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Guideline on Influenza Vaccines – Quality Module

Draft

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Once finalised, this Quality Module will replace the Quality requirements of the following guidelines:

- Note for guidance on harmonisation of requirements for influenza vaccines (CPMP/BWP/214/96)
- Cell culture inactivated influenza vaccines - Annex to note for guidance on harmonisation of requirements for influenza vaccines (CPMP/BWP/214/96)
- Points to consider on the Development of Live Attenuated Influenza Vaccines (EMEA/CPMP/BWP/2289/01)
- Procedural advice on the submission of variations for annual update of human influenza inactivated vaccines applications in the centralised procedure (EMA/CHMP/BWP/99698/2007 Rev. 1)
- Annex I variation application(s) content for live attenuated influenza vaccines (EMA/CHMP/BWP/577998/2010)
- Guideline on Dossier Structure and Content for Pandemic Influenza Vaccine Marketing Authorisation Application (EMEA/CPMP/VEG/4717/03 rev. 1)

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- Guideline on Submission of Marketing Authorisation Applications for Pandemic Influenza Vaccines through the Centralised Procedure (EMEA/CPMP/4986/03)
- Guideline on Influenza vaccines prepared from viruses with the potential to cause a pandemic and intended for use outside of the core dossier context (CHMP/VWP/263499/06)
- Guideline on quality aspects on the isolation of candidate influenza vaccine viruses in cell culture (EMA/CHMP/BWP/368186/2011)

Comments should be provided using this template. The completed comments form should be sent to quality-influenzaguideline@ema.europa.eu

Keywords	***Quality requirements, influenza vaccines, inactivated, LAIV, seasonal, pre-pandemic, pandemic, mock up dossier, annual strain update, pandemic strain update***

Guideline on Influenza Vaccines – Quality Module

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1. Introduction (background)

The need to update the current guidelines regarding the quality, non-clinical and clinical development of influenza vaccines was recognised in the wake of the 2009-2010 influenza pandemic, as the Agency conducted its "lessons learnt" exercise. Since then, experience has also been gained through requests for CHMP scientific advice and the processing of Marketing Authorisation Applications for influenza vaccines.

As announced in a concept paper published in September 2011[1], the revision of guidelines on influenza vaccines is intended to appear under the form of a single guideline, developed according to a modular approach i.e. with distinct Modules covering each relevant topic.

The present Module compiles the quality requirements for the different types of influenza vaccines, in line with the scope described under section 2. A Separate Module for the non-clinical and clinical requirements is intended to be published at a later stage.

It should be noted that the present Module may be subject to changes as regards its structure, due to its interdependency with the other Modules under development.

2. Scope

The guideline provides guidance on Marketing Authorisation Applications for influenza vaccines based on the types for which ample experience has been gained during seasonal vaccination campaigns as well as the 2009 H1N1 pandemic, namely inactivated non-adjuvanted vaccine and inactivated vaccine with squalene based adjuvant to be used in the seasonal, pre-pandemic or pandemic setting and live attenuated seasonal influenza vaccines, and for strain updates of authorised influenza vaccines.

Many elements of this guideline will, however, be applicable to novel types of inactivated vaccine (for example those based on alternative vaccine antigens) or for novel constructs of live attenuated influenza vaccines.

Other vaccine concepts such as recombinant constructs combining many different influenza virus epitopes in a single expression product, or influenza vaccines based on nucleic acids are not covered by this guideline.

Applicants are encouraged to seek individual scientific advice when developing a medicinal product.

3. Legal basis and relevant guidelines

This guideline should be read in conjunction with the Annex I to Directive 2001/83/EC. Applicants should also refer to other relevant European and ICH guidelines and European Pharmacopoeia Monographs and Chapters.

[1] Concept paper on the revision of guidelines for influenza vaccines (EMA/CHMP/VWP/734330/2011)

4. Quality requirements for influenza vaccines

4.1. Inactivated influenza vaccines

4.1.1. Seasonal influenza vaccines

4.1.1.1. Marketing Authorisation application for a seasonal influenza vaccine

Seasonal influenza vaccines are produced either in embryonated hens' eggs or on a cell substrate. Seasonal influenza vaccines shall be compliant with the Ph. Eur. monograph for Vaccines for human use[i] and the relevant Ph. Eur. monographs for inactivated egg-derived[ii] and inactivated influenza vaccines produced in cell cultures[iii], as appropriate.

Data deriving from multiple strains should be used to develop a knowledge database which could be useful to describe in more detail, quality requirements following strain-specific adaptations to the manufacturing process during seasonal updates.

4.1.1.1.1. Candidate Vaccine Virus

Definition

Candidate vaccine viruses (CVV) represent influenza strains recommended by WHO/CHMP and which are suitable for seasonal vaccine production. They are supplied by a WHO Collaborative Centre (CC), a WHO Essential Regulatory Laboratory (ERL) or an otherwise approved laboratory to influenza vaccine manufacturers for establishment of their seed lots. They are characterised antigenically, genetically and phenotypically. It is the responsibility of the vaccine manufacturer to establish the suitability of a CVV for vaccine production and to establish a vaccine seed lot, in line with EU recommendations for seasonal influenza vaccine composition.

Candidate Vaccine Virus – development

Viruses to be used in vaccine manufacture may be isolated in one of the following substrates:

- embryonated hens' eggs
- cells derived from embryonated hens' eggs
- mammalian cells (see also Annex 1)

The CVV is likely to be one of the following:

- A high yielding reassortant virus generated by classical reassorting. The virus contains the haemagglutinin (HA) and neuraminidase (NA) genome segments of the WHO recommended strain and one or more of the remaining genome segments from the high yielding donor strain PR8. The genome constellation is serendipitous and is defined as, for example, 5:3 where the first number refers to the number of genome segments from PR8 and the second from the recommended wild type virus. At minimum, the reassortant must have the HA and NA from the wild type strain and will have been shown to be antigenically similar to the WHO recommended strain by a WHO CC.
- A reassortant derived by reverse genetics. These are constructed with a defined genome constellation and typically would contain the HA and NA genome segments from the wild type strain and the remaining six genome segments from PR8; alternative combinations or modifications of genome segments could be constructed. As above the CVV will have been shown to be antigenically unchanged from the recommended wild type virus by a WHO CC.

- A non-reassortant wild-type influenza virus.

Candidate Vaccine Virus - Quality and control

In accordance with the Ph. Eur. monographs for egg-derived inactivated influenza vaccines and cell culture derived inactivated influenza vaccines, the origin and passage history of virus strains shall be approved by the competent authority.

A general description of the vaccine virus development (seed lot history, passage level) should be provided.

Where the preparation of the CVV involves reverse genetics, there are quality considerations as a result of genetic modification(s) and derivation in animal cells beyond those for classical reassortants (see 4.1.2.1.1)

Detailed laboratory records are maintained. The laboratory records should include documentation that no other influenza viruses or their genetic material are handled at the same time as the rescue work in order to avoid cross contamination.

If a manufacturer develops its own reassortant from a wild type virus, appropriate tests on the reassortant should be performed and reported including a demonstration of its antigenic similarity, or of the subsequent seed lot, to the WHO/CHMP recommended strain by a WHO CC.

4.1.1.1.2. Vaccine seed lots

Production

A vaccine seed lot system should be employed. The vaccine seed lots should be prepared in SPF embryonated hens' eggs or on a qualified cell line, as specified by the Ph. Eur[2]. Such a seed lot system is likely to be based on a CVV issued by a WHO ERL (see 4.1.1.1.1).

Qualification

The HA and NA antigens of each seed lot are identified by suitable methods. Usually, specific antisera obtained from a WHO Collaborating Centre for Influenza are used for determination of HA and NA identity. In the event that reagents are not available, alternative tests to identify the seed virus (e.g. PCR) should be developed; however, antigenic confirmatory tests for identity are the preferred option.

Testing for extraneous agents

The seed virus shall be tested for freedom from extraneous agents according to the Ph. Eur. monographs for egg-derived inactivated influenza vaccines and cell culture derived inactivated influenza vaccine, as appropriate. It remains possible that reagents and substrates, which are of animal origin and are used for the development of the vaccine seed lot, could pose a viral safety risk.

Whilst the details of the extraneous risk evaluation for viral seeds prepared either using fertilised hens' eggs or cell cultures and originating from classical or reverse genetics techniques may be different, a risk assessment should be made.

Such a risk assessment could include:

- information about (new/emerging) viruses that could potentially be present in clinical specimen/isolates used for production of the CVV. Pathogens to be considered could include

[2] Influenza virus used in the preparation of seed lots is propagated in fertilised eggs from chicken flocks free from specified pathogens (SPF) (Ph. Eur. 5.2.2) or in suitable cell cultures (Ph. Eur. 5.2.3), such as chick-embryo fibroblasts, chick kidney cells obtained from SPF chicken flocks (Ph. Eur. 5.2.2), or a diploid or continuous cell line.

respiratory syncytial virus, adenovirus, parainfluenza virus, coronavirus, rhinovirus, enterovirus, EBV, HSV, CMV and mycoplasmas.

- susceptibility of the production substrate and of the substrate used to isolate the strain
- viral safety risks associated with the use of raw materials, reagents and substrates of animal origin used during the preparation of the seed lot
- historical records of virus seed extraneous agents testing

This risk assessment should be the basis for setting vaccine seed lot specifications for extraneous agents testing.

If the substrate proves to be susceptible to a contaminating agent detected in the seed, the seed is not acceptable. If the substrate is not susceptible to a detected contaminating agent, steps should be in place to ensure that the contaminating agent in the working seed is removed and/or inactivated by the production process.

As there may be time constraints for the strain change[12], manufacturers of influenza vaccines are encouraged to develop assays for potential contaminating human pathogens, e.g. multiplex PCR, which could be applied effectively within the time constraints of annual vaccine manufacturing, notwithstanding the obligation to complete the testing according to the Ph. Eur. monograph on extraneous agents.

In addition to seed lot testing for extraneous agents, appropriate and specific downstream testing at the level of each inactivated monovalent bulk as well as process validation should ensure that the removal and/or inactivation processes are effective and that any contaminant which may subsequently be identified in the seed virus is absent from the vaccine.

4.1.1.1.3. Substrate for vaccine virus manufacture

For routine production, the virus of each strain is propagated in the allantoic cavity of fertilised hens' eggs from healthy flocks or in a diploid or continuous cell line[iv].

Testing for extraneous agents

In accordance with current requirements on cell substrates[v,v,vi] and in addition to the general testing for extraneous agents, the cell substrate used for production of monovalent bulks should be tested for relevant extraneous agents, such as those specific to the species of origin of the cells and those which may have been introduced from biological reagents used during establishment of the cell banks.

4.1.1.1.4. Manufacturing development

The manufacturing development should be detailed in the dossier. The process may have to be tailored / technically adapted annually due to specific strain characteristics to fulfil the requirements for vaccine production. Efforts should be made to gain enhanced process and product knowledge based on historical production experience and state-of the-art process and product characterisation studies. This may allow better prediction of the potential impact of process changes on product quality. Experience with multiple strains could be used to build a knowledge database to provide insight into strain-specific process adaptations during annual strain variation that will ensure quality attributes are unaffected.

4.1.1.1.5. Process validation

Process validation data should be generated to demonstrate that critical processes, operated within established parameters, can perform effectively and reproducibly to produce a medicinal product

meeting its predetermined specifications and quality attributes. The inactivation of the vaccine virus is considered to be a critical process. A Ph. Eur. requirement of inactivated influenza vaccines is that the inactivation process is shown to be capable of inactivating the influenza vaccine virus without destroying its antigenicity; the process should cause minimum alteration of the HA and NA antigens. Inactivation kinetics studies should be carried out using material from normally three commercial scale production batches. Where justified, material from pilot scale batches may be used e.g. where equivalence between the pilot and commercial process is demonstrated. Consistency of the inactivation process should be demonstrated.

For split or subunit vaccines, the splitting of the influenza virus is also considered a critical process step which needs to be included in the process validation programme. Splitting efficiency should be demonstrated using suitable analytical methods (e.g. SDS-PAGE, isopycnic gradient ultracentrifugation analysis of pre-/post-splitting process samples). Process validation data should be submitted for at least three consecutive batches.

The inactivation process shall have been shown to be capable of inactivating avian leucosis virus and mycoplasmas. Where the conditions of inactivation have been modified, the impact on the inactivation capacity for avian leucosis and mycoplasma should be discussed.

The influenza vaccine inactivation step, along with any other steps considered to contribute to virus inactivation/removal should be evaluated for the inactivation/removal of a range of potential contaminants of the vaccine seeds in accordance with existing guidance on virus validation[vii,viii]. The need for such studies should be based on a risk assessment as outlined in section 4.1.1.1.2.

Tests for the effectiveness of vaccine virus inactivation may be performed using the cell substrate or any other cell system provided that there is adequate validation of this test for sensitivity.

The occurrence of protein aggregation either in the Drug Substance or Drug Product should be evaluated. If this would occur, information should be provided about the root cause (strain specific characteristic, specific process step transport, shaking stress, temperature) of this aggregate formation as well as an appropriate control strategy. Considerations should be given to the safety and immunogenicity of a formulation containing such particles.

4.1.1.1.6. Characterisation

While it is appreciated that certain characteristics may be strain specific, extended characterisation studies can contribute to an enhanced process and product understanding and may provide information about product consistency from one season to another. This enhanced product knowledge may allow relevant specifications to be established and may support the scientific evaluation of comparability after product or process changes have been introduced.

The kind of characterisation studies needed will depend on the nature of the vaccine, e.g. whole virion, split virion or subunit.

The components, i.e. active substance(s) and process related impurities, contained in the drug substance should be investigated and characterised as appropriate.

The biological, immunological and physicochemical properties of the HA antigen should be verified using a wide range of state-of-the-art analytical methods[3]. As required by Ph. Eur., the presence and type of NA antigen should be confirmed by suitable enzymatic or immunological methods on the

[3] The following analytical methods have been described for chemical, physical and biological characterization of HA antigens: HA titre, HI, Western Blot, epitope scanning, immunogenicity in mice, ferret challenge, SDS-PAGE, MALDI/MS, HPLC, transmission electron microscopy, isopycnic gradient ultracentrifugation, dynamic light scattering, tryptic peptide mapping, amino acid sequencing.

monovalent pooled harvests. Considerations should be given to characterise in nature and quantity the relevant antigens as far as technically feasible.

New analytical technology and modifications to existing technology are continually being developed and should be utilised when appropriate.

Where present, aggregates should be investigated, e.g. in terms of diameter, composition, content and dissolution profile. Considerations should be given to the safety and immunogenicity of a formulation containing such particles.

Process related impurities (e.g. ovalbumin / host cell protein, residual host cell DNA), downstream-derived impurities such as reagents used for inactivation/splitting) should be identified, quantified and data used to set release specifications.

4.1.1.1.7. Presentation

Where there is a need for a preparation tailored to a specific target population, its introduction should be supported by appropriate quality data, including but not limited to compatibility, process manufacturing validation and stability data.

4.1.1.1.8. Vaccine standardisation

Quantification of HA by the immunological SRD assay is currently the internationally recognised method to measure the potency of inactivated influenza vaccines. There is not an exact correlate between vaccine potency and clinical outcome, as this will depend on the nature of the vaccine (e.g. whole vs. split vs. subunit vaccine), its formulation (e.g. the presence or not of adjuvant), differing manufacturing processes, route of administration, the match of the vaccine with the disease-causing strain, the absence of proper dose response studies, variability of serological assays and a lack of knowledge on the true serological correlates of protection. Rather, the intent of the SRD assay is to assure a consistent HA antigen content and antigenicity. For an inactivated seasonal vaccine, the current international consensus for a vaccine dose is 15 SRD µg of HA antigen.

From that perspective, information on the quality characteristics, such as the amount, antigenicity, of the relevant influenza antigens and their formulation (e.g. adjuvanted or not) needs to be improved and the significance of these on immunogenicity, efficacy and safety needs to be understood and controlled.

The SRD method requires strain-specific reagents and the timing of their availability may impact on the availability of the early batches of vaccine. The need for the use of alternative assays (ELISA, HPLC etc) that can be applied prior to the availability of SRD reagents, is recognised and encouraged.

Therefore, vaccine manufacturers are encouraged to investigate alternative methods and also improved SRD potency assays in collaboration with regulatory and academic laboratories. Specific attention should be given to those methods providing a biologically relevant potency measure (functionally active protein) and/or which can be stability-indicating. Ideally, potency values obtained with an alternative assay should correspond to those measured in the SRD test but it is accepted that a complete calibration across methods may not be achievable, especially for all strains. This can be the case for HA quantification assays that are limited to measuring physical properties of the HA and that cannot unequivocally confirm antigenicity or immunogenicity. Therefore, a manufacturer will be expected to justify and validate the use of such techniques for in-process control testing, release testing (before/after SRD reagents are available) and/or as stability indicating tests. This is especially so where separate methods for identity testing, on one hand, and for IPC/release assay/stability assays, on the other, may be required. The strategy for the use of an antibody-independent alternative

assay should take into consideration how immunogenicity of the antigen (e.g. specificity, antigenicity) and continued consistency of immunogenicity between production lots will be assured.

Thus, the validity, usefulness and applicability of alternative assays could be further demonstrated by process validation and characterisation studies. If the alternate assay is intended to be used for release, then the method should be validated against SRD based on the experience gained with these assays using multiple strains.

These considerations are important factors when deciding on the strategy of use of an alternative assay once SRD reagents become available.

There is preliminary evidence that it is possible to use current 'egg-derived' SRD standards for cell culture vaccines manufactured using a virus seed derived and passaged in eggsix. However, in future, problems may arise if a vaccine strain is isolated, passaged and produced on a mammalian cell substrate, as this is a process that can select for viruses that are antigenically distinct. Studies to evaluate the need for "cell-derived" standards by using them in parallel with "egg-derived" SRD standards (antigens and antisera) are in progress. At least one 'cell-derived' reagent has been made available by a WHO ERL.

4.1.1.1.9. Adjuvants

Where an adjuvant system is used, reference for the quality aspects is be made to the CHMP Guideline on Adjuvants in Vaccines for Human Use[x]. The dossier should contain detailed information on the adjuvant on the origin of starting materials, its production process, physical and chemical characterisation, control testing, stability testing, and on the interaction between the vaccine antigen and adjuvant.

The development of manufacture of the adjuvant system should be detailed.

Where appropriate, detailed information should be provided on extemporaneous mixing of adjuvant with antigen. The effect of mixing time and conditions on the essential characteristics of the antigen/adjuvant combination should be considered. The proposed in-use shelf-life should be appropriately validated. Considerations should be made on issues related to multiple puncture of stoppers for multidose containers (i.e. issue of coring, appropriate gauge and length of needles to be used for withdrawal and administration). The description of appearance of each component should be adequately detailed and information given relating to acceptability of use if particles/aggregates are observed. Filling overages are likely to be used in each container – these should be adequately justified and taken into account in mixing/administration instructions.

4.1.1.1.10. Stability / Shelf life

Stability data for the influenza vaccine should be developed as described in Ph. Eur monograph of Vaccines for Human Use and ICH Q5C[xi]. Stability data, comprising studies under real-time and accelerated conditions, should be presented for drug substance (monovalent bulk) and drug product (final formulated vaccine) in support of the maximum storage time and shelf life, respectively.

A protocol for testing vaccine stability should be developed. The procedure for assignment of a shelf life / expiry date should be outlined and justified. In accordance with the core SmPC for Trivalent Influenza Vaccines[xii], the drug product shelf life should not exceed one year to avoid confusion between vaccine composition of subsequent vaccination campaigns. However, data covering extended time periods and under accelerated conditions may provide useful information about the vaccine's stability profile, if the testing frequency is sufficient to allow conclusions to be drawn.

4.1.1.2. 'Annual update' application for a seasonal vaccine

IMPORTANT REMARK

Please note that only relevant and adequate sections of the CTD variation application should be submitted. All sections not felt to be necessary should however be justified adequately in the Summary/Overview.

4.1.1.2.1. First step submission – "Quality" Variation Application

MAHs shall submit a Type II variation application containing the adequate quality documentation in accordance with Article 18 of Commission Regulation (EC) No 1234/2008, by the Agency recommended target annual deadline, which will be published every year together with the EU Annual strain(s) recommendations.

4.1.1.2.1.1. Candidate Vacccine Virus Quality and control

The guidance provided in section 4.1.1.1.1 applies.

4.1.1.2.1.2. Vaccine seed lots

The guidance provided in 4.1.1.1.2 applies. Where the seed virus is tested for extraneous agents using PCR, and if further to discussion with the Agency and Rapporteurs the need for additional PCR testing of the seed has been agreed, these data should be included in the application (3.2.S.2.3)

4.1.1.2.1.3. Manufacturing development

Any optimization in the vaccine production process (within the limits of the core dossier) needed due to specific strain characteristics should be clearly indicated and justified (3.2.S.2).

The formulation development (actual formula with new season's strains to be provided in 3.2.P.3.2)) and vaccine composition (3.2.P.1) and should be provided and the Certificate of Analysis of batch(es) used in clinical trial(s) when available (either in quality or in clinical submission) (3.2.P.2.2.1).

4.1.1.2.1.4. Process validation / process consistency

Critical manufacturing steps should be re-evaluated for the newly strain(s). Adequate inactivation and, as appropriate, the splitting efficiency should be demonstrated (3.2.S.2.5).

Batch analysis results of the first three monovalent bulks (including test for neuraminidase) should be presented from each working seed lot of a new master seed lot of new strains or from each working seed lot derived from a previously approved master seed lot where the procedure of working seed lot preparation is different from the approved procedure (3.2.S.4.4)

4.1.1.2.1.5. Vaccine standardisation

Validation of analytical procedures should be shown where they are potentially impacted by the strain change(s), e.g. validation of the SRD test. Validation data are expected for the monovalent bulk (3.2.S.4.3), as well as trivalent bulk or drug product (3.2.P.5.3).

A copy of the approved specifications for the monovalent bulk(s) (3.2.S.4.1) and drug product (3.2.P.5.1) as well as an overview of the analytical procedures (3.2.S.4.2) should be presented in tabular format.

4.1.1.2.1.6. Stability / Shelf life

Stability studies for monovalent bulks under real-time and accelerated storage conditions should support the claimed maximum storage time and may provide further evidence of vaccine quality between different vaccine strains. Therefore, it is recommended to perform such studies for each new vaccine strain to build up the vaccine quality database. In any case, stability test results from monovalent bulks should be presented where they are used for more than one year (3.2.S.7)

For the drug product, stability data from the previous season should be submitted as well as a stability commitment to put a number of drug product lots on a stability program detailed in a post-approval stability protocol (3.2.P.8).

4.1.1.2.2. Second step submission – "clinical" Variation Application

Where applicable, an updated Quality Overall Summary should be submitted.

4.1.2. Pre-pandemic influenza vaccines

4.1.2.1. Marketing Authorisation application for a pre-pandemic influenza vaccine

4.1.2.1.1. Candidate Vaccine Virus

The guidance provided in section 4.1.1.1.1 applies.

The choice of strain should be justified by the applicant. For example, reference is made to the information published by WHO on Antigenic and genetic characteristics of A(H5N1), A(H7N3), A(H9N2) and variant influenza viruses and candidate vaccine viruses developed for potential use in human vaccines[xiii].

When the CVV is derived from a highly-pathogenic H5 or H7 subtype, *in vitro* and *in vivo* testing should have demonstrated elimination of the high pathogenicity phenotype[4] (see Annex 2 and also in Non-clinical section).'

The vaccine CVV for the pandemic dossier is likely to be derived from an avian, porcine or human source by one of the procedures outlined in section 4.1.1.1.1. A non-reassortant wild-type influenza virus (highly pathogenic or low pathogenic) can be used.

Examples of CVV suitable for use as vaccine strains:

An H5N1 reassortant derived from a highly pathogenic strain by reverse genetics; the WHO website provides a list of available H5N1 CVVs. In view of the continued prevalence of human H5 virus infections, such a choice has the advantage of being a potential pandemic strain and being produced by reverse genetics, the most likely method of pandemic CVV development from a highly pathogenic H5 or H7 subtype.

An H7N1 reassortant derived from a highly pathogenic avian virus by reverse genetics. H7N1 and H7N7 viruses have been associated with European poultry outbreaks in recent years and H7N7 viruses have been associated with human infections.

An H5N3 avian virus. Vaccines produced from the H5N3 strain A/Duck/Singapore/97 have already been tested clinically. This strain is antigenically close to the highly pathogenic H5N1 strain, A/Hong Kong/156/97. Other low-pathogenic H5 virus subtypes could be considered.

An H9N2 virus. Human H9N2 viruses such as A/Hong Kong/1073/99 have already been used for experimental vaccine production and have been tested clinically. Sporadic H9N2 infections in humans continue to be reported and there is preliminary evidence that individuals born before 1968 may have residual immunity that enhances H9N2 vaccine immunogenicity. Consideration should therefore be given to clinical trials of H9N2 vaccines that are stratified by age.

An H2N2 human virus. A/Singapore/1/57 is the 1957 pandemic strain and has been recently used for experimental vaccine production. Clinical trials of H2N2 vaccine should take account of residual immunity in persons born before 1968.

Where the preparation of the CVV involves reverse genetics, there are quality considerations beyond those involved with seasonal vaccine production.

Reverse genetics requires the use of mammalian cells for development of a CVV and this imposes additional requirements to assure the safety and quality of the product. The use of mammalian cells

[4] The virus will be tested for pathogenicity in chickens and ferrets according to protocols approved by the OIE (www.oie.int) and WHO respectively.

for the development of CVV by reverse genetics requires the following minimum set of parameters to be met:

- The cell substrate used to develop the CVV 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. A bank of cells approved for human vaccine production will be suitable for use for this purpose.
- Materials used in generating a CVV via reverse genetics process must be compliant with the current version of the Transmissible Spongiform Encephalopathy Note for Guidancexiv.
- Materials used in generating a CVV via reverse genetics process may affect the safety of the vaccine in terms of viral, bacterial, fungal and mycoplasma contamination. Potential safety risks associated with these materials should be taken into account in the applicant's overall safety evaluation for the vaccine.

4.1.2.1.2. Vaccine seed lots

The guidance provided in section 4.1.1.1.2 applies.

Where the seed virus has been genetically modified to remove the highly pathogenic trait of specific H5 and H7 viruses, or where the seed virus derives for a low pathogenicity H5 or H7 virus, the sequence of the HA at the cleavage site from virus comprising the seed lot should be verified and compared to that of the CVV to confirm maintenance of the low pathogenic trait (i.e. absence of the polybasic amino acid stretch at the HA cleavage site). This should also be performed at the passage level representing the final vaccine for three batches. Collaboration between manufacturers and WHO/ERLs, OMCLs and/or qualified national reference centres is encouraged wherever possible to characterise the seed lot on a mutual basis (e.g. identity, titre, molecular/genetic characterisation).

4.1.2.1.3. Substrate for virus propagation

Guidance provided in section 4.1.1.1.3 applies.

4.1.2.1.4. Manufacture development

The manufacturing process for pre-pandemic influenza vaccine could be based either on an established and licensed process (e.g. seasonal vaccine) or on a newly designed process. In any case, the manufacturing development should be detailed in a self-standing dossier, but it is expected that the data requirements for any newly designed process would be more extensive. The process may have to be tailored / technically adapted to fulfil the requirements for vaccine production in a pre-pandemic situation. These adaptations should be fully explored and validated, as appropriate. Efforts should be made to gain an enhanced product and process knowledge based on historical production experience and state-of the-art process and product characterisation studies. The potential impact of process changes on product quality should be verified in terms of Critical Quality Attributes based on (pre-) pandemic and seasonal vaccine development studies. Experience with multiple pandemic and seasonal strains could be used to build a knowledge database that might be useful to describe in more detail quality requirements following strain-specific adaptations during pre-pandemic strain variation.

4.1.2.1.5. Process validation

The guidance provided in section 4.1.1.1.5 applies.

4.1.2.1.6. Characterisation

The guidance provided in section 4.1.1.1.6 applies.

4.1.2.1.7. Presentation

Pre-pandemic influenza vaccines can be presented either as multi-dose or single dose preparations.

For multidose preparations, the need for an effective antimicrobial preservative should be evaluated, taking into account possible contamination during use and the maximum recommended period after first use (in-use shelf life). The use of a preservative may allow for maximal use of the doses within a multi-dose presentation by maximising the in-use shelf life.

Tests for the antimicrobial preservative should be included for the bulk vaccine if appropriate. The applicant should investigate the possible interference of the antimicrobial preservative with other tests.

If the influenza vaccine contains Thiomersal as a preservative, the applicant should justify the final Thiomersal content of the vaccine, in line with the established CHMP guidance[xv].

The proposed in-use shelf-life should be appropriately validated. Considerations should be made on issues related to multiple puncture of stoppers for multidose containers (i.e. issue of coring, appropriate gauge and length of needles to be used for withdrawal and administration).

Where there is a need for a preparation tailored to a specific target population, such a preparation (e.g. a paediatric presentation containing a half-dose of antigen with a full dose of adjuvant, or any other combination) may be different in terms of approved formulation and primary container. Its introduction should be supported by appropriate quality data, including but not limited to compatibility, process manufacturing validation and stability data.

4.1.2.1.8. Vaccine standardisation

In general, the guidance provided in section 4.1.1.1.8 applies.

Special emphasis should be placed on accurate determination of low quantities of HA antigen since a Pre-pandemic influenza vaccine might contain a significantly lower quantity of HA compared to seasonal vaccines.

4.1.2.1.9. Adjuvants

Where an adjuvant system is used, reference for the quality aspects is made to the CHMP Guideline on Adjuvants in Vaccines for Human Use[xi]. The dossier should contain detailed information on the adjuvant on the origin of starting materials, its production process, physical and chemical characterisation, control testing, stability testing, and on the interaction between the vaccine antigen and adjuvant.

The development of manufacture of the adjuvant system should be detailed.

Where appropriate, detailed information should be provided on extemporaneous mixing of adjuvant with antigen. The effect of mixing time and conditions on the essential characteristics of the antigen/adjuvant combination should be considered.

The description of appearance of each component should be adequately detailed and give information related to acceptability of use if particles/aggregates are observed. Filling overages are likely to be used in each container – these should be adequately justified and taken into account in mixing/administration instructions.

4.1.2.1.10. Stability / Shelf life

Stability data for the influenza vaccine should be developed as described in Ph. Eur monograph of Vaccines for Human Use (01/2009:0153) and ICH Q5C. Stability data, comprising studies under real-

time and accelerated conditions, should be presented for drug substance (monovalent bulk) and drug product (final formulated vaccine) in support of the maximum storage time and shelf life, respectively.

A minimum of 6 months real time stability data need to be included in the application. Any extension of the shelf life up to one year should be based on real-time stability data.

Vaccine components (e.g. bulk antigen and adjuvant) might be stored separately.

In-use stability testing

Stability and characterisation studies should be presented to support the in-use shelf life as claimed in the SmPC. State-of-the-art methods should be used to demonstrate that the vaccine meets the specifications for critical quality attributes (such as stability indicating and microbial parameters) after formulation. The in-use stability studies should reflect a worst-case scenario that includes real or simulated withdrawing of doses from a multi-dose vial such that the vial is punctured a number of times corresponding to the maximum number of withdrawals under normal environmental conditions.

Useful information is provided in the CHMP Note for Guidance on in-use stability testing for human medicinal products[xvi] and CHMP Note for guidance on maximum shelf-life for sterile products for human use after first opening or following reconstitution[xvii].

4.1.2.2. Strain update of a pre-pandemic influenza vaccine

It is possible that MAHs might wish to propose replacement of the strain in an approved vaccine. For example, this might occur if sequential studies show low or negligible cross-reactivity and crossprotection to drift variants and/or if expert opinion suggests alternative HA subtypes of influenza virus most likely to trigger a pandemic. Two scenarios could occur and have different regulatory implications as follows:

a. Replacement of the strain in the approved vaccine with a different strain of the same subtype (e.g. supplanting the original H5N1 with another H5N1 strain). In this case the MAH would have to submit all manufacturing and quality data related to the new strain. The information required would resemble the information needed for an annual update' application for seasonal vaccine (section 4.1.1.2)

b. Replacement of the HA/NA subtype of strain (e.g. supplanting an original H5N1 strain with an H7N7 strain). Advice from EU competent authorities should be sought on the regulatory framework and data requirements for such a change as additional non-clinical and clinical data may be required.

4.1.3. Pandemic influenza vaccines

As for seasonal influenza vaccines, most pandemic influenza vaccines will be produced in either embryonated hens' eggs or on a cell substrate. An Influenza vaccine intended to mimic a pandemic "mock-up" vaccine and a pandemic vaccine itself shall be compliant with the relevant Ph. Eur. monographs for egg-derived and cell derived inactivated influenza vaccines, as appropriate. For testing for freedom from extraneous agents of the seed virus for the pandemic vaccine (viruses, mycoplasma, bacteria and fungi) alternative approaches may have to be undertaken in view of time constraints.

4.1.3.1. Marketing authorisation granted under the 'Mock-up' procedure

4.1.3.1.1. First phase: 'Mock up' application

4.1.3.1.1.1. Candidate Vaccine Virus

The guidance provided in sections 4.1.1.1.1 and 4.1.2.1.1 applies.

4.1.3.1.1.2. Vaccine seed lots

The guidance provided in sections 4.1.1.1.2 and 4.1.2.1.2 applies.

4.1.3.1.1.3. Substrate for virus propagation

The guidance provided in section 4.1.1.1.3 applies.

4.1.3.1.1.4. Vaccine Production

Manufacturing development

The guidance in 4.1.2.1.4 applies.

4.1.3.1.1.5. Process validation

The guidance provided in section 4.1.1.1.5 applies.

4.1.3.1.1.6. Characterisation

The guidance provided in section 4.1.1.1.6 applies.

4.1.3.1.1.7. Presentation

Pandemic vaccines can be presented either as multi-dose or single dose preparations.

The guidance provided in section 4.1.2.1.7 applies.

4.1.3.1.1.8. Vaccine standardisation

The guidance provided in sections 4.1.1.1.8 and 4.1.2.1.8 applies.

4.1.3.1.1.9. Adjuvants

The guidance provided in section 4.1.2.1.9 applies.

4.1.3.1.1.10. Stability / Shelf life

The guidance provided in section 4.1.2.1.10 applies.

A protocol for testing pandemic vaccine stability should be developed. The procedure for assignment of a shelf life / expiry date for the pandemic vaccine and its intermediates should be outlined and justified. In principle, an extension of the shelf life should be based on real-time stability data

In-use stability testing

The guidance provided in section 4.1.2.1.10 applies.

4.1.3.1.2. Second phase: Pandemic strain update

4.1.3.1.2.1. Candidate Vaccine Virus

The guidance provided in sections 4.1.1.1.1 and 4.1.2.1.1 applies.

It is acknowledged that full information as described in sections 4.1.1.1.1 and 4.1.2.1.1 may not necessarily be available at the time of the variation application to introduce the pandemic strain, but this should be provided within the shortest possible time lines. Where information is not available for materials which have a potential safety risk, a risk assessment for the vaccine should be provided taking into account the control strategy applied and production process characteristics.

4.1.3.1.2.2. Vaccine seed lots

The guidance provided in sections 4.1.1.1.2 and 4.1.2.1.2 applies.

Until specific antisera are available from a WHO Collaborating Centre, alternative tests to confirm the identity of the seed virus (e.g. PCR) as developed for the mock-up vaccine, shall be used. When such reagents become available, HI tests should be used to confirm the identity of the seed virus.

Any change to the vaccine virus seed introduced during the pandemic vaccine production campaign, e.g. a change to more productive reassortant virus, or additional passaging of the same production strain seed, should be justified. A summary of the accompanying data is shown in Table 1.

Table 1: Accompanying data to support change to the vaccine virus seed introduced during pandemic vaccine production campaign

Stage of production	Data requirements
Seed virus	Confirmation to specifications, e.g. identity (HI, NAI). For RG strains, seeds developed with an additional passage should be confirmed as possessing the same genetic sequence of the gene to the Master Seed and of reference virus stain received from the WHO CC to ensure safety (and immunogenicity) remains intact.
Harvest (pool)	HA antigen yield
Drug Substance (monovalent pool)	Process validation of critical steps which are shown to be strain specific Characterisation data Batch analyses; first three monobulks derived from a new working seed should be tested for the presence and type of NA antigen. Stability data in support of the claimed holding period (time lines to be indicated in case full data set not yet available)
Drug Product	Batch analyses Stability in support of the claimed shelf life (time lines to be indicated in case full data set not yet available)

Any differences seen in comparability studies (i.e. in yield, content of residuals e.g. sodium deoxycholate) should be critically discussed and supported (safety, immunogenicity) based on the manufacturer's prior production experience with influenza vaccines. The extent of the differences seen in strain characteristics between seeds should form the basis for the extent of additional data requirements (i.e. inactivation studies).

4.1.3.1.2.3. Vaccine production

The guidance provided in section 4.1.2.1.4 applies.

Amendments to the production process (e.g. to improve yield, scale up), should be detailed, justified and validated.

In case human immunogenicity data are not (yet) available, appropriate immunogenicity data in animals on at least one batch should support the change of the vaccine virus from the mock-up to the pandemic vaccine (see also the Non-clinical section).

4.1.3.1.2.4. Process validation

The guidance provided in section 4.1.1.1.5 applies.

4.1.3.1.2.5. Characterisation

The guidance provided in section 4.1.1.1.6 applies.

It is not expected that full comparability of the pandemic vaccine with the mock-up vaccine is established. Nevertheless, the Critical Quality Attributes of the pandemic vaccine should be compared to the mock-up vaccine. Any differences should be discussed in terms of safety and immunogenicity.

4.1.3.1.2.6. Vaccine standardisation

Depending on the results from the clinical trials with the mock-up vaccine, a pandemic vaccine may contain a different quantity of HA than the 15 µg per strain contained in the seasonal influenza vaccine.

Any alternative tests for vaccine potency, validated for the mock-up vaccine, should be used as long as SRD reagents are not available. When SRD reagents become available, they shall be used for potency testing.

4.1.3.1.2.7. Adjuvants

The guidance provided in section 4.1.1.1.9 applies.

4.1.3.1.2.8. Stability/ Shelf life

Vaccine stability testing for drug substance and drug product is to be performed according to the protocol in the mock-up dossier. A shelf life and storage conditions for the pandemic vaccine should be proposed. It is acknowledged that full real time data from commercial lots may not be available at the time of submission of the pandemic variation application. Supporting stability data may be used from small-scale development and commercial batches if these materials are representative of the final full scale manufacturing process. The shelf life claim may be supported by comparison of available real time and accelerated stability data for the pandemic strain and mock-up strain.

Out of specification results or significant trending result in any the quality attribute are to be reported to the authorities.

In principle, an extension of the shelf life should be based on real-time stability data.

In-use stability testing

Vaccine in-use stability testing is to be performed according to the protocol in the mock-up dossier to support the claimed utilisation period for the pandemic vaccine after mixing of antigen and adjuvant system preparations.

4.1.3.2. Marketing authorisation granted under the 'Emergency' procedure

Essentially, the guidance provided in section 4.1.3.1 applies albeit that the data should concern the declared pandemic strain.

4.2. Live attenuated influenza vaccines

Live attenuated influenza vaccines are produced in fertilised hens' eggs under appropriate conditions defined by the phenotypic characteristics of the attenuated master strain. In contrast to currently available inactivated influenza vaccines which are purified, very limited downstream processing can be performed on live vaccines. Therefore, only high quality starting materials tested or certified for compliance with all regulations pertinent to the manufacture of vaccines for human use should be used during production in order to eliminate any potential source of contamination that might affect the final vaccine. In particular, these issues relate to the egg substrate, to the attenuated parent strain, to the donor strain of the relevant haemagglutinin (HA) and neuraminidase (NA) and to the resulting attenuated reassortant. Influenza vaccines are updated periodically to include new influenza strains. Whenever this occurs, there are severe constraints on the time available for quality tests.

4.2.1. Seasonal vaccines

4.2.1.1. Marketing Authorisation application for a seasonal vaccine

4.2.1.1.1. Live attenuated parent strain

4.2.1.1.1.1. Development

Detailed documentation is requested on the history of all biological agents used, i.e. viruses and cells, the method of preparation and a complete list of all starting materials used for construction of the attenuated parent strain.

4.2.1.1.1.2. Characterisation

Phenotypic and genetic properties of the attenuated parent strain need intensive investigation and should be documented in detail in the dossier.

The phenotypic characterisation includes studies on the markers for attenuation as, for example, on the cold-adapted (ca) or temperature sensitive (ts) phenotype, performed in vivo and/or in vitro under conditions allowing the detection of revertants to wild type, or reassortant strain with wild virus. The absence of any neurovirulent capacity of the attenuated parent strain should be demonstrated. These studies should be performed in appropriate animal models and cover issues raised by the potential direct neurovirulence caused by the vaccine virus strains themselves or on the potential indirect neurovirulence due to secondary infections. The relevant guidance[xviii] applies. The suitability of small animal species for the test of neurovirulence should be explored[xix].

The genetic characterisation of the attenuated parent strain includes (i) determination of the nucleotide sequence of the complete viral genome, (ii) reports on the analysis of the molecular basis of the attenuated phenotype and (iii) demonstration of the genetic stability of the attenuated parent strain by comparison of the nucleotide sequence of the viral genome at different passage levels.

In particular, attention should be paid to the testing for extraneous agents. These tests are performed according to the relevant requirements and guidelines on extraneous agents of viral seeds[xx], on cell substrates[v,vi,vii], on tests for sterility[xxi] and on tests for mycoplasmas[xxii].

4.2.1.1.1.3. Vaccine seed lots

For production of live attenuated influenza vaccine, a seed lot system of the attenuated parent strain should be established using eggs from specific pathogen free (SPF) flocks[xxiii]. The method of

preparation should be described in detail and storage conditions of seed lots should be validated with respect to infectivity, genetic stability and sterility.

Genetic stability parameters comprise a demonstration of the retention of the defined phenotypic properties and the genetic structure of the attenuated parent strain throughout seed lot production beyond (at least five passages) production level, as evidenced according to the requirements described under point 4.2.1.1.2 A stability program for the seed lot system should be established.

Extraneous agent testing of seed lots of the attenuated parent strain should be performed according to relevant Ph Eur monographs on extraneous agents of viral seeds, on tests on sterility and on tests on mycoplasmas as indicated above (see 4.2.1.1.2).

4.2.1.1.2. Wild type Influenza HA & NA donor strain

4.2.1.1.2.1. Isolate and passage history

The antigenic identity of the influenza HA & NA donor virus should be in accordance with the seasonal recommendations of the WHO and the origin and history of the donor strain should be documented.

4.2.1.1.2.2. Seed Lots

Seed lots of the donor strain should be derived from the primary isolate by propagation on eggs or in controlled and certified cell substrates. Eggs used for propagation of the donor strain should be from hens certified to be free from specified pathogens (SPF).

4.2.1.1.2.3. Testing of Seed Lots

The following tests should be performed on the final seed lot of an influenza HA & NA donor strain that will be used for reassortment with the live attenuated parental influenza strain. If agreed with the competent authority, these tests can also be performed at the level of the reassortant Working Virus Seed.

Tests should demonstrate the absence of extraneous agents in the donor strain seed lot according to current Ph Eur monographs. Specific screening for human respiratory agents able to replicate in eggs should also be performed. In addition to tests already described in relevant guidelines, a multiplex PCR could be developed that is validated to detect genomes of human respiratory agents which may replicate in the egg substrate used for production of the seeds.

Since removal or inactivation of microbial contaminants is unlikely to be possible at any level of the production process of live attenuated influenza vaccine, the presence of any microbial agent in the seed lots of the attenuated parent strain and of the donor strain is not acceptable.

4.2.1.1.3. Preparation of the live attenuated reassortant virus

Reassortment between the live attenuated parent strain and the influenza HA & NA donor strain using classical techniques or reverse genetics methodology should be performed with biological reagents, for example antisera, enzymes, etc., certified to be free from infectious agents. With the use of reverse genetics methodology, the principles and recommendations of the Note for Guidance on Gene Transfer Medicinal Products[xxiv] should be adhered to. The presence of the correct HA and NA derived from the influenza donor strain should be demonstrated; this can be achieved by using specific antisera and certified reference reagents and/or other methods.

4.2.1.1.3.1. Generation and development of seed lots from live attenuated reassortant virus

A characterised clone of live attenuated reassortant virus is used for propagation in specific pathogen free embryonated eggs. A Master Virus Seed Lot and, optionally, a Working Virus Seed Lot derived from the Master Seed Lot should be established.

4.2.1.1.3.2. Characterization of Master and Working Virus Seeds Lots

Controls applied for identification and characterization of live attenuated reassortant virus seeds include those described under 4.2.1.1.1.2. The presence of genes associated with attenuation as well as the identity of HA and NA must be demonstrated. Lack of neurovirulence of the live attenuated reassortant virus should be demonstrated. Justification should be given if the test is not performed or replaced by an alternative test.

4.2.1.1.4. Substrate for virus propagation

Influenza virus used in the preparation of seed lots is propagated in fertilised eggs from chicken flocks free from specified pathogens (SPF) (Ph.Eur. 5.2.2) or in suitable cell cultures (Ph.Eur. 5.2.3), such as chick-embryo fibroblasts, chick kidney cells obtained from SPF chicken flocks (Ph.Eur. 5.2.2), or a diploid or continuous cell line.

4.2.1.1.5. Vaccine Production

In general, special attention should be given to aseptic production conditions strictly avoiding contamination of the production system with extraneous agents. Since it may be difficult to perform a test for extraneous agents on single vims harvests, uninoculated control eggs are incubated in parallel to production eggs and tested for the absence of extraneous agents. Alternatively, single harvests may be tested for extraneous agents on suitable cell substrates in the presence of influenza HA neutralizing antibodies.

4.2.1.1.5.1. Egg substrate used for production

Flocks of hens used for egg production should be stringently controlled for the presence and maintenance of the SPF status at regular time intervals. Only controlled eggs from such flocks should be used for production of the live attenuated influenza vaccine.

4.2.1.1.5.2. Virus harvest

As for any live viral vaccine, the addition of preservatives such as thiomersal is contraindicated. Only virus harvests that comply with the tests for HA identity, and which are within an acceptable specification of bioburden should be used for further propagation. If agreed with the competent authority, tests for sterility and mycoplasmas can be performed at an appropriate stage following downstream processing of the single virus harvest. A specification should be provided for potency, e.g. determination of the egg infectious dose (EID50) of live attenuated virus/ml of chorio-allantoic fluid or assay specific unit such as Fluorescence Focus Units.

4.2.1.1.5.3. Monovalent bulk vaccine

Tests on the monovalent bulk vaccine include tests on the retention of phenotypic and genetic markers of the live attenuated virus described under point 4.2.1.1.1.2.

4.2.1.1.5.4. Trivalent final bulk vaccine

Trivalent bulks fall within tight specifications for potency. Potency, ovalbumin content and bacterial endotoxin concentration should fall within tight specifications. Lack of pyrogenicity of the trivalent live attenuated influenza vaccine following intranasal applications should be demonstrated in a suitable animal species on a limited number of final bulks. Thermal stability of the final vaccine should be adequately documented in real time stability studies and in studies at elevated temperatures. An end of shelf life specification should be defined and adequately justified.

4.2.1.1.6. Process validation

Process validation data should be generated to demonstrate that critical processes, operated within established parameters, can perform effectively and reproducibly to produce a medicinal product meeting its predetermined specifications and quality attributes.

4.2.1.1.7. Characterisation

Characterisation is necessary to allow relevant specifications to be established and may support the scientific evaluation of comparability after product or process changes have been introduced.

The biological, immunological and physicochemical properties of the HA antigen should be verified using a wide range of state-of-the-art analytical methods. For example, particle aggregation, phenotype/genotype, virus morphology, potency, percentage of infectious particles, should be evaluated.

Process related impurities (e.g. ovalbumin / host cell protein), downstream-derived impurities should be identified, quantified and data used to set release specifications.

4.2.1.1.8. Presentation

Where there is a need for a preparation tailored to a specific target population, its introduction should be supported by appropriate quality data, including but not limited to compatibility, process manufacturing validation and stability data.

4.2.1.1.9. Vaccine standardisation

For determination of potency the number of infectious virus particles per vaccine dose (e.g. EID50 or TCID 50 (tissue culture infectious dose or other assay specific unit) should be determined using an appropriate virus reference preparation. Particular consideration should be given to assay specificity, e.g. suitable reference reagents for each subtype should be used.

4.2.1.1.10. Stability / Shelf life

Stability data for the influenza vaccine should be developed as described in Ph. Eur monograph of Vaccines for Human Use[i] and ICH Q5C[xii]. Stability data, comprising studies under real-time and accelerated conditions, should be presented for drug substance (monovalent bulk) and drug product (final formulated vaccine) in support of the maximum storage time and shelf life, respectively.

A protocol for testing vaccine stability should be developed. The procedure for assignment of a shelf life / expiry date should be outlined and justified.

Any extension of the shelf life up to one year should be based on real-time stability data.

4.2.1.2. 'Annual update' application for a seasonal vaccine

IMPORTANT REMARK

Please note that only relevant and adequate sections of the CTD variation application should be submitted. All sections not felt to be necessary should however be justified adequately in the Summary/Overview.

4.2.1.2.1. First step submission – "Quality" Variation Application

4.2.1.2.1.1. Wild type Influenza HA & NA donor strain / Preparation of the live attenuated reassortant virus

Production history of the seed including:

- description of the derivation of the seed starting from master attenuated donor virus and WHO recommended strain(s);
- passage history;
- genetic sequence of the seed;
- phenotypic characterisation (including attenuation test and haemagglutinin and neuraminidase antigenicity);
- genetic stability for the seed lot including relevant genotypic and phenotypic markers (e.g. full genetic sequencing);
- analytical protocols (including extraneous agents safety test); Where the seed virus is tested for extraneous agents using PCR, and if in discussion with the Agency and Rapporteurs the need for additional PCR testing of the seed has been agreed, these data should be included in this application.
- neurovirulence test; Neurovirulence testing of annual updates (i.e. antigenically drifted strains) is normally not required. Neurovirulence testing will be required if a new HA subtype of influenza A virus (i.e. non-H1, non-H3 subtype) or a novel influenza B virus type differing from the currently circulating genetic lineages is included in the vaccine or in case specific safety concerns arise.

4.2.1.2.1.2. Manufacturing development

Any optimization in the vaccine production process (within the limits of the mock-up dossier) needed due to specific strain characteristics should be clearly indicated and justified (3.2.S.2).

The formulation development (actual formula (new season's strains) and Certificate of Analysis when available (either in quality or in clinical submission) of batch(es) used in clinical trial(s), where these are required.

4.2.1.2.1.3. Process validation / process consistency

Critical manufacturing steps should be re-evaluated for the newly strain(s).

Batch analysis results of first three monovalent bulks from each new seed lot intended for commercial production should be presented.

Batch analysis results of Drug Product including thermal stability should be provided.

4.2.1.2.1.4. Vaccine standardisation

Validation of analytical procedures should be shown where they are potentially impacted by the strain change(s), e.g. validation of the potency assay. Validation data are expected for the monovalent bulk (3.2.S.4.3), as well as trivalent bulk or drug product (3.2.P.5.3).

A copy of the approved specifications for the monovalent bulk(s) (3.2.S.4.1) and drug product (3.2.P.5.1) as well as an overview of the analytical procedures (3.2.S.4.2) should be presented in tabular format.

4.2.1.2.1.5. Stability / Shelf life

Stability test results from monovalent bulks should be presented where they are used for more than one year (3.2.S.7)

For the drug product, stability data from the previous season should be submitted as well as a stability commitment to put a number of drug product lots on a stability program detailed in a post-approval stability protocol (3.2.P.8). Accelerated stability trends can be used to show that new strains are likely to have same stability characteristics as those used in stability studies on which shelf-life is based.

Annex 1

Guideline on quality aspects on the isolation of candidate influenza vaccine viruses in cell culture

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 above 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 [2] 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 verified[5].

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 and should comply, in principal, with the Note for guidance on the use of bovine serum in the manufacture of human biological medicinal products [3]. Animal-derived materials used in cell culture

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

manipulations must be compliant with the current version of the Transmissible Spongiform Encephalopathy Note for Guidance [4].

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

[3] 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

[4] Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary products (EMEA/410/01 Rev. 3), http://www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webContentId=WC500003700

Annex 2

Influenza Virus Produced by Reverse Genetics Derivation from a highly pathogenic precursor (Example)

Virus description: *a reverse genetics derived 2:6 reassortant between A/abc/123/04 and A/PR/8/34*

Passage history: Vero x, Egg x

Parameter	SOP/Method	Results/Comments
A/abc/123/03 virus growth in eggs	SOP xyz, at BSL4 containment	Original egg grown virus from ...
Cloning and genetic modification of A/abc/123/04 HA segment	Standard molecular biological techniques	A/abc/123/04 HA segment cloned with polybasic cleavage site excised and stabilising mutations introduced
Sequencing of cloned HA	Plasmid DNA cycle sequening	A/abc/123/04-like with absence of polybasic amino acids at cleavage site
Cloning of A/abc/123/04 NA segment	Standard molecular biological techniques	A/abc/123/04 NA segment cloned unmodified
Sequencing of cloned NA	Plasmid DNA cycle sequening	A/abc/123/04-like
PR8 plasmids	Standard molecular biological techniques	Prepared by ...or Provided by ...
Plasmid preparation	SOP xyz	Plasmids HAxx and NAzz used plus six PR8 'backbone' and four helper plasmids
Vero cells	SOP xyz	Cells are validated for human vaccine manufacture
Reverse genetics	SOP xyz	The Vero cell rescued virus was passaged twice in eggs; HA titre abc

Influenza Virus Produced by Reverse Genetics Finished Product Specification (Example)

Virus description: *a reverse genetics derived 2:6 reassortant between A/abc/123/04 and A/PR/8/34*

Passage history: Vero x, Egg x

Parameter	SOP/Method	Specification	Result
Antigenic analysis of virus	SOP xyz	A/abc/123/04-like	Complies with specification
Virus titre	SOP xyz	N/A	HA titre of ...
Infectivity in eggs	SOP xyz	N/A	10x EID50/ml
HA sequence of virus	RT-PCR/cycle sequencing	A/abc/123/04-like with absence of polybasic amino acids at cleavage site	Complies with specification
NA sequence of virus	RT-PCR/cycle sequencing	A/abc/123/04-like	Complies with specification
Chicken	SOP xyz	IVPI 1.2 or less	Result ...

pathogenicity test			Complies with specification
Ferret pathogenicity test	SOP xyz	Viral titres in respiratory tissue no greater than parental viruses. Virus replication restricted to respiratory tract. Clinical symptoms indicative of attenuation.	Complies with specification
Egg embryo test	SOP xyz	Does not kill embryos	Complies with specification
Sterility	SOP xyz	Meets requirement	Complies with specification
Contamination of virus with plasmid DNA	PCR	N/A	Result ...
Animal materials used during derivation of virus	Traceability of materials	Compliance with EU Guideline on TSE	Complies with specification

954 N/A: not applicable

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956 # References

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xiv CPMP Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Medicinal Products (EMEA/410/01 Rev. 3)

xv CPMP/CVMP Note for Guidance on Inclusion of Antioxidants and Antimicrobial Preservatives in Medicinal Products (CPMP/CVMP/QWP/115/95)

xvi CPMP Note for Guidance on in-use stability testing for human medicinal products (CPMP/QWP/2934/99)

xvii CPMP Note for guidance on maximum shelf-life for sterile products for human use after first opening or following reconstitution (CPMP/QWP/159/96 corr)

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xx European Pharmacopoiea Commission 2011. Tests for extraneous agents in viral vaccines for human use (2.6.16)

xxi European Pharmacopoiea Commission 2011. Sterility (2.6.1)

xxii European Pharmacopoiea Commission 2008. Mycoplasmas (2.6.7)

xxiii European Pharmacopoiea Commission 2010. Chicken flocks free from specified pathogens for the production and quality control of vaccines (5.2.2.)

xxiv CPMP guideline on Quality, Preclinical and Clinical Aspects of Gene Transfer Medicinal Products (CPMP/BWP/3088/99)