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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 Master 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 published 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 may prefer to use a cell-only passaged virus instead of 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 [1]. 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 more related to the virus found in clinical specimens in contrast to egg-adapted variants in which specific HA amino acid substitutions have been identified [1]. Thus a cell-isolated virus might be more clinically relevant for vaccine than an egg isolate although to date this has not been fully demonstrated scientifically.

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 general 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.

The major concern in isolating vaccine viruses in cells is the possibility of adventitious agent contamination that might derive from the cells, the environment or materials used during isolation and propagation of the viruses. Thus, the purpose of this document is to provide regulatory guidance on the quality of 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 master seed is prepared according to GMP. Normally, regulatory guidance is directed towards the vaccine manufacturers as it is they who have the responsibility for ensuring that their vaccine seed is suitable for the production of a human influenza vaccine. However, it is appreciated that the isolation of influenza candidate vaccine viruses will take place in WHO Collaborating Centres and as such, from a practical point of view, these laboratories should be familiar with the EU recommendations presented in this document.

The quality aspects of the establishment of a manufacturer's Master 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. 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. It should be reminded however that influenza viruses used in vaccines should also follow the recommendations published by the WHO on this matter.

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 [3] 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 is good experience in the use of certain cell substrates in influenza virus research and vaccine development, such as MDCK, Vero and primary cells of chick origin. Where a cell line is used, cells should be derived from a cell banking system.

The main 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 verified¹.

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 a cell bank system approved for use for human vaccine manufacture that comply with Ph. Eur. general chapter 5.2.3 would be acceptable for virus isolation.

It should be noted that some cell lines, e.g. Vero cells, are able to propagate a wide range of (human) viruses and there is an increased 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. The use of animal-free components is recommended. If substances of human or animal origin are used they should be free from infectious agents. Bovine serum used for the preparation and maintenance of cell cultures should be irradiated

¹ Where a cell banking system is in operation, identity and purity would normally be assessed for the Master Cell Bank.

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].

Handling of viruses should take place within a dedicated microbiological safety cabinet (MSC). Only one virus isolate should be handled at any one time and the MSC should be sprayed with ethanol or other disinfectant before and after use. The MSC should be run for a minimum period before a second virus is handled. Greater segregation of different subtypes of virus should be considered. A dedicated storage system for cells and for candidate vaccine viruses should be in place and the distribution of viruses should be recorded.

4.3. Quality assurance

Assurance should be provided that the propagation of cells and viruses involves the use of dedicated facilities and that staff are fully trained (or undergoing training) in all procedures. Documentation should allow full traceability of procedures, equipment performance, origin of materials and training competency of staff. While manufacturers may source candidate vaccine viruses from WHO laboratories which have optimised their suitability for use in vaccine production, marketing authorisation holders are reminded that they are responsible for the suitability of their Master Seed for use in their individual production systems.

Working to GMP/GLP is not expected for the virus isolation process.

Where a cell-isolated virus is used in the manufacture of vaccine in eggs, if the virus has been derived in accordance with this guidance, there should be no impact on the quality requirements of the egg-manufactured 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 (EMA/410/01 Rev. 2 – October 2003)
<http://www.emea.europa.eu/pdfs/human/bwp/TSE%20NFG%20410-rev2.pdf>