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## 4 Guideline on Influenza Vaccines – Quality Module

5 Draft

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

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



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

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Comments should be provided using this [template](#). The completed comments form should be sent to [quality-influenzaguideline@ema.europa.eu](mailto:quality-influenzaguideline@ema.europa.eu)

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<b>Keywords</b>	<i>Quality requirements, influenza vaccines, inactivated, LAIV, seasonal, pre-pandemic, pandemic, mock up dossier, annual strain update, pandemic strain update</i>
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## 56 **1. Introduction (background)**

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

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

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

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

## 70 **2. Scope**

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

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

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

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

## 83 **3. Legal basis and relevant guidelines**

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

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<sup>1</sup> Concept paper on the revision of guidelines for influenza vaccines (EMA/CHMP/VWP/734330/2011)

## 87 **4. Quality requirements for influenza vaccines**

### 88 **4.1. Inactivated influenza vaccines**

#### 89 **4.1.1. Seasonal influenza vaccines**

##### 90 **4.1.1.1. Marketing Authorisation application for a seasonal influenza vaccine**

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

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

##### 98 **4.1.1.1.1. Candidate Vaccine Virus**

###### 99 **Definition**

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

###### 107 **Candidate Vaccine Virus – development**

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

- 109 • embryonated hens' eggs
- 110 • cells derived from embryonated hens' eggs
- 111 • mammalian cells (see also Annex 1)

112 The CVV is likely to be one of the following:

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

- 125 • A non-reassortant wild-type influenza virus.

## 126 **Candidate Vaccine Virus - Quality and control**

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

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

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

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

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

### 141 **4.1.1.1.2. Vaccine seed lots**

#### 142 **Production**

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

#### 146 **Qualification**

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

#### 151 **Testing for extraneous agents**

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

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

159 Such a risk assessment could include:

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

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<sup>2</sup> 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.

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

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

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

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

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

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

#### 183 **4.1.1.1.3. Substrate for vaccine virus manufacture**

184 For routine production, the virus of each strain is propagated in the allantoic cavity of fertilised hens'  
185 eggs from healthy flocks or in a diploid or continuous cell line<sup>iv</sup>.

#### 186 **Testing for extraneous agents**

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

#### 191 **4.1.1.1.4. Manufacturing development**

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

#### 199 **4.1.1.1.5. Process validation**

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

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

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

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

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

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

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

#### 229 **4.1.1.1.6. Characterisation**

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

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

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

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

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<sup>3</sup> 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.

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

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

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

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

#### 252 **4.1.1.1.7. Presentation**

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

#### 256 **4.1.1.1.8. Vaccine standardisation**

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

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

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

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

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

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

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

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

#### 300 **4.1.1.1.9. Adjuvants**

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

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

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

#### 316 **4.1.1.1.10. Stability / Shelf life**

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

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

327 **4.1.1.2. 'Annual update' application for a seasonal vaccine**

328 **IMPORTANT REMARK**

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

332 **4.1.1.2.1. First step submission – “Quality” Variation Application**

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

337 **4.1.1.2.1.1. Candidate Vaccine Virus Quality and control**

338 The guidance provided in section 4.1.1.1.1 applies.

339 **4.1.1.2.1.2. Vaccine seed lots**

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

343 **4.1.1.2.1.3. Manufacturing development**

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

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

349 **4.1.1.2.1.4. Process validation / process consistency**

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

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

356 **4.1.1.2.1.5. Vaccine standardisation**

357 Validation of analytical procedures should be shown where they are potentially impacted by the strain  
358 change(s), e.g. validation of the SRD test. Validation data are expected for the monovalent bulk  
359 (3.2.S.4.3), as well as trivalent bulk or drug product (3.2.P.5.3).

360 A copy of the approved specifications for the monovalent bulk(s) (3.2.S.4.1) and drug product  
361 (3.2.P.5.1) as well as an overview of the analytical procedures (3.2.S.4.2) should be presented in  
362 tabular format.

363 **4.1.1.2.1.6. Stability / Shelf life**

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

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

372 **4.1.1.2.2. Second step submission – “clinical” Variation Application**

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

374

375 **4.1.2. Pre-pandemic influenza vaccines**

376 **4.1.2.1. Marketing Authorisation application for a pre-pandemic influenza vaccine**

377 **4.1.2.1.1. Candidate Vaccine Virus**

378 The guidance provided in section 4.1.1.1.1 applies.

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

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

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

389 Examples of CVV suitable for use as vaccine strains:

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

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

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

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

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

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

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

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

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

- 415 • The cell substrate used to develop the CVV should, in principle, meet the requirements of Ph. Eur.  
416 general chapter 5.2.3. on cell substrates for the production of vaccines for human use. A bank of  
417 cells approved for human vaccine production will be suitable for use for this purpose.
- 418 • Materials used in generating a CVV via reverse genetics process must be compliant with the  
419 current version of the Transmissible Spongiform Encephalopathy Note for Guidance.
- 420 • Materials used in generating a CVV via reverse genetics process may affect the safety of the  
421 vaccine in terms of viral, bacterial, fungal and mycoplasma contamination. Potential safety risks  
422 associated with these materials should be taken into account in the applicant's overall safety  
423 evaluation for the vaccine.

#### 424 **4.1.2.1.2. Vaccine seed lots**

425 The guidance provided in section 4.1.1.1.2 applies.

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

#### 434 **4.1.2.1.3. Substrate for virus propagation**

435 Guidance provided in section 4.1.1.1.3 applies.

#### 436 **4.1.2.1.4. Manufacture development**

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

#### 449 **4.1.2.1.5. Process validation**

450 The guidance provided in section 4.1.1.1.5 applies.

#### 451 **4.1.2.1.6. Characterisation**

452 The guidance provided in section 4.1.1.1.6 applies.

453 **4.1.2.1.7. Presentation**

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

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

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

461 If the influenza vaccine contains Thiomersal as a preservative, the applicant should justify the final  
462 Thiomersal content of the vaccine, in line with the established CHMP guidance<sup>xv</sup>.

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

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

471 **4.1.2.1.8. Vaccine standardisation**

472 In general, the guidance provided in section 4.1.1.1.8 applies.

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

476 **4.1.2.1.9. Adjuvants**

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

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

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

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

490 **4.1.2.1.10. Stability / Shelf life**

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

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

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

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

#### 498 **In-use stability testing**

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

505 Useful information is provided in the CHMP Note for Guidance on in-use stability testing for human  
506 medicinal products<sup>xvi</sup> and CHMP Note for guidance on maximum shelf-life for sterile products for  
507 human use after first opening or following reconstitution<sup>xvii</sup>.

508

#### 509 **4.1.2.2. Strain update of a pre-pandemic influenza vaccine**

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

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

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

522

523 **4.1.3. Pandemic influenza vaccines**

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

530 **4.1.3.1. Marketing authorisation granted under the 'Mock-up' procedure**

531 **4.1.3.1.1. First phase: 'Mock up' application**

532 **4.1.3.1.1.1. Candidate Vaccine Virus**

533 The guidance provided in sections 4.1.1.1.1 and 4.1.2.1.1 applies.

534 **4.1.3.1.1.2. Vaccine seed lots**

535 The guidance provided in sections 4.1.1.1.2 and 4.1.2.1.2 applies.

536 **4.1.3.1.1.3. Substrate for virus propagation**

537 The guidance provided in section 4.1.1.1.3 applies.

538 **4.1.3.1.1.4. Vaccine Production**

539 **Manufacturing development**

540 The guidance in 4.1.2.1.4 applies.

541 **4.1.3.1.1.5. Process validation**

542 The guidance provided in section 4.1.1.1.5 applies.

543 **4.1.3.1.1.6. Characterisation**

544 The guidance provided in section 4.1.1.1.6 applies.

545 **4.1.3.1.1.7. Presentation**

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

547 The guidance provided in section 4.1.2.1.7 applies.

548 **4.1.3.1.1.8. Vaccine standardisation**

549 The guidance provided in sections 4.1.1.1.8 and 4.1.2.1.8 applies.

550 **4.1.3.1.1.9. Adjuvants**

551 The guidance provided in section 4.1.2.1.9 applies.

552 **4.1.3.1.1.10. Stability / Shelf life**

553 The guidance provided in section 4.1.2.1.10 applies.

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

557 **In-use stability testing**

558 The guidance provided in section 4.1.2.1.10 applies.

559

560 **4.1.3.1.2. Second phase: Pandemic strain update**

561 **4.1.3.1.2.1. Candidate Vaccine Virus**

562 The guidance provided in sections 4.1.1.1.1 and 4.1.2.1.1 applies.

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

568 **4.1.3.1.2.2. Vaccine seed lots**

569 The guidance provided in sections 4.1.1.1.2 and 4.1.2.1.2 applies.

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

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

576

577 **Table 1:** Accompanying data to support change to the vaccine virus seed introduced during pandemic  
 578 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)

579

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

585 **4.1.3.1.2.3. Vaccine production**

586 The guidance provided in section 4.1.2.1.4 applies.

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

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

592 **4.1.3.1.2.4. Process validation**

593 The guidance provided in section 4.1.1.1.5 applies.

594 **4.1.3.1.2.5. Characterisation**

595 The guidance provided in section 4.1.1.1.6 applies.

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

599 **4.1.3.1.2.6. Vaccine standardisation**

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

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

606 **4.1.3.1.2.7. Adjuvants**

607 The guidance provided in section 4.1.1.1.9 applies.

608 **4.1.3.1.2.8. Stability/ Shelf life**

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

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

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

619 **In-use stability testing**

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

623 **4.1.3.2. Marketing authorisation granted under the 'Emergency' procedure**

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

626

627 **4.2. Live attenuated influenza vaccines**

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

638 **4.2.1. Seasonal vaccines**

639 **4.2.1.1. Marketing Authorisation application for a seasonal vaccine**

640 **4.2.1.1.1. Live attenuated parent strain**

641 **4.2.1.1.1.1. Development**

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

645 **4.2.1.1.1.2. Characterisation**

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

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

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

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

663 **4.2.1.1.1.3. Vaccine seed lots**

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

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

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

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

#### 675 **4.2.1.1.2. Wild type Influenza HA & NA donor strain**

##### 676 **4.2.1.1.2.1. Isolate and passage history**

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

##### 679 **4.2.1.1.2.2. Seed Lots**

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

##### 683 **4.2.1.1.2.3. Testing of Seed Lots**

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

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

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

##### 696 **4.2.1.1.3. Preparation of the live attenuated reassortant virus**

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

704 **4.2.1.1.3.1. Generation and development of seed lots from live attenuated reassortant virus**

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

708 **4.2.1.1.3.2. Characterization of Master and Working Virus Seeds Lots**

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

714 **4.2.1.1.4. Substrate for virus propagation**

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

719 **4.2.1.1.5. Vaccine Production**

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

726 **4.2.1.1.5.1. Egg substrate used for production**

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

730 **4.2.1.1.5.2. Virus harvest**

731 As for any live viral vaccine, the addition of preservatives such as thiomersal is contraindicated. Only  
732 virus harvests that comply with the tests for HA identity, and which are within an acceptable  
733 specification of bioburden should be used for further propagation. If agreed with the competent  
734 authority, tests for sterility and mycoplasmas can be performed at an appropriate stage following  
735 downstream processing of the single virus harvest. A specification should be provided for potency, e.g.  
736 determination of the egg infectious dose (EID<sub>50</sub>) of live attenuated virus/ml of chorio-allantoic fluid or  
737 assay specific unit such as Fluorescence Focus Units.

738 **4.2.1.1.5.3. Monovalent bulk vaccine**

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

741 **4.2.1.1.5.4. Trivalent final bulk vaccine**

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

748 **4.2.1.1.6. Process validation**

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

752 **4.2.1.1.7. Characterisation**

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

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

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

761 **4.2.1.1.8. Presentation**

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

765 **4.2.1.1.9. Vaccine standardisation**

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

770 **4.2.1.1.10. Stability / Shelf life**

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

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

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

778 **4.2.1.2. 'Annual update' application for a seasonal vaccine**

779 **IMPORTANT REMARK**

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

783 **4.2.1.2.1. First step submission – “Quality” Variation Application**

784 **4.2.1.2.1.1. Wild type Influenza HA & NA donor strain / Preparation of the live attenuated**  
785 **reassortant virus**

786 Production history of the seed including:

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

803 **4.2.1.2.1.2. Manufacturing development**

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

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

809 **4.2.1.2.1.3. Process validation / process consistency**

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

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

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

814 **4.2.1.2.1.4. Vaccine standardisation**

815 Validation of analytical procedures should be shown where they are potentially impacted by the strain  
816 change(s), e.g. validation of the potency assay. Validation data are expected for the monovalent bulk  
817 (3.2.S.4.3), as well as trivalent bulk or drug product (3.2.P.5.3).

818 A copy of the approved specifications for the monovalent bulk(s) (3.2.S.4.1) and drug product  
819 (3.2.P.5.1) as well as an overview of the analytical procedures (3.2.S.4.2) should be presented in  
820 tabular format.

821 **4.2.1.2.1.5. Stability / Shelf life**

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

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

828

829 **Annex 1**

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

832 ***Executive summary***

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

836 **1. Introduction (background)**

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

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

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

859 The major concern in isolating vaccine viruses in cells is the possibility of adventitious agent  
860 contamination that might derive from the cells, the environment or materials used during isolation and  
861 propagation of the viruses. Thus, the purpose of this document is to provide regulatory guidance on  
862 the quality of the cells used to isolate the virus, the conditions under which viruses are isolated and the  
863 subsequent passage of these viruses until the manufacturer's master seed is prepared according to  
864 GMP. Normally, regulatory guidance is directed towards the vaccine manufacturers as it is they who  
865 have the responsibility for ensuring that their vaccine seed is suitable for the production of a human  
866 influenza vaccine. However, it is appreciated that the isolation of influenza candidate vaccine viruses  
867 will take place in WHO Collaborating Centres and as such, from a practical point of view, these  
868 laboratories should be familiar with the EU recommendations presented in this document.

869 The quality aspects of the establishment of a manufacturer's Master Seed lot and subsequent use in a  
870 cell vaccine manufacturing process have been described above and will not be further addressed in this  
871 document.

872 **2. Scope**

873 An influenza virus isolated on cell culture could be used to derive a seed virus for either a cell culture  
874 or an egg vaccine production process for the manufacture of inactivated or live attenuated influenza  
875 vaccines. Thus, the scope of this document is to provide guidance for the isolation on cell culture of  
876 any potential influenza vaccine virus intended for cell culture or egg-based influenza vaccine  
877 manufacture. It should be reminded however that influenza viruses used in vaccines should also follow  
878 the recommendations published by the WHO on this matter.

879 **3. Legal basis**

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

882 This guideline should be read in conjunction with all other relevant guidelines, especially those  
883 pertinent to the production and quality control of influenza vaccines. Furthermore, reference is made to  
884 the Ph. Eur. General chapter 5.2.3 on cell substrates for the production of vaccines for human use [2]  
885 and to the Influenza vaccine Ph. Eur. monographs which state the following: "The origin and passage  
886 history of virus strains shall be approved by the competent authority."

887 **4. Main guideline text**

888 **4.1. Cell substrate used for the isolation**

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

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

895 The origin, source and history of the cells should be available (including the nature of media used in  
896 their propagation) and the identity and purity of the cells should be verified<sup>5</sup>.

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

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

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

904 **4.2. Cell manipulation, virus isolation and virus propagation**

905 The composition and source of media used for all cell culture manipulations including cell passaging,  
906 virus isolation and virus propagation should be recorded in detail. The use of animal-free components  
907 is recommended. If substances of human or animal origin are used they should be free from infectious  
908 agents. Bovine serum used for the preparation and maintenance of cell cultures should be irradiated  
909 and should comply, in principal, with the Note for guidance on the use of bovine serum in the  
910 manufacture of human biological medicinal products [3]. Animal-derived materials used in cell culture

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<sup>5</sup> Where a cell banking system is in operation, identity and purity would normally be assessed for the Master Cell Bank.

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

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

### 919 **4.3. Quality assurance**

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

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

928 Where a cell-isolated virus is used in the manufacture of vaccine in eggs, if the virus has been derived  
929 in accordance with this guidance, there should be no impact on the quality requirements of the egg  
930 manufactured vaccine.

### 931 **References**

932 [1] Robertson, J.S. (1993) Clinical influenza virus and the embryonated hen's egg. Reviews in Medical  
933 Virology 3, 97-106

934 [2] European Pharmacopoeia general chapter 5.2.3. Cell substrates for the production of vaccines for  
935 human use, 01/2009:50203

936 [3] Note for guidance on the use of bovine serum in the manufacture of human biological medicinal  
937 products. CPMP/BWP/1793/02, <http://www.emea.europa.eu/pdfs/human/bwp/179302en.pdf>

938 [4] Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents  
939 via human and veterinary products (EMA/410/01 Rev. 3),  
940 [http://www.ema.europa.eu/ema/pages/includes/document/open\\_document.jsp?webContentId=WC500](http://www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webContentId=WC500003700)  
941 [003700](http://www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webContentId=WC500003700)

942 **Annex 2**

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

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

946 **Passage history:** Vero x, Egg x

947

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 sequencing	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 sequencing	A/abc/123/04-like
PR8 plasmids	Standard molecular biological techniques	Prepared by ...or Provided by ...
Plasmid preparation	SOP xyz	Plasmids HAx 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

948

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

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

952 **Passage history:** Vero x, Egg x

953

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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- i European Pharmacopoeia Commission 2012. Vaccines for human use, Monograph 0153
- ii European Pharmacopoeia Commission 2012. Influenza Vaccine (Split Virion, Inactivated), Monograph 0158. Influenza Vaccine (Surface Antigen, Inactivated), Monograph 0869. Influenza Vaccine (Whole Virion, Inactivated), Monograph 0159, Influenza vaccine (surface antigen, inactivated, virosome), Monograph 2053
- iii European Pharmacopoeia Commission 2008. Influenza vaccine (whole virion, inactivated, prepared in cell cultures), Monograph 2308. Influenza vaccine (surface antigen, inactivated, prepared in cell cultures), Monograph 2149
- iv European Pharmacopoeia Commission 2011. Cell Substrates for the Production of Vaccines for Human Use, Monograph 50203
- v Q5D CPMP Note for Guidance on Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates used for the Production of Biotechnological/Biological Products (CPMP/ICH/294/95)
- vi WHO Expert Committee on Biological Standardisation 1998. Requirements for the Use of Animal Cells as *in vitro* Substrates for the Production of Biologicals (Requirements for Biological Substances N° 50). WHO Technical Report Series 878
- vii CPMP Note for Guidance on Virus Validation Studies. The Design, Contribution and Interpretation of Studies Validating the Inactivation and Removal of Viruses (CPMP/BWP/268/95)
- viii Q5A (R1) CPMP Note for Guidance on Quality of Biotechnological Products: Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin, (CPMP/ICH/295/95)
- ix J. M., Dunleavy U., Newman R. W., Riley A. M., Robertson J. S. and Minor P. D. 1999. The influence of the host cell on standardisation of influenza vaccine potency. In "Inactivated Influenza Vaccines Prepared in Cell Culture". Eds. Brown F., Robertson J. S., Schild G. C. and Wood J. M. Dev. Biol. Stand. 98, Karger, Basel, pp183 – 188
- x Guideline on Adjuvants in Vaccines for Human Use (EMA/CHMP/VEG/134716/2004)
- xi Q5C CPMP Note for Guidance on Quality of Biotechnological Products: Stability testing of biotechnological/biological products (CPMP/ICH/138/95)
- xii Core SmPC for Trivalent Influenza Vaccines (CMDh/128/2003/Rev5 December 2011)
- xiii [http://www.who.int/influenza/vaccines/virus/characteristics\\_virus\\_vaccines/en/](http://www.who.int/influenza/vaccines/virus/characteristics_virus_vaccines/en/)
- xiv CPMP Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Medicinal Products (EMA/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)
- xviii European Pharmacopoeia Commission 2008. Tests for neurovirulence of live virus vaccines (2.6.18)
- xix War, A.C. Neurovirulence of Influenza A virus. Journal of Neurovirology 1996, 2: 139-151

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

xxi European Pharmacopoeia Commission 2011. Sterility (2.6.1)

xxii European Pharmacopoeia Commission 2008. Mycoplasmas (2.6.7)

xxiii European Pharmacopoeia 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)