25 April 2014
EMA/CHMP/BWP/78207/2014
Committee for Medicinal Products for Human Use (CHMP)

Overview of comments received on 'Guideline on Influenza Vaccines – Quality Module'

Interested parties (organisations or individuals) that commented on the draft document as released for consultation.

Stakeholder no.	Name of organisation or individual
1	Vaccines Europe
2	CSL Behring
3	Health Canada

7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7418 8416
E-mail info@ema.europa.eu **Website** www.ema.europa.eu

1. General comments – overview

A BWP Drafting Group Meeting with Stakeholders was held at EMA on 19 November 2013. During this meeting, some of the comments put forward by stakeholders were discussed in more detail.

Stakeholder no.	General comment (if any)	Outcome (if applicable)
1	Vaccines Europe welcomes the opportunity to provide comments to the Quality Module and looks forward to collaborating with the European Medicines Agency in the process of revision of the Influenza Guidelines.	
	In general, the document combines the requirements for egg-derived and cell-derived seasonal influenza vaccines without making a clear distinction, which leads to lack of clarity/confusion. In this regard, it should be clearly specified which requirements apply to which type of vaccine in each section.	Although there are certain quality related areas that overlap, differences exist between quality aspects for egg-derived and cell culture - derived influenza vaccines. The document will be reviewed and amended, as appropriate, to better distinct requirements for egg-derived vs. cell culture derived influenza vaccines.
	In addition, a number of tests described in this guideline are new requirements (e.g. PCR detection of adventitious agents, identification and assay of host cell proteins, etc.). Analytical development will be necessary before their routine implementation. Therefore, a minimum of 3 years should be granted before the entry into force of these new requirements.	It is acknowledged that the development of a new analytical method, if not already required by Ph. Eur., will take time. However, timelines cannot be given as it these will have to be considered on a case-by-case basis taking into account for example, the complexity of the method, new technology concepts, and (cross-)validation. It will be clarified what quality data is expected as part of the Annual Update variation.
	Finally, it is important to note that the comments that were provided	Comments previously received in the context of the fast-

Stakeholder no.	General comment (if any)	Outcome (if applicable)
	to the EMA in the context of the public consultation of the fast-track procedure for the annual update of human influenza vaccines are also applicable for this Quality Module of the Guideline on Influenza Vaccines.	track procedure will be taken into account and the document will be amended as appropriate.
2	CSL Behring thanks the EMA for an opportunity to comment on the Guideline for Influenza Vaccines – Quality Module. CSL Behring supports and appreciates the initiatives of the EMA in increasing clarity around the quality-related requirements for different influenza vaccine types. CSL Behring does not object to publication of the comments in this submission.	

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
Lines 70-82	1	**Comment:** We suggest to extend the scope to other vaccines concepts such as influenza vaccines based on new technology, nucleic acids, synthetic seed approach... or to write a separate annex dedicated to any new technology.	Partly accepted. It has been decided to limit the scope to vaccines for which ample experience has been gained, also taking in to account that the scope of the Quality module should be kept in line with the guidance provided on non-clinical and clinical aspects. Reverse genetics technology using synthetic influenza virus gene sequences will be included in the guideline, as the approach to prepare and use seeds derived with this technology can be considered similar to that for strains prepared by reverse genetics. It is possible that the guideline will be updated in the future when more information comes forward to provide additional scientific guidance on other vaccine concepts as mentioned by the stakeholder.
Lines 100-103	1	**Comment:** Line 138 establishes that “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”. Therefore the possibility for manufacturers to establish their CVV should also be mentioned here. **Proposed change:** Add in line 103 the text in track changes:	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		"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. Vaccine manufacturers may also establish their own CVV provided that these seeds are demonstrated to represent the influenza strains recommended by WHO/CHMP."	
Lines 113-119	1	**Comment:** It is mentioned in line 115 that the high yielding donor strain PR8 is being used. Nevertheless, to date also B/Lee/40/90 and B/Panama/45/90 are being used. **Proposed change:** Modify the text as follows: "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 a high yielding donor strain ~~PR8.~~"	Accepted.
Line 119	1	**Comment:** Please clarify the meaning of "antigenically similar.	Accepted.
Line 126	1	**Comment:** It is not clear to which laboratories these requirements apply. It has to be clear that these requirements apply to the laboratories preparing vaccine strains / reassortants. **Proposed change:** Add in the title: "Candidate Vaccine Virus - Quality and control at	Not accepted. Vaccine candidate vaccine virus may also be prepared by manufacturers. In this case, the requirements would also be applicable to companies.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		WHO-CC or WHO-ERL"	
Lines 127-129	1	**Comment:** In case a manufacturer establishes its own CVV, requirements from competent Authorities in terms of origin and passage history of virus strains, would need to be clarified.	Partly Accepted. It should be noted that information related to the preparation and testing of CVV is already presented in Annex 1 and 2.
Line 145	1	**Comment:** In line with the comment on lines 100-103 **Proposed change:** Introduce the following changes: "Such a seed lot system ~~is likely to~~ may be based on a CVV issued by a WHO ERL, WHO CC or other approved laboratory or may be based on a CVV established by a vaccine manufacturer that has been demonstrated to represent the influenza strains recommended by WHO/CHMP (see 4.1.1.1.1)."	Accepted.
Lines 147-148	3	**Comment:** "*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.*" BGTD feels that it might be useful to add that seeds should be produced in a sterile environment. Some sponsors prepare their seeds in class B or lower and perform a sterility test.	The environmental control is considered sufficiently covered by EU GMP legislation / guidelines.
Lines 149-150	1	**Comment:** Introduce PCR testing as general alternative possibility for HA and NA identification	Partly accepted. In case reagents are available an immunological test remains the preferred option. PCR may be considered if justified, i.e. in case insufficient reagent specificity.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Proposed change: Change text as follows: Alternative tests to identify the seed virus (e.g. PCR) ~~should~~ may be developed and used to confirm HA and NA identity	
Line 150	3	**Comment:** "*Qualification*" A requirement for an Antigenic Drift Analysis if the passage number between Master and Working Seeds is greater than 1 could be added herein.	Accepted. The guideline text has been revised to include evaluation of the occurrence of aggregation by appropriate analytical methods, e.g. Dynamic Light Scattering.
Lines 152,175	2	**Comment:** CSL Behring agrees that testing of the seed virus for freedom from extraneous agents according to Ph. Eur. Monographs for egg-derived inactivated influenza vaccine is appropriate. However, CSL Behring feels that additional testing is unnecessary for egg-derived reassortants as demonstrated by many years of use and to the species barrier imposed by the use of eggs for the process. **Proposed change:** Please consider exemption of egg-derived reassortants from any additional testing for extraneous agents.	Not accepted. Whether or not egg-derived seeds should be tested for extraneous agents and the extent needed, will be based on the outcome of the risk assessment as outline in the document.
Lines 160-164	1	**Comment:** It is assumed that the risk assessment related to production of the CVV will be performed by the organisation producing the CVV (WHO ERL or industry) and this assessment would be provided to vaccine manufacturer together with the CVV.	Partly accepted. Whilst the risk assessment should be performed for the manufacturer's seed lot, information about its history should be used. In case the CVV is obtained from the WHO ERL or CC, companies should obtain information relevant for such a risk assessment (i.e. cell substrates, raw materials from animal origin), the risk assessment should however be done by the vaccine manufacturer. In case the

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Proposed change: Clarify that the two bullet points refer to the organisation responsible for producing the CVV.	vaccine virus is being developed by the company itself, the risk assessment should include all relevant information as mentioned in the bullet points.
Lines 168-169	1	**Comment:** Vaccines Europe understands that this applies to first applications, not to seasonal updates (Cf. 4.1.1.2). **Proposed change:** Please clarify	Not accepted. As outlined in the document emerging viruses should be part of the risk assessment. As such, whether or not the seed should be tested and the panel of potential extraneous viruses will depend on the outcome of the annually updated risk assessment.
Lines 174-178	1	**Comment:** According to the current Ph.Eur. Monograph 2149 on cell derived influenza vaccine; rapid assays (e.g. multiplex PCR) may be applied as alternatives to general chapter 2.6.16 when agreed with the competent authority. **Proposed change:** Add in line 178 "For cell-derived influenza vaccines rapid assays (e.g. multiplex PCR) may be applied as alternatives to general chapter 2.6.16 when agreed with the competent authority." Furthermore, "The obligation to complete the testing according to the Ph.Eur 2.6.16'', can be removed when it is agreed by the competent authority.	Accepted.
Line 175	1	**Comments:** As specified in the general comments, there is no distinction between egg-derived and cell-derived influenza vaccines. The risks and testing requirements are quite different at this step for cell- and egg-derived	Partly accepted. The risk may be different but this will be based on the risk assessment performed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		vaccines. **Proposed change:** Reflect this in the text.	
Lines 179-182	1	**Comment:** The appropriateness of monovalent bulk testing in addition to viral clearance validation should be discussed on a case-to-case basis depending on individual product and process. **Proposed change:** Update text in track changes: "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~~ and/or 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."	Not accepted. In line with ICH Q5A 3 complementary approaches to control the potential viral contamination are adhered, i.e. a) selecting and testing cell lines and other raw materials, including media components, for the absence of undesirable viruses which may be infectious and/or pathogenic for humans; b) assessing the capacity of the production processes to clear infectious viruses; c) testing the product at appropriate steps of production for absence of contaminating infectious viruses. The strategy chosen to ensure freedom from extraneous agents in the final vaccine should be duly justified for the applied production platform.
Lines 202, 206, 350	2	**Comment:** CSL Behring agrees that the inactivation of the vaccine virus is a critical process parameter. CSL Behring questions the need however to perform inactivation kinetics studies on new strains using (normally) three commercial scale production batches if a knowledge database has been developed for the inactivation process over many years and the inactivation process has been validated and approved. **Proposed change:**	Partly accepted. The inactivation kinetics is strain dependent and the need for annual revalidation does not rely on process robustness/experience. However, the number of batches to be used in the inactivation kinetics may be reduced based on the experience gained.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		If an inactivation process has been validated and this validation is approved by the regulatory authorities and where an extensive knowledge database is available to demonstrate consistency of the inactivation process, CSL Behring believes that inactivation kinetics could be replaced by Residual Infectious Virus testing when introducing new virus strains.	
Lines 215-217	1	**Comment:** As chapter 4 is about seasonal flu vaccine (egg and cell derived) it should be better specified if requirements are for both or one of the vaccines. In the future manufacturers of cell derived flu vaccines might use seeds from cell derived origin where testing or removal validation of ALV will be redundant. **Proposed change:** Specify that removal of validation of ALV is only necessary with egg derived seed viruses	Partly accepted. Validation of ALV inactivation capacity will have to be shown in case egg-derived seed viruses and/or egg-based production system are used, or where a cell culture-derived seed virus derived from a egg based CVV is used.
Lines 224-228	1	**Comment:** This is a new chapter/requirement and (if any) should be described in the section on Process Development (S.2.6. and P.2.3).	Accepted.
Line 229	3	**Comment:** "*The occurrence of protein aggregation either in the Drug Substance or Drug Product should be evaluated.*" BGTD suggests that it should be clarified how this is assessed. By visual inspection? Does the *Eur. Ph. 2.9.19 Particulate Contamination: sub-visible particles*	Accepted. The guideline text has been revised to include evaluation of the occurrence of aggregation by appropriate analytical methods, e.g. Dynamic Light Scattering.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		apply to influenza vaccines?	
Line 232	3	**Comment:** "…*as well as an appropriate control strategy*." BGTD would like to discuss what constitutes an appropriate control strategy. Is visual inspection sufficient?	See previous comment.
Lines 239-243	1	**Comment:** So far in many MA's HA characterisation is based on SRD/SDS-PAGE. It is unclear if new characterisation should be implemented. **Proposed change:** New information cannot be submitted during an Annual Update. Update of HA characterisation implies submission of a variation.	Not accepted. The guideline is applicable to new and existing MAs. For existing MA, additional characterisation studies could be implemented via variation procedure. Once registered, the characterisation tools could be applied for new strains, if feasible, as part of the Annual Update package or to build up the knowledge database depending on the quality attribute.
Line 242	1	**Comment:** Clarification would be needed on what is intended by the sentence "Considerations should be given to characterise in nature and quantity the relevant antigens as far as technically feasible."	Accepted.
Lines 246-248	1	**Comment:** This is a new chapter/requirement. See also line 224-228 **Proposed change:** We would like to suggest describing it only in Process development and not in characterisation	Not accepted. The extent and composition of aggregates is considered a (potential) vaccine characteristic and therefore a quality attribute to be discussed in the characterisation section. The need for process amendments to limit the presence of aggregates should be part of process development.
Line 249	1	**Comment:** We would suggest to either introduce the need of	Accepted. This will have to be harmonised with Ph. Eur. requirements as well.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		testing for process related impurities as release specification or as part of the process validation	
Line 253	1	**Comment:** The wording “preparation” should be replaced by “presentation” (as in line 252) as it is not clear what is meant by “preparation”.	Accepted.
Lines 256 - 292	2	**Comment:** CSL Behring acknowledges the difficulties in the timing of availability of SRD assay reagents in the event of new strains being introduced. CSL Behring endorses the EMA’s position that there is a need for alternative assays that can be applied prior to the availability of SRD reagents.	Accepted. No change needed.
Lines 264 - 265	1	**Comment**: The expression of the vaccine dose should follow the European Pharmacopoeia requirements. 15 µg/dose per strain is a target value. However, the definition of the European Pharmacopoeias is “For an inactivated vaccine, the current European Pharmacopoeia dose definition is 15 µg of haemagglutinin antigen per dose for each strain present in the vaccine determined by SRD, where the confidence limits (P=0.95) are not less than 80 per cent (12 µg) and not more than 125 per cent of the estimated haemagglutinin antigen content. The lower confidence limit (P=0.95) is not less than 80 per cent of the amount stated on the label for each strain.” **Proposed change:**	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		We propose to add the European Pharmacopoeia definition as a footnote of page 9	
Line 270	3	**Comment:** "*...For an inactivated seasonal vaccine, the current international consensus for a vaccine dose is 15 SRD µg of HA antigen.*" Some manufacturers report potency values for release and stability with no decimals based on the fact that guidance documents indicate potency with no decimals	Agreed. No change proposed.
Line 294	1	**Comment:** Typographical error **Proposed change:** Change 'eggsix' to 'eggs'	Accepted. 'ix' is a reference.
Line 296	1	**Comment:** All vaccine candidates should be antigenically like the reference strain. The principle that SRD reagents should be prepared using the exact matching strain/reassortant will apply equally to egg and cell derived strains. It could lead to misunderstandings to refer to antigenic differences between vaccine candidates.	Accepted. Wording will be harmonised and further clarified.
Lines 294-296	1	**Comment:** Seems to be contradictory with the statement below in line 848 where antigenically mutations in isolates are assigned to eggs. **Proposed change:** Harmonise wording in the document	Accepted. Wording will be harmonised and further clarified.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
Line 298	1	**Comment:** The reason why it is mentioned that at least one 'cell-derived' reagent has been made available by a WHO ERL is unclear **Proposed change:** Propose to delete this sentence	Accepted.
Line 323	2	**Comment:** Regarding the duration of the product shelf life, CSL Behring agrees that it is important to ensure that vaccine from one season is removed from the market prior to launch of the next season's vaccine. However, as an alternative to restricting vaccine to a 12-month shelf life, a common "cut-off" date for each season could be implemented. For example, if vaccine lots for a given season were given a common end June expiry date, it would be possible to extend the shelf life to 15 months, such that vaccine manufactured in April or May of a given year could be given an expiry date of end June of the following year. **Proposed change:** Please consider alternatives to the application of a 12-month shelf life in order to avoid seasonal vaccine cross-over. Introduction of an "end of season" date, being the latest date of expiry applied within a given season, could be considered.	Not accepted. The proposed common "cut-off" is not generally supported by industry as clarified during the Drafting Group meeting with Stakeholders.
Line 329	1	**Comment:**	Not accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		In general, reference should be made to the CMDh best practice guide on Fast Track Procedure for the annual update of Human Influenza Vaccines, Chapter 9 in order to avoid repeated requirements	The Quality Module of the revised influenza guideline is intended to cover all influenza vaccines for which ample experience has been gained, irrespective of the registration route.
Lines 328-331	1	**Comment:** A definition of what "relevant and adequate sections" are is not provided in the guideline. We are of the opinion that for the variations dossier the relevant information will be the information that has been updated. Therefore, when providing the Quality Documentation only the information that has been changed should be included. **Proposed change:** *Amend the first paragraph of Section 4.1. – Module 3 as follows:* "Please note that for this Module only the updated / new information on the different sections of the CTD dossier ~~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."~~	The paragraph has been removed as guidance is provided elsewhere in the document. See also the overview of comments related to the procedural guidance on annual strain updates.
Lines 332-336	1	**Comment:** There is only one step in the annual update submission. In addition the legal reference to Article 18 is outdated since there has been an update on the Variations legislation.	Partly accepted. The Quality Module is intended to provide scientific guidance. Reference is made to the latest procedural guidance on annual strain updates for guidance on procedural aspects.
Line 345	1	**Comment:**	Partly accepted. Agreed that process optimisations studies

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Optimisations are usually described in 3.2.S.2.6 Process Development. As it would only be applicable for a specific/ new strain it cannot be described in the other chapters. **Proposed change:** Change from 3.2.S.2 to 3.2.S.2.6	should be described in 3.2.S.2.6 Process Development. However, based on development studies, the process description and its control, as described in 3.2.S.2.2, may have to be amended.
Lines 347-348	1	**Comment:** Inclusion of the Certificate of Analysis (CoA) for batches used in clinical trial(s) (if a clinical trial is requested) in the initial data package submitted for the quality submission may lead to undue delay in procedure start. In the event that Clinical Trials are not requested, manufacturers will not be able to send the CoA of the clinical material along with the Quality Parts of the Annual update submission. **Proposed change:** Submission of the CoA is acceptable for the clinical dossier	Accepted.
Line 352	3	**Comment:** "*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….*" The label claim is, as per guidance, 15 µg HA per dose. However, in recent years, the potency of influenza vaccines has increased significantly due to overages applied at formulation to account for loss of potency	Comment noted. As for any other medicinal products, the use of overages should be justified. No change needed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		during storage. We now have vaccines with 20 µgHA/dose per strain. There is currently no upper limit. These overages have an impact on the interpretation of immunogenicity and efficacy data generated in clinical trials. BGTD has observed situations where the sponsor used clinical trial lots with potencies much higher than the label claim. The overages used in the clinical trials are not reflected in the commercial lot specifications, which are based on the pharmacopoeia.	
Lines 352-355	1	**Comment:** In case more than one working seed lot has been manufactured from a new master seed lot of a new strain it should only be necessary to provide batch analysis results on three consecutive monovalent bulks for one working seed lot, if there is no difference in passage level. **Proposed change:** The section should be re-written as it is confusing.	Partly accepted. Sentence will be revised to better clarify when batch analysis results of the first three monovalent bulks are required.
Lines 355-356	3	**Comment:** "*Critical manufacturing steps should be re-evaluated for the newly strain(s). Adequate inactivation and, as appropriate, the splitting efficiency should be demonstrated"* BGTD has noted that data demonstrating adequate inactivation are provided (Viral inactivation and virus infectious titer) in the strain updates but most sponsors do not provide supporting data on three	In the guideline, reference is made to the Ph.Eur. monographs for inactivated influenza vaccine which require 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. No change needed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		consecutive batches to demonstrate that the antigenic properties of the virus were not altered (HI titer - HA titer). Would this be useful?	
Lines 357-359	1	**Comment:** "Validation of analytical procedures": the wording "validation" should be changed by "re-qualification", as full re-validation is not done every year. Note that the term "re-qualification" is used in US. Additionally, a full re-validation is not done for new reagents (validation or re-validation of the SRD test is independent from the reagents). Vaccines Europe would prefer also to place the data for this re-qualification on S.5 (not S.4.3) and P.6 (not P.5.3), because it is confusing – it is a change in the reference materials, not in the validation. Additionally, it is not really clear, what kind of "re-qualification" is expected. Finally, validation data for SRD testing for cannot be complete for the initial quality submission as drug product formulation is generally in parallel to the quality submission. **Proposed changes:** The wording "validation" should be changed by "re-qualification". MA are allowed to submit information about method validation from critical process steps as part of the clinical dossier to enable in time submission of the	Not accepted. It is agreed that further guidance on the data required for re-validation is needed. It is acknowledged that full validation of SRID with respect to trivalent product may not be finalised. The data should then be submitted as part of the request for supplementary information and/or request for additional data response package.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		quality dossier.	
Lines 361-362	1	**Comment:** "Overview of the analytical procedures (3.2.S.4.2) should be presented in tabular format": Vaccines Europe does not agree to provide this as part of annual strain variation (not done currently), and analytical methods listed in "A copy of the approved specifications for the monovalent bulk(s) (3.2.S.4.1) and drug product 360 (3.2.P.5.1)". Indeed, this section is new in the list for Annual Update requirements. We would like to ask for further clarification on the need to request this information as it was not requested in the previous guideline and as for (e)CTD submissions this section contains separate documents of approved/registered Analytical Procedures. Furthermore, this section should not change for the Annual Update. **Proposed change:** Add clarification that 3.2.S.4.2 only applies when there are changes in analytical procedures due to the introduction of new strains.	Accepted.
Lines 362-363	3	**Comment:** "*Validation of analytical procedures should be shown where they are potentially impacted by the strain change(s), e.g. validation of the SRD test.*" It is useful to obtain the Qualification reports of the	Agreed. The guideline has been updated to include guidance on the re-validation of the SRD, comprising e.g. serum qualification.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		reference reagents used in the SRID.	
Line 364	1	**Comment:** "Stability studies for monovalent bulks under real-time and accelerated storage conditions". Currently manufacturers do not provide the accelerated stability data on monovalent bulks. **Proposed change:** Remove this requirement.	Not accepted. It is recommended to perform such study in order to build up the quality knowledge database.
Lines 371-372	3	**Comment:** "*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)"* BGTD would like to point out that there are no requirements for the stability specification for monovalent bulk HA content (e.g. % decrease from initial value) or an acceptance criterion associated to the re-testing policy prior to formulation of the trivalent bulk. The shelf-life of the product cannot be evaluated with a "to report" acceptance criterion. If the SRID test is not directly correlated to clinical outcomes (see above in 4.1.1.1.8), should there be a required to test the immunogenicity of vaccines formulated with "aged" bulk to support their use? This is also relevant to pre-pandemic vaccines, where antigens are stored in bulk awaiting formulation.	Immune response as expected in humans cannot be fully assured by immunogenicity testing in animals. Testing of immunogenicity (e.g. in mice) is not a sufficiently sensitive tool. For seasonal influenza vaccines, the SRD assay will assure a HA content that is expected to induce an immune response in humans. For (pre-)pandemic influenza vaccines additional aspects may be important to induce an appropriate immune response (e.g. class and amount of adjuvanting system). No change needed.
Line 373	1	**Comment:** "Where applicable, an updated Quality Overall	The title of this section was revised to "second step submission – Additional data" in line with the revised

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Summary should be submitted." As this sentence falls in the section "Second step submission – "clinical" Variation Application", it should be clarified in which circumstances an updated QOS applies.	procedural guidance on annual strain updates. An updated QOS may be needed if Quality data are submitted in this step.
Lines 383-385	1	**Comment:** It would be helpful to have some guidance what tests are required for viruses derived from low pathogenic pre-/pandemic vaccine candidate strains, e.g. H9N2 or H3N2v.	Accepted. Additional guidance will be provided or reference made to relevant WHO documents.
Line 473	2	**Comment:** The SRD assay may not be highly accurate for potency measurement of HA quantities significantly lower than those of seasonal vaccines. In the event of a pre-pandemic or pandemic vaccine containing low HA quantities, an alternative assay may be more appropriate.	Accepted. *(No change needed as 4.1.1.1.8 already mentions alternative methods)*
Line 579	1	**Comment:** The requirement for genetic sequencing should be limited to RG strains that are derived from highly pathogenic strains that have been genetically modified to reach attenuation.	Information about sequencing analysis is requested for RG strains, for which limited production experience is as yet obtained. In addition, sequence analysis for non-RG seasonal strains may be of interest to build the knowledge database.
Lines 600 - 601	1	**Comment**: The expression of the vaccine dose should comply with the European Pharmacopoiea requirements. **Proposed change:** We propose to add the European Pharmacopoeia definition as a footnote of page 20	Not accepted. Not needed in this context.
Lines 664 -	1	**Comment:**	Partly accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
667		The virus seed lots are assessed for potency rather than infectivity. See comment below regarding genetic stability. **Proposed change:** Change sentence as follows in line 665: "The method of preparation should be described in detail and storage conditions of seed lots should be validated with respect to ~~infectivity~~ potency~~, genetic stability~~ and sterility."	Actually, virus titer was meant, potency is acceptable. Genetic stability is requested.
Lines 668-671	1	**Comment:** Genetic stability testing is a one-off test on each new virus seed lot and is not part of a stability program per se. The outcome of previous discussion with the agency is that it is only necessary to assess retention of phenotypic parameters if changes in the genetic sequence are seen in the sites that control phenotype. **Proposed change:** "Each new virus seed lot is assessed for genetic stability. The genetic stability parameters comprise a demonstration of the retention of the ~~defined phenotypic properties and the~~ genetic sequence of the known gene segments of the attenuated parent strain conferring the desired phenotypic properties 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.	Partly accepted. It is agreed that genetic stability testing is a one-off test on the virus seed and not (necessarily) part of a stability program. A more extensive consideration on phenotypic parameters would be warranted if changes in the genetic sequence are seen. However, confirmation of the phenotypic characteristics is part of the routine testing. A stability program for the seed lot system is required but it is not directly linked to the discussion on genetic stability in this paragraph.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Phenotypic testing is only required if sequence changes are seen in the sites that control phenotype. ~~A stability program for the seed lot system should be established."~~	
Line 722	1	**Comment:** Typographical error **Proposed change:** Replace 'vims' with 'virus'.	Accepted.
Line 732	1	**Comment:** HA identity testing is performed on the Master Virus Seed (MVS) and on the initial monovalent bulk lots. **Proposed change:** Only virus harvests manufactured from seed lots that comply with the tests for HA identity, and which are within an acceptable specification of bioburden should be used for further propagation.	Accepted.
Lines 743-745	1	**Comment:** Endotoxin levels are controlled in the final bulk trivalent vaccine therefore it is not necessary to test further for "lack of pyrogenicity." Furthermore a study has been conducted which demonstrated that administration of a dose of endotoxin much higher than the upper limit of the specification to rabbits, did not cause fever in the animals. **Proposed change:** Remove sentence in lines 743-745 ~~"Lack of pyrogenicity of the trivalent live attenuated~~	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		~~influenza vaccine following intranasal applications should be demonstrated in a suitable animal species on a limited number of final bulks."~~	
Line 848	1	**Comment:** It may cause misunderstandings to talk about changes to antigenicity. All vaccine candidate strains are tested and released by WHO as "antigenically equivalent to the reference strain". Although there may be changes selected during passaging in eggs/cells, any change that alters antigenicity would render the strain unsuitable as a vaccine strain.	Accepted.
Lines 852-853	1	**Comment:** It is stated that "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." **Proposed change:** Since there is no scientific proof Vaccines Europe would suggest deleting the sentence above.	Partly accepted. Text has been amended.
Lines 866-868	1	**Comment:** While the isolation of influenza candidate vaccine viruses normally take place in WHO CC it should be acknowledged that manufacturers may also isolate/establish their own CVV. **Proposed change:** "However, it is appreciated that the isolation of influenza candidate vaccine viruses ~~will take~~ normally	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		takes place in WHO Collaborating Centers.”	