance on harmonisation of requirements for influenza vaccines' [2] and will not be further addressed in this document.

2. Scope

An influenza virus isolated on cell culture could be used to derive a seed virus for either a cell culture or an egg vaccine production process for the manufacture of inactivated or live attenuated influenza vaccines.
Classically, an egg isolate is derived directly from a clinical specimen. Introduction of an intermediate step consisting of a passage in cell culture (essentially an amplification step) prior to egg inoculation enhances the probability of isolating (or recovering) an appropriate 'egg' variant.
Consequently, the use of a cell-isolate to derive an egg-isolate is likely to result in a greater efficiency of obtaining an initial egg-isolate. Thus, the scope of this document is to provide guidance for the isolation on cell culture of any potential influenza vaccine virus intended for cell culture or egg-based influenza vaccine manufacture.

3. Legal basis

This guideline has to be read in conjunction with the introduction and general principles (4) and Part 1 of the Annex I to Directive 2001/83 as amended.

This guideline should be read in conjunction with all other relevant guidelines, especially those pertinent to the production and quality control of influenza vaccines. Furthermore, reference is made to the Ph. Eur. General chapter 5.2.3 on cell substrates for the production of vaccines for human use (01/2009:50203) and to the Influenza vaccine Ph.Eur. monographs which state the following: “The origin and passage history of virus strains shall be approved by the competent authority.”

4. Main Guideline Text

4.1. Cell substrate used for the isolation

There should be good quality control of the cells to be used for the isolation of an influenza virus destined to be a candidate vaccine virus. There is good knowledge of certain cell substrates used in influenza virus research and development, such as MDCK, Vero and primary cells of chick origin. Regardless of the cell type used, where a cell line is used cells should be derived from a cell banking system.

The principle concern regarding the cells is their microbial and viral safety and the cells should, in principle, meet the requirements of Ph. Eur. general chapter 5.2.3. on cell substrates for the production of vaccines for human use in this respect.

The origin, source and history of the cells should be available (including the nature of media used in their propagation) and the identity and purity of the cells should be verified.

Tumourigenicity testing would not be required for cell lines for which relevant information is available such as MDCK, Vero, PerC.6 or for primary cells of chick origin.

Cells from an approved cell bank system used for human vaccine manufacture are likely to comply with general chapter 5.2.3 and would be acceptable for virus isolation. Similarly, cell substrates that comply with ICH Q5D would be acceptable for use in virus isolation.

It should be noted that some cell lines, e.g. Vero cells, are able to propagate a very wide range of (human) viruses and there is a greater risk of isolating a co-infecting human virus from a clinical specimen in addition to an influenza virus (where such co-infections exist).

4.2. Cell manipulation, virus isolation and virus propagation

The composition and source of media used for all cell culture manipulations including cell passaging, virus isolation and virus propagation should be recorded in detail. If substances of human or animal origin are used they should be free from extraneous agents. Bovine serum used for the preparation and maintenance of cell cultures should be irradiated and should comply, in principal, with the Note for guidance on the use of bovine serum in the manufacture of human biological medicinal products [4]. Animal-derived materials used in cell culture manipulations must be compliant with the current version of the Transmissible Spongiform Encephalopathy Note for Guidance [5].

Only one virus should be handled in an open system at any one time.

4.3. Quality assurance

The propagation of cells and viruses should be performed under a quality system. This should involve the use of dedicated facilities, and staff should be fully trained (or undergoing training) in all procedures. Documentation should allow full traceability of procedures, equipment performance, materials and training competency of staff. The distribution of viruses should be recorded.

It is the responsibility of the manufacturer to ensure that their vaccine seed is suitable for the production of a human influenza vaccine.

References

[1] Robertson, J.S. (1993) Clinical influenza virus and the embryonated hen's egg. *Reviews in Medical Virology* **3**, 97-106.

[2] Cell Culture Inactivated Influenza Vaccines. Annex to note for guidance on harmonisation of requirements for influenza vaccines (CPMP/BWP/214/96). CPMP/BWP/2490/00.
http://www.emea.europa.eu/pdfs/human/bwp/249000en.pdf

[3] European Pharmacopoeia general chapter 5.2.3. Cell substrates for the production of vaccines for human use, 01/2009:50203.

[4] Note for guidance on the use of bovine serum in the manufacture of human biological medicinal products. CPMP/BWP/1793/02.
http://www.emea.europa.eu/pdfs/human/bwp/179302en.pdf

[5] Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary products (EMEA/410/01 Rev. 2 – October 2003)
http://www.emea.europa.eu/pdfs/human/bwp/TSE%20NFG%20410-rev2.pdf