Australian Government
Department of Health, Disability and Ageing
Therapeutic Goods Administration

Seasonal influenza vaccines - Quality module

Explanatory document for the EMA guideline on influenza vaccine (EMA/CHMP/BWP/310834/2012 Rev.1) adopted by TGA, overseas effective date 1 February 2018

Version 3.0, August 2026

Contents

Introduction

The TGA adopted the EMA Guideline on Influenza Vaccines – Quality Module (EMA/CHMP/BWP/310834/2012) 1 November 2014, with Rev.1 which came into effect 1 February 2018. The quality modules submitted to the TGA as part of registration applications for influenza vaccines (Category 1 applications) and/or variation applications for annual strain updates to seasonal influenza vaccines (Category 3 applications) are expected to meet the EMA guidelines.

Scope

This explanatory document:

- Provides further guidance/clarification on the data/information to be included in the quality modules for seasonal influenza vaccine submissions, based on TGA's interpretation of the EMA Guideline on Influenza Vaccines – Quality Module
- Contains information to ensure effective and efficient evaluation of the quality aspects of the primary application and any subsequent applications to vary the conditions of registration i.e. the annual strain updates (ASU)
- Does **not** contain explanation on pre-pandemic and pandemic influenza vaccines, covered under sections, 4.1.2 and 4.1.3 and of the EMA guideline, respectively.

Appendix 1 provides additional information on procedural issues relating to the ASU and seasonal sampling requirements to help ensure efficient review and batch release of products.

You are encouraged to discuss your concerns, alternative methods or request further guidance by contacting Biomedicines.Evaluation@Health.gov.au.

This is an explanatory document that includes clarification on only some sections of the EMA guideline. However, it is expected that quality modules will comply with all sections of the EMA guideline that are applicable to the product.

The same section numbering and headings are used as in the current version of EMA/CHMP/BWP/310834/2012 Rev.1.

Legal basis and relevant guidelines

You should also refer to other relevant European and ICH guidelines, European Pharmacopoeia Monographs and Chapters, World Health Organization (WHO) Technical Report Series for the Recommendations for the production and control of influenza vaccine (inactivated), Recommendations to assure the quality, safety and efficacy of influenza vaccines (human, live attenuated) for intranasal administration and the Minor variations to prescription medicines: biological medicines.

4. Quality requirements for influenza vaccines

4.1 Inactivated influenza vaccines

4.1.1 Seasonal influenza vaccines

4.1.1.1 Marketing Authorisation application for a seasonal influenza vaccine

This section outlines the data that are expected to form the core of the primary submission for the vaccine and which consequently form the basis for subsequent updates (see Section 4.1.1.2 'Annual update' application for a seasonal vaccine). Data derived from multiple strains should be used to provide a framework against which seasonal strain changes can be assessed and to which data from additional seasonal strain changes will be added as they become available.

Seasonal influenza vaccines are produced either in specific pathogen free (SPF) embryonated hens' eggs or on cell culture substrates. Seasonal influenza vaccines shall be compliant with the European Pharmacopoeia (Ph. Eur.) general monograph 0153 Vaccines for human use, as well as the relevant Ph. Eur. specific monographs for inactivated egg-derived[1] and inactivated cell culture-derived[2] influenza vaccines, as appropriate.

Wherever possible, tables should be used to summarise the available data for a given process/parameter across all strains. Further details of the summarised data expected in each section of the dossier are shown in the relevant sections below.

4.1.1.1.1 Candidate Vaccine Virus (CVV)

The Australian Influenza Vaccines Committee meets annually to make recommendations for the composition of influenza vaccines for the Southern Hemisphere (SH) season. Following this, the TGA makes a decision regarding the vaccine composition for Australia.

Consequent to these recommendations, the TGA considers viruses or reassortants identified from global surveillance data and recommendations from the WHO Influenza vaccine website to be suitable vaccine strains for the relevant SH season.

In accordance with EMA/CHMP/BWP/310834/2012 Rev.1, antigenic similarity between the wild-type donor strain and the Master Virus Seed should be demonstrated by two-way Hemagglutination Inhibition (HI) testing against the WHO recommended strain. Two-way HI testing should be performed by a WHO Collaborating Centre (CC) and certification should be provided confirming the virus is like the WHO recommended strain.

4.1.1.1.2 Vaccine seed lots

Production

Data should be provided on the source of the Candidate Vaccine Virus (CVV) and the passage history of the virus in the manufacturer's facility. All available information on the CVV should be included in the dossier. Note that the passage level for the working seed is calculated from the number of passages after the passage at which the antigenic/genetic characterisation of the CVV was completed. If the adopted standard is the European Pharmacopeia[1,2] then the following applies:

[1] Ph. Eur. 0158 Influenza Vaccine (Split Virion, Inactivated), Ph. Eur. 0869 Influenza Vaccine (Surface Antigen, Inactivated), Ph. Eur. 0159 Influenza Vaccine (Whole Virion, Inactivated), Ph. Eur. 2053 Influenza Vaccine (Surface Antigen, Inactivated, Virosome).

[2] Ph. Eur. 2149 Influenza Vaccine (Surface Antigen, Inactivated, Prepared in Cell Cultures)

> 'The production of vaccine is based on a seed-lot system. Working seed lots represent not more than 15 passages from the approved reassorted virus or the approved virus isolate. The final vaccine represents 1 passage from the working seed lot.'

Qualification

The EMA guideline indicates that the haemagglutinin (HA) and neuraminidase (NA) antigens from each seed lot are identified by suitable methods. Ph. Eur. 0158 and Ph. Eur. 0869 states the following:

> 'The haemagglutinin and neuraminidase antigens of each seed lot are identified as originating from the correct strain of influenza virus by suitable methods.'

It also states for the monovalent pooled harvests that:

> 'The presence and type of neuraminidase antigen are confirmed by suitable enzymatic or immunological methods on the first 3 monovalent pooled harvests from each working seed lot.'

Based on the above guidance, it is TGA's expectation that suitable characterisation of HA and NA be provided for all working seed lots. It is recommended that characterisation of the NA be performed on at least the first three (3) batches of monovalent pooled harvest (MPH) produced from all working seed lots.

Testing for extraneous agents

Please refer to Appendix 3: Control of contamination with extraneous agents

4.1.1.1.3 Substrate for vaccine virus manufacture

For routine production, the virus of each strain is propagated in SPF embryonated hens' eggs from healthy flocks or in a diploid or continuous cell line.

Testing for extraneous agents

Please refer to Appendix 3: Control of contamination with extraneous agents

4.1.1.1.4 Manufacturing development

The EMA guideline discusses the occurrence of aggregation in either the drug substance or drug product in this section. This is discussed further in the current document under Section 4.1.1.1.6.

4.1.1.1.5 Process validation

Safety risks associated with influenza vaccines are most likely to arise from incomplete inactivation and splitting of the vaccine virus; both inactivation and splitting are identified as critical process steps in the EMA guideline. Inactivation is carried out to ensure that the product contains no live virus and splitting to ensure that whole virus is 'split' into less reactogenic structures. Effective inactivation of the virus under the manufacturing conditions should be clearly demonstrated.

Likewise, acceptable splitting of the virus should be demonstrated under the selected manufacturing conditions. Any inactivation that occurs as a result of the splitting process should also be detailed/quantitated if this is used as part of any risk assessment.

Process validation data should be submitted for at least three (3) consecutive commercial scale production batches. Where justified, pilot scale batches may be used if equivalence between the pilot and commercial processes is demonstrated.

It is advisable, that the following data be summarised in this section for all available strains:

- Inactivation of the vaccine virus - data on the maximum reduction in titre observed during the inactivation step for all seasonal strains to which the current production process applies.
- Splitting of the virus – quantitative (ideally percentage of split virus) data on the proportion of whole virus in the zonal pool/inactivated zonal pool that is split by the current production process. If electron microscopy is used as the primary method to determine the level of splitting, then the quantitation approach must be clearly described in the Validation of Analytical Methods section.

Similarly, it is expected that for any other analytical methods, the approach used to quantify the test should be clearly and adequately described within the Validation of Analytical Methods section.

- Splitting of the virus – data on the maximum reduction in titre observed during the splitting step for all seasonal strains to which the current production process applies (if applicable).

4.1.1.1.6 Characterisation

The EMA guideline provides the general framework against which characterisation studies are to be undertaken:

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

As indicated, strain- and product-specific characteristics may exist; therefore, not all characteristics are expected to apply to every strain, subtype or product. As indicated in the EMA guidelines *'Marketing authorisation holders should take account of scientific and technical progress'* and so, as new data becomes available it is possible that characterisation studies may evolve over time. Nevertheless, characteristics that are chosen should generally, be readily quantifiable and of relevance to the categories of characteristics outlined below. For clarity, the type of studies expected for drug substance and drug product are shown separately below.

Drug substance

Primary characterisation would generally be more extensive for the drug substance than the drug product. The following characteristics provide a starting point for the primary characterisation of the drug substance:

- Antigen content for the major viral antigens. While the HA content of drug substance is routinely estimated from single-radial immunodiffusion (SRID), consideration should also be given to estimating the total content of HA by other methods along with verifying 'the biological, immunological and physiochemical properties of the HA antigen' (EMA guideline page 9).
- Attempts should also be made to characterise the NA antigen beyond confirmation of its presence and identity. Ideally the NA antigen would be quantified and consideration given to its biological, immunological and physiochemical properties.
- Total protein content and the concentration of impurities derived from both the vaccine-virus and the production substrate should be estimated.
- Aggregates should be investigated as indicated on page 9 of the EMA guideline, 'Where present in the drug substance and/or drug product, aggregates should be investigated, e.g. in terms of diameter, composition, content and dissolution profile. Considerations should be given to the safety and immunogenicity of a formulation containing such particles.'
- It is recommended that the presence of other process related impurities (for example, residual formaldehyde from inactivation) be also identified and wherever possible quantified.

The original submission would as a minimum address all of the above categories for the drug substance and provide a comprehensive primary characterisation. If certain characteristics do not apply to a specific substance, then the submission should include supporting justification and/or evidence.

Consideration of the above characteristics should also be given to the drug substance through the duration of its shelf life.

Drug product

The primary characterisation of the drug substance will invariably inform characteristics of the drug product that should be considered during its primary characterisation. In the absence of data from the primary characterisation of the drug substance, the following would generally be expected to apply to the characterisation of the drug product i.e. those characteristics of the drug substance that may be expected to change through the formulation and filling of the drug product:

- Aggregates should be investigated as indicated on page 9 of the EMA guideline, 'Where present in the drug substance and/or drug product, aggregates should be investigated, e.g. in terms of diameter, composition, content and dissolution profile. Considerations should be given to the safety and immunogenicity of a formulation containing such particles.'

The original submission is expected to address all of the above categories for the drug product and provide primary characterisation. Where certain characteristics are not applicable to a particular product, supporting justification and/or evidence should be provided.

Consideration of the above characteristics should also be given to the drug product through the duration of its shelf life and in both the final bulk and filled-product stages of the production process.

Seasonal characterisation protocols

The primary characterisation of the drug substance and drug product should serve as the baseline for determining parameters to be assessed as part of the Annual Strain Update (ASU). The characterisation protocol established for the drug substance is expected to be applied to process changes and may also be used within the stability program. It is advisable to use the baseline to define the parameters for initial characterisation studies of the drug product. Additional characterisation studies should also be considered following process changes to the drug substance or drug product and as part of the drug product stability program.

In some instances, it may also be necessary to assess the effect of storage of the drug substance and the drug product on some characterisation parameters. This is particularly important for parameters expected to change over time, such as aggregate size and distribution and levels of potentially unstable impurities.

It is advisable to provide a characterisation protocol summarising the studies conducted for the drug substance and drug product as part of the ASU. This protocol is expected to be applied to the first three (3) batches of drug substance produced from each new strain. Depending on the chosen characteristics, its application (or parts thereof) should also be considered for manufacturing process changes.

4.1.1.1.7 Presentation

If there is a need for a presentation adapted to target a specific population this should be supported by appropriate quality data which should include compatibility, process manufacturing validation and stability data.

4.1.1.1.8 Vaccine standardisation

As indicated in the EMA guideline, the quantification of HA by SRID is the internationally recognised method to estimate vaccine antigen content and 'the intent of the SRID assay is to assure a consistent HA antigen content and antigenicity'. Towards this end, it is expected that the primary validation of the SRID should demonstrate that the specific method chosen (including the stipulated method for zone size measurement and analysis) is able to deliver the required specificity, accuracy, precision (intermediate precision and repeatability) and linearity across a suitable range of drug substances and drug products, using a range of reference reagents. The robustness of the assay should also be examined to ensure it is able to deliver comparable results across a range of small variations in the method parameters.

The primary validation should be designed to show that the basic method is able to deliver the required results within the context of its normal use, verification of the assay will be required as the result of any seasonal strain changes (see Section 4.1.1.2.1.6). The primary validation should also aim to highlight any potential features of the assay which may introduce bias into the assay as a result of a seasonal strain change.

The verification of the assay as part of the ASU is detailed in Section 4.1.1.2.1.6 and Appendix 2.

4.1.1.1.9 Adjuvants

Where an adjuvant system is used, reference should be made to the Committee for Medicinal Products for Human Use (CHMP) Guideline on Adjuvants in Vaccines for Human use (EMEA/CHMP/VEG/134716/2004). Since the origin and nature of adjuvants currently being used or developed is highly diverse, where relevant monographs of the Ph. Eur. exist, they should be adhered to. Manufacturing quality related data for the adjuvant should be presented in a self-standing section of the CTD dossier (preferably in 3.2.P) using the same relevant subsections as for the active substance. A risk-based approach will be applied to assess data acceptability which is dependent on the complexity of the adjuvant. The Sponsor is responsible for determining whether a GMP clearance or licence is required for the manufacture of the adjuvant.

4.1.1.1.10 Stability / Shelf life

Guidance for the studies to be undertaken to support storage times and shelf life are detailed in the EMA guideline. In addition to stability data supporting the drug product shelf life, data are also required to support excursions from the approved 2-8°C storage conditions, particularly during transportation. Particular attention should be given to the TGA's guidance on Stability testing for prescription medicines (see particularly 14.4 Biological medicines: specific requirements). It is highly recommended that stability studies be designed to include excursions at temperatures both above 8°C and below 2°C. It is also highly recommended to include stability data performed under real time conditions from a minimum of three (3) batches to support these temperature excursions.

The TGA will not accept shelf life claims or proposed temperature excursions using data from accelerated stability studies since the degradation of biological medicines is not usually amenable to kinetic analysis and extrapolation from accelerated testing. These should be submitted as variations under s9D(3) and be reflected on the ARTG and in an updated CPD.

The relevant sections of the dossier should include a commitment to a post-approval stability protocol for both the drug substance (3.2.S.7) and the drug product (3.2.P.8) and indicate the number of batches to be tested annually for continuing and new strains.

4.1.1.2 'Annual update' application for a seasonal vaccine

Only changes related to the new influenza strains may be introduced. No other changes are allowed to be processed via this 'fast track' procedure.

4.1.1.2.1.2 Vaccine seed lots

Updated risk assessment – Please refer to Appendix 3: Control of contamination with extraneous agents

4.1.1.2.1.3 Manufacturing development

Formulation targets should be included in the quality module (i.e. in Section 3.2.P.3.2 Batch Formula). It is advisable that the actual formulation target for each drug substance in the drug product be presented rather than the nominal content. Justification for any overage applied to each strain should be provided, taking into account the known or predicted stability of the drug substance and drug product as well as other relevant factors.

4.1.1.2.1.4 Process validation / process consistency

As indicated in Section 4.1.1.1.5, inactivation and splitting are critical processes in the manufacture of safe and efficacious influenza vaccines and need to be satisfactorily validated for new strains. Validation is expected to comprise at least three (3) batches for each new strain.

Batch analysis results for the first three (3) batches produced from a new working seed should be provided in the quality module (Section 3.2.S.4.4). Ideally, these batches should also be used for the characterisation studies outlined below in Section 4.1.1.2.1.5.

4.1.1.2.1.5 Characterisation

It is advisable that at least three (3) batches of drug substance from new vaccine strains are assessed according to the Characterisation protocol described in Section 4.1.1.1.6. Ideally these three (3) batches of drug substance would be the same batches used for the batch analysis described in Section 4.1.1.2.1.4. It is recommended that characterisation data should be provided in Section 3.2.S.3 of the dossier. Where possible, Section 3.2.S.3 should also contain a summary of all data previously generated under the characterisation protocol indicated in Section 4.1.1.1.6 above.

4.1.1.2.1.6 Vaccine standardisation

The EMA guideline indicates that verification of the SRID test should be undertaken when the analytical procedures are potentially impacted by strain changes. For new strains, full verification is required in accordance with the EMA guideline (a more detailed description of the verification is provided in Appendix 2). Ideally, the verification should be performed for both drug substance and drug product. In the case of the drug product, verification needs to be performed for all strains as changes to vaccine strain-composition may impact the analytical procedure for continuing strains. It is therefore highly advisable that verification is performed to assess the impact on all strains in the final product.

The verification process should focus on ensuring that the inclusion of new strains does not introduce any assay bias.

Reagents may also change between seasons, and this can result in changes in assay performance when either the antigen and/or the antiserum are involved. When either antigen and/or antisera are changed for a particular strain, it is highly recommended that, as a minimum, a bridging study be carried out. The bridging study should be designed to show the effective equivalence of the old and the new reagents for the assay of the drug product. However, as indicated in Appendix 2, bridging studies alone are unlikely to provide sufficient verification of the assay when there have been strain changes.

4.1.1.2.1.7 Stability / Shelf life

The EMA guideline indicates that both real-time (at recommended storage temperature) and accelerated data are provided for the drug substance MPH. This data supports the shelf life of the MPH and contributes to the vaccine quality database. Manufacturers are therefore encouraged to summarise data from previous vaccine strains in the relevant section of the dossier.

Stability studies on the drug product should be carried out using the vaccine filled into the container closure system intended for release into the market. These studies should include both real-time and accelerated data and are intended to support the shelf life of the drug product.

The relevant sections in the Module 3 should include a summary of stability studies currently being conducted and the final report(s) from any studies completed since the last annual strain update. Interim reports do not need to be included with the annual strain update.

It is advisable that final reports present HA stability data both as absolute amounts and as percentage loss relative to the initial HA content. The final study report analysis should also include an estimate of the rate of HA loss through the study and a description of the method used to calculate the rate. This data should ideally be provided in the Summary Tables in Sections 3.2.S.7.3./3.2.P.8.3 (% loss data at each time point) or as footnotes to those tables (% rate-loss and method of calculation). Ideally, the

summary table should include a reference to the source report for the data, along with the reagents used during testing. The report should specify the reagents used for HA stability testing and note any changes in reagents used over the course of the testing program. Preferably, the details of the method of container storage should be included for example needle up, plunger up etc. and whether any of the packing was retained during storage.

4.2 Live attenuated influenza vaccines

4.2.1 Seasonal influenza vaccines

Live attenuated influenza vaccines are produced in SPF embryonated hens' eggs. Live attenuated influenza vaccines shall be compliant with the Ph. Eur. monograph 0153 Vaccines for human use and the Ph. Eur. monograph 2772 Influenza Vaccine (Live, Nasal).

4.2.1.1 Marketing Authorisation application for a seasonal influenza vaccine

This section outlines the data that are expected to form the core of the primary submission for the vaccine and which consequently form the basis for subsequent updates (see Section 4.2.1.2 'Annual update' application for a seasonal vaccine).

Please also refer to Section 4.1.1.1 Marketing Authorisation application for a seasonal influenza vaccine of this document.

4.2.1.1.1 Live attenuated parent strain

The Australian Influenza Vaccines Committee meets annually to make recommendations for the composition of influenza vaccines for the Southern Hemisphere (SH) season. Following this, the TGA makes a decision regarding the vaccine composition for Australia.

Consequent to these recommendations, the TGA considers viruses or reassortants identified from global surveillance data and recommendations from the WHO Influenza vaccine website to be suitable vaccine strains for the relevant SH season.

In accordance with EMA/CHMP/BWP/310834/2012 Rev.1, antigenic similarity between the wild-type donor strain and the Master Virus Seed should be demonstrated by two-way Hemagglutination Inhibition (HI) testing against the WHO recommended strain. Two-way HI testing should be performed by a WHO Collaborating Centre (CC) and certification should be provided confirming the virus is like the WHO recommended strain.

4.2.1.1.1.1 Development

Sponsors are requested to provide detailed documentation on the history of all biological agents used, i.e. viruses, cells and raw biological materials, the method of preparation and a complete list of all starting materials used for construction of the attenuated parent strain.

If the adopted standard is the Ph. Eur. monograph 2772, then the following applies:

The WHO reviews the world epidemiological situation annually and if necessary, recommends new strains corresponding to this epidemiological evidence.

Such strains are used in accordance with the regulations in force in the signatory States of the Convention on the Elaboration of a European Pharmacopoeia (noting that Australia is an observer of the European Pharmacopoeia Commission).

The attenuated donor virus strain and the attenuated vaccine virus strain may be generated by the manufacturer itself by classical reassortment methods or reverse genetics (e.g. plasmid rescue). The wild type virus strains used for the production of the attenuated vaccine virus seed lots must have been approved by the TGA.

The complete history of production of the attenuated vaccine virus strain including description of the derivation of the seeds from the attenuated donor virus strain(s) and the WHO recommended wild virus strain(s) shall be approved by the TGA.

4.2.1.1.1.2 Characterisation

The EMA guideline provides the general framework against which characterisation studies are to be undertaken:

The comprehensive investigation and detailed documentation of the phenotypic and genetic properties of the attenuated parent strain should be documented in detail in the dossier.

Phenotypic characterisation should include studies on the markers for attenuation (performed *in vivo*) and on the cold-adapted (*ca*) or temperature sensitive (*ts*) phenotype (performed *in vitro*). Such studies, conducted under conditions that enable the detection of revertants to wild type, are essential for demonstrating the absence of neurovirulent capacity. It is expected that these investigations will be performed in suitable animal models, addressing both the potential for direct neurovirulence from the vaccine virus and the risk of indirect neurovirulence arising from secondary infections (Ph. Eur. 2.6.18). The suitability of small animal species for neurovirulence testing should also be carefully considered. The genetic characterisation of the attenuated parent strain includes:

- Determination of the nucleotide sequence of the complete viral genome
- Analysis of the molecular basis of the attenuated phenotype
- Demonstration of the genetic stability of the attenuated parent strain by comparison of the nucleotide sequence of the viral genome at different passage levels.

Where initial characterisation studies of naturally cloned variants reveal issues that may adversely affect the manufacture of LAIV, alternative approaches may be considered to improve the biological characteristics of the parent virus strain, provided the approach has prior TGA approval.

Issues identified in the naturally cloned variants may include but not be limited to: poor receptor binding as determined by a relevant human cell model for Tissue Culture Infectious Dose ($TCID_{50}$), suboptimal HA/NA balance, or antigenic mismatch resulting from egg adaptation. Alternative methods to improve biological characteristics will be considered on a case-by-case basis, provided it can be demonstrated that the modified cloned variant is antigenically similar to the reference wild-type virus. This should be confirmed by two-way HAI antigenicity test conducted at a WHO CC and a certification provided that the virus is like the WHO recommended strain.

Particular attention should also be given to the testing for extraneous agents. These assessments must be conducted in accordance with the prevailing requirements and guidelines, including those pertaining to viral seeds, cell substrates, sterility, and mycoplasma testing, to ensure the integrity and safety of the material under investigation. Please refer to Appendix 3: Control of contamination with extraneous agents.

4.2.1.1.1.3 Vaccine seed lots

For production of live attenuated influenza vaccine, information should be provided regarding the establishment of the seed lot system, including the use of eggs sourced from SPF flocks in accordance with Ph. Eur. 5.2.2. The dossier should contain a comprehensive description of the methods used for preparation, as well as details on the validation of storage conditions, specifically with respect to virus concentration, genetic stability and sterility. Where reverse genetics technology is employed, it is recommended that cDNA clone banks for the six RNA segments derived from the parent strain (M, NS, NP, PA, PB1 and PB2) be established for the manufacture of reassortant viruses.

A stability program should be implemented for the seed lot system to monitor quality attributes, especially where long term storage is anticipated.

Testing for extraneous agents in seed lots of the attenuated parent strain should be conducted in accordance with the relevant Ph. Eur. monographs, including those addressing extraneous agents in viral seeds, sterility and mycoplasma testing. Subject to agreement with the TGA, these assessments may alternatively be performed at the level of the reassortant working virus seed.

It is TGA's expectation that suitable characterisation of HA and NA be provided for all vaccine seed lots.

Testing for extraneous agents

Please refer to Appendix 3: Control of contamination with extraneous agents.

If the adopted standard is the Ph. Eur. monograph 2772, then the following applies:

The production of the attenuated master seed lot (MSL) must be approved by the TGA. The attenuated MSL must have the same characteristics as the attenuated donor virus strain. The number of passages required to produce the attenuated MSL from the attenuated donor virus strain is limited and approved by the TGA. Unless otherwise justified and authorised, the inoculum for infecting the eggs used in the production of a vaccine lot shall be a virus harvest without intermediate passage, so that no vaccine virus is more than 1 passage from an attenuated MSL that has passed all safety tests.

4.2.1.1.2 Wild type influenza HA and NA donor strain

4.2.1.1.2.1 Isolate and passage history

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

Antigenic similarity of the wild-type (WT) donor strain (by two-way HI testing) to the WHO/CHMP recommended strain will have been performed and documented by a WHO CC.

4.2.1.1.2.2 Seed Lots

Seed lots of the donor strain should be derived from the primary isolate by propagation in eggs or in controlled and certified cell substrates (refer to Ph. Eur. 5.2.3). Eggs used for propagation of the donor strain should be from hens certified to be free from specified pathogens (SPF).

4.2.1.1.2.3 Testing of seed Lots

The following tests should be performed on the final seed lot of an influenza HA and NA donor strain that will be used for reassortment with the live attenuated parental influenza strain:

- Identity test for HA and NA
- Test to demonstrate the absence of extraneous agents in the donor strain seed lot according to current Ph. Eur. monographs.
- Specific screening for human respiratory agents able to replicate in eggs should also be performed. For this, a validated multiplex PCR could be developed. If agreed with the TGA, these tests can also be performed at the level of the reassortant working virus seed.
- Confirmation of no microbial contaminants, as the presence of any microbial agent in the seed lots of the attenuated parent strain and of the donor strain is not acceptable.

If the adopted standard is the Ph. Eur. monograph 2772, then the following applies:

The attenuated MSL must express the haemagglutinin and neuraminidase from the wild type virus strain and other proteins from the attenuated donor virus strain.

The attenuated MSL characterisation shall include the following tests:

- genotype analyses using validated nucleic acid amplification techniques (2.6.21)

- virus sequencing of the seed lot and comparison of the coding sequences as follows; the sequences of the haemagglutinin and neuraminidase genes with those of the recommended strains and the sequences of the 6 remaining genes with those of the attenuated donor strain
- genetic stability by sequencing, cold adapted and temperature sensitive phenotypes determination and attenuation test upon several passages in the substrate.

Only an attenuated MSL that complies with the requirements described in the Ph. Eur. monograph 2772 may be used in the preparation of the harvest.

4.2.1.1.3 Preparation of the live attenuated reassortant virus

Detailed information should be provided regarding the process of reassortment between the live attenuated parent strain and the influenza HA and NA donor strain, whether utilising classical techniques or reverse genetics methodology. The dossier should specify that only biological reagents—such as antisera and enzymes—certified to be free from infectious agents are used throughout the procedure. When reverse genetics methodology is employed, compliance with the principles and recommendations outlined in the Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014) is required. Additionally, documentation must demonstrate the presence of the correct HA and NA derived from the influenza donor strain, which may be confirmed using specific antisera, certified reference reagents, or other validated methods.

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

A characterised clone of live attenuated reassortant virus is used for propagation in SPF embryonated eggs. A MSL and optionally a Working Virus Seed Lot (WSL) derived from the MSL should be established.

4.2.1.1.3.2 Characterisation of master and working virus seeds lots

Controls applied for identification and characterisation of live attenuated reassortant virus seeds include those outlined in Section 4.2.1.1.1.2 Characterisation.

Antigenic similarity of the MSL (by two-way HI testing) to the WHO/CHMP recommended strain will have been performed and documented by a WHO CC.

The genetic stability of each new virus seed lot requires assessment by sequence analysis. Genetic stability parameters include retention of the defined phenotypic properties and the genetic structure of the attenuated parent strain throughout seed lot production and for at least five passages at the production level.

Lack of neurovirulence of the live attenuated reassortant virus should be demonstrated. Justification should be given if the test is not performed or replaced with an alternative test.

If the adopted standard is the Ph. Eur. monograph 2772, then refer to Section 4.2.1.1.2.3 Testing of Seed Lots for the required tests.

4.2.1.1.4 Substrate for virus propagation

Influenza virus used in the preparation of seed lots is propagated in fertilised eggs from SPF chicken flocks (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.

Testing for extraneous agents

Please refer to Appendix 3: Control of contamination with extraneous agents.

4.2.1.1.5 Vaccine production

Aseptic production conditions strictly avoiding contamination of the production system with extraneous

agents must be adhered to. Testing for the absence of extraneous agents can be performed on uninoculated control eggs that are incubated in parallel to production eggs or single harvests on suitable cell substrates in the presence of influenza HA neutralizing antibodies.

4.2.1.1.5.1 Egg substrate used for production

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

4.2.1.1.5.2 Virus harvest

Virus harvests may only be used for further production if they meet acceptable bioburden specifications and are derived from seed lots that comply with HA identity testing.

With prior agreement from the TGA, tests for bioburden and mycoplasmas can be performed at an appropriate stage following downstream processing of the single virus harvest. A specification should be provided for potency, e.g. determination of the egg infectious dose (EID_{50}) of live attenuated virus/mL of chorio-allantoic fluid or assay specific unit such as Fluorescence Focus Units.

If the adopted standard is the Ph. Eur. monograph 2772, then the following applies:

Only a single harvest or a MPH that complies with the following requirements may be used in the preparation of the monovalent bulk:

- Extraneous agents (Ph. Eur. 2.6.16)
- Avian leucosis viruses (Ph. Eur. 2.6.16)
- Microbiological contamination (total viable aerobic count and verify the absence of yeast and mould. Absence of *Vibrio*, *Shigella* and *Salmonella* is carried out using supplementary specific validated techniques agreed by the TGA).

4.2.1.1.5.3 Monovalent bulk vaccine

Tests on the monovalent bulk vaccine include:

- Identity testing for HA and NA
- Tests on the retention of genetic markers
- Confirmation of the phenotype of the live attenuated virus.

If the adopted standard is the Ph. Eur. monograph 2772, then the following applies:

Only a monovalent bulk that complies with the following requirements may be used in the preparation of the final bulk vaccine:

- Identification
- Virus concentration
- Cold adapted and temperature sensitive phenotype
- Attenuation test
- Genotyping
- Bacterial and fungal contamination
- Total protein content.

Seasonal characterisation protocols

The primary characterisation of the drug substance and drug product should serve as the baseline for determining parameters to be assessed as part of the annual strain update (ASU). The characterisation protocol established for the drug substance is expected to be applied to process changes and may also be used within the stability program. It is advisable to use the baseline to define the parameters for initial characterisation studies of the drug product. Additional characterisation studies should also be considered following process changes to the drug substance or drug product, and as part of the drug product stability program.

In some instances, it may also be necessary to assess the effect of storage of the drug substance and the drug product on some characterisation parameters. This is particularly important for parameters expected to change over time, such as aggregate size and distribution and levels of potentially unstable impurities.

It is advisable to provide a characterisation protocol summarising the studies conducted for the drug substance and drug product as part of the ASU. This protocol is expected to be applied to the first three (3) batches of drug substance produced from each new strain. Depending on the chosen characteristics, its application (or parts thereof) should also be considered for manufacturing process changes.

4.2.1.1.5.4 Trivalent bulk vaccine / final vaccine

Trivalent bulks must meet tight specifications for potency and both ovalbumin content and bacterial endotoxin concentration must also comply with defined specifications. Thermal stability of the final vaccine should be adequately documented in real time stability studies and in studies at elevated temperatures. An end of shelf life specification should be defined and adequately justified.

If the adopted standard is the Ph. Eur. monograph 2772, then the following applies:

A final bulk vaccine is formulated aseptically from appropriate quantities of the monovalent bulks of each virus strain.

The final bulk vaccine is distributed aseptically into sterile, tamper-evident containers. Where a final bulk vaccine is formulated as a release intermediate, it complies with the following requirements and is within the limits approved for the particular product. A suitable stabiliser may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot:

- Bacterial and fungal contamination (Ph. Eur. 2.6.1)

4.2.1.1.6 Process validation

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

4.2.1.1.7 Characterisation

The EMA guideline provides the general framework against which characterisation studies are to be undertaken.

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

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

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

4.2.1.1.8 Presentation

If there is a need for a presentation adapted to target a specific population this should be supported by appropriate quality data which should include compatibility, process manufacturing validation and stability data.

4.2.1.1.9 Vaccine standardisation

As indicated in the EMA guideline, the determination of potency, the number of infectious virus particles per vaccine dose e.g. EID_{50} or $TCID_{50}$ (tissue culture infectious dose or other assay specific unit) should be determined using an appropriate virus reference preparation. Particular consideration should be given to assay specificity, e.g. suitable reference reagents for each subtype should be used.

Towards this end, it is expected that the primary validation of the potency assay should demonstrate that the method chosen is able to deliver the required specificity, repeatability, intermediate precision and reproducibility across a suitable range of drug substances and drug products, using a range of reference reagents. The potency assay should be evaluated over an appropriate range to demonstrate a proportional relationship between the measured results and the true (known or theoretical) sample values. It should be noted that specificity is determined by the qualification of the specificity of the polyclonal and monoclonal antibodies for subtypes used in the potency assay.

The primary validation should be designed to show that the basic method is able to deliver the required results within the context of its normal use, verification of the assay will be required as the result of any seasonal strain changes (see Vaccine standardisation in Section 4.2.1.2.1.4). The primary validation should also aim to highlight any potential features of the assay which may introduce bias into the assay as a result of a seasonal strain change.

4.2.1.1.10 Stability / Shelf life

Guidance for the studies to be undertaken to support storage times and shelf life is detailed in the EMA guideline and recommend stability data be developed according to Ph. Eur. monograph 0153 *Vaccines for Human Use* and ICH Q5C Stability data. Stability data, comprising studies under real-time and accelerated conditions, should be presented for drug substance (monovalent bulk) and drug product (final formulated vaccine) in support of the maximum storage time and shelf life, respectively.

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

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

In addition to stability data to support the shelf life of the drug product, data are also required to support excursions from the approved storage conditions (e.g. 2-8°C) particularly excursions during transportation after thawing. Attention is drawn to the TGA's guidance on Stability testing for prescription medicines (see particularly 14.4 Biological medicines: specific requirements). It is highly recommended that studies be designed to include excursions at temperatures both above and below the approved storage conditions. These should be submitted as variations under s9D(3), if not included during registration and be reflected in an updated CPD.

The relevant sections of the dossier should include a post approval stability protocol for the drug substance (Section 3.2.S.7) and drug product (Section 3.2.P.8) and indicate the number of batches to be tested annually for both continuing and new strains.

4.2.1.2 'Annual update' application for a seasonal vaccine

4.2.1.2.1 First step submission – “Quality”

4.2.1.2.1.1 Wild type influenza HA and NA donor strain / preparation of the live attenuated reassortant virus

Updated risk assessment - Please refer to Appendix 3: Control of contamination with extraneous agents.

Please also refer to the Guideline on influenza vaccines – submission and procedural requirements - Regulatory and procedural requirements module (EMA/56793/2014)[3].

Production history of the seed should be included in Section 3.2.S.2.3 Control of Materials. Relevant information includes:

- Description of the derivation of the seed starting from master attenuated donor virus and WHO recommended strain(s)
- Passage history
- Genetic sequence of the seed
- Phenotypic characterisation (including studies on the markers for attenuation (performed in vivo) and on the cold-adapted (ca) or temperature sensitive (ts) phenotype (performed in vitro) under conditions allowing the detection of revertants to wild type and identity tests for HA and NA)
- Genetic stability for the seed lot including relevant genotypic and phenotypic markers (e.g. full genetic sequencing)
- Antigenic similarity of the WT donor strain and the Master Virus Seed (by two-way HI testing) to the WHO/CHMP recommended strain, as performed and documented by a WHO CC
- Analytical protocols (including extraneous agents safety test); where the seed virus is tested for extraneous agents using PCR, and if in discussion with the TGA the need for additional PCR testing of the seed has been agreed, these data should be included in this application
- Neurovirulence test; neurovirulence testing of annual updates (i.e. antigenically drifted strains) is normally not required. Neurovirulence testing will be required if a new HA subtype of influenza A virus (i.e. non-H1, non-H3 subtype) or a novel influenza B virus differing from the currently circulating genetic lineages is included in the vaccine or in case specific safety concerns arise.

4.2.1.2.1.2 Manufacturing development

Any change to the vaccine production process due to specific new season strain characteristics should be included in the quality module in Section 3.2.S.2 Manufacture.

The formulation development and targets, including actual formulas for the new season’s strains and Certificate of Analysis when available should be included in Section 3.2.P.3.2 Batch Formula.

4.2.1.2.1.3 Process validation / process consistency

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

Batch analysis results of first three (3) monovalent bulks from each new seed lot intended for commercial production should be provided in the quality module in Section 3.2.S.4.4 Batch Analyses. Any alternative approach or deviation from relevant guidelines, including

[3] While it is acknowledged that this guideline has been superseded by Guideline on influenza vaccines - submission and procedural requirements, the TGA undertakes an extensive process of internal and external consultations to ensure the Guideline is consistent with prevailing requirements in Australia prior to adoption. It is advised that you check the TGA website for the adoption of the updated guideline (see International scientific guidelines adopted in Australia | Therapeutic Goods Administration (TGA)).

EMA/CHMP/BWP/310834/2012 Rev.1, may be considered on a case-by-case basis, provided the Sponsor submits a valid scientific justification. This must be discussed with and approved by the TGA prior to ASU submission.

Batch analysis results of Drug Product should be provided in Section 3.2.P.5.4 Batch Analyses.

4.2.1.2.1.4 Vaccine standardisation

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

For potency testing using the Fluorescent Focal Assay (FFA), the primary or secondary antibodies may require updating to match the new season's strains. When either the primary or secondary antibodies are changed for a particular strain, qualification certificates are required. Justification of suitability of selected antibodies for the new strain needs to be demonstrated (i.e. provision of validation data).

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

4.2.1.2.1.5 Stability / shelf life

Please refer to Section 4.1.1.2.1.7. Stability / Shelf life as the general requirements are applicable for stability testing of the monovalent bulks and drug product filled into the container closure system except for reporting % losses against HA content.

The shelf life of the drug product filled into the container closure is determined from real-time stability studies. Potency of all influenza virus strains in the drug product should be determined according to the stability protocol approved by the marketing authorisation holder.

Thermal stability should be investigated according to Ph. Eur. 2772 to ensure that for each virus strain, the virus concentration does not decrease by more than the approved amount during the period of exposure.

Appendix 1: Procedural and sampling requirements for Annual Strain Update (ASU) submissions for seasonal influenza vaccines

For changes that require submission of data under s 9D(3), the quality updates to the Module 3 included in the submission should meet the requirements of TGA's Minor variations to prescription medicines - biological medicines. In addition, the information provided should be consistent with the guidance provided in the EMA Guideline on Influenza Vaccine – Quality Module (EMA/CHMP/BWP/310834/2012) as outlined above.

TGA prefers electronic submissions for influenza annual strain updates (ASUs). Please refer to TGA's guidance for electronic submissions. Ideally a baseline submission in e-CTD format should be made for the product to ensure that a full copy of the Module 3 is available at the time of evaluation thereby potentially reducing the number of requests for information and evaluation timeframes.

Annual Strain Updates (ASUs)

Any changes to the recommended strains for SH vaccines will result in a change to at least one of the approved reassortants or viruses used for production. It is recommended that Sponsors provide Category 3 submissions with the relevant details on the strain change(s) and other quality changes associated with the strain change(s) including labelling and PI changes to reflect the new influenza strain/s.

Generally, it is recommended that submissions of quality data (outside of the labelling and PI update) be submitted as a single package as it ensures efficient evaluation and issues arising can be clarified earlier rather than later within the evaluation period. If a separate strategy is to be taken, then the Sponsor should approach the TGA as early as possible with a summary of the proposed strategy and the associated timeframes.

It is preferable that only updated sections of the CTD are submitted with the application and that a full copy of the current Module 3 is available at the time of the evaluation. In the absence of a full copy of the Module 3 being available through an e-CTD baseline submission, then the Sponsor may **either**:

- submit the complete Module 3 with one of the Category 3 submission indicated above. However, if this is to be done then the covering letter should include a statement that the full Module 3 has been supplied and a list of the updated sections for the forthcoming season has been provided; or
- submit the updated sections of the module only and then, after approval of all variations, submit a full e-copy of the Module 3.

The above guidance will facilitate an efficient review of the submission.

Even in the absence of changes to the recommended strains for production of SH vaccines there may be changes to the approved reassortant or virus to be used for recommended strains. In such cases, a Category 3 submission is required. However, if the change is only in the passage or lot number of the approved reassortant or virus then the conditions of TGA's Minor variations to prescription medicines - biological medicines for Self-Assessable Requests (SARs) may apply.

In some instances, there may be no changes to the strains recommended for SH vaccine but there may be a change to the working seeds previously approved e.g. generation of a new working seed lot. In such instances, the requirements of TGA's Minor variations to prescription medicines - biological medicines for SARs may apply for new seeds generated by a previously approved procedure. However, the seeds still need to meet the requirements of any relevant standards and data should be supplied.

Certified product details

As soon as is practicable after the approval of the ASU a copy of the Certified Product Details should be provided. Requests for a suitable template should be made to vaccines@health.gov.au and returned to the same address.

Annual product quality review

It is expected that at the completion of seasonal supply into the Australian market, an electronic copy of the Annual Product Quality Review is provided to the TGA. The completed review should be sent as a **single PDF** document to vaccines@health.gov.au

Sampling requirements

The current Official Control Authority Batch Release (OCABR) guidelines for Influenza Vaccine have been adopted by TGA (Batch release assessment of vaccines) with the following notation:

> 'Although released on OCABR certification, our laboratories may still test these batches as part of our batch release program. The sponsor must still supply samples, batch details and evidence maintaining acceptable shipping conditions for the batch under this pathway.'

The OCABR process involves assessment of manufacturing documentation (summary protocol review) and laboratory testing guidelines for specific influenza vaccine types (e.g., inactivated, split, live attenuated etc.). When the Sponsor provides evidence that the batch supplied in Australia has passed OCABR testing, the vaccine can be released without the TGA conducting a manufacturing protocol assessment or (potentially) laboratory testing. The Sponsor must still supply samples, batch details and evidence of the maintenance of adequate shipping conditions for the batch under this pathway.

In relation to the above, sponsors are requested to submit the following samples and information to assist in the batch release process based on vaccine type:

Inactivated influenza vaccines

a. Details of reference antigen/antisera to be used for the release testing for each subtype. These details should also be included in the manufacturer's batch release protocol supplied to the Laboratories Branch. If possible, reagents should be used as a 'kit', i.e. either TGA or NIBSC reagents are acceptable.

b. For non-TGA reagents, supply a minimum of 20 vials of reference antigen and 20 vials of antisera. **Additionally**, supply 1 vial of reference antigen and antisera for each batch that is intended for release in Australia. This should be supplied as one consignment. **Please note that sponsors should not have reference reagents shipped directly to TGA** and cannot import reference reagents using TGA's import permit, i.e. sponsors must obtain their own permit(s) and have reagents shipped *via* the sponsor.

c. Supply 3 batches of Monovalent Bulk (5 mL) for each strain included in the vaccine. These should be from recent batches and should be provided along with the associated protocol or Certificate of Analysis that states the HA content of the bulk.

d. For each batch that you intend to release in Australia supply the batch release protocol; the protocol need only be provided with the first consignment for release.

e. For all consignments for release complete the TGA 'Request for Release.' Copies of the 'Request for Release' template are available on request from vaccines@health.gov.au.

Where there are excursions outside of the approved storage conditions of 2-8°C the submission number under which such excursions have been previously approved should be quoted. Refer to the TGA guidance Stability testing for prescription medicines (Specifically, 14.4 Biological medicines: specific requirements). Where prior approval of temperature excursions have not been approved then additional data may be requested to support the release of the product into the market.

f. For each batch that is intended for release in Australia provide 20 samples in final packaging for 0.5 mL formulations and 40 samples for 0.25mL formulations from the first consignment received in Australia. Note that delivery of samples to TGA can only be accepted between the hours of 08:30 and 15:30 on normal business days.

g. For each subsequent consignment of a batch into Australia i.e. only differ in shipping details, additional samples are not required by the TGA. However, the TGA may request 10 additional samples of further consignments if they are associated with a quality issue, such as if the samples experience temperature deviations.

h. For any 'reworking' of samples, provide 20 samples from the first re-working operation. Samples are not required for any further re-working of the same final packaged lot. In addition to samples, Time Out of Refrigeration (TOR) documentation should be provided for each re-working operation.

i. The supply of pre-consignment samples in the same quantities as stated in part f. is optional. The provision of pre-consignment samples may expedite the release process by reducing the optimisation time for the assay of the drug product. However, pre- consignment samples are not usually accepted as replacements for any of the other samples requested above.

j. If the manufacturing batch has been released in Europe or United Kingdom a copy of the EU OCABR certificate (or equivalent from the UK) must be provided.

When an OCABR certificate has been provided for batches supplied in Australia, supply of samples, batch details and evidence of the maintenance of adequate shipping conditions for the batch are still required.

Live attenuated influenza vaccines

a. Details of reference LAIV Blend Control material and qualified primary/secondary antibodies to be used for the release testing for each subtype. These details should also be included in the manufacturer's batch release protocol supplied to the Laboratories Branch.

b. For reference LAIV Blend Control material, supply a minimum of 20 vials (100 µL) of control material. **Additionally**, supply 2 vials of Blend Control material for each batch that is intended for release in Australia. This should be supplied as one consignment. **Please note that sponsors should not have reference reagents shipped directly to TGA** and cannot import reference reagents using TGA's import permit, i.e. sponsors must obtain their own permit(s) and have reagents shipped via the sponsor. The corresponding qualification certificates should also be provided that states the included strains, target mean range for each strain and expiry date.

c. For FFA testing, supply a minimum of 20 vials (50 µL) of each qualified primary antibody and 5 vials (500 µL) of each qualified secondary antibody. Additionally, supply 1 vial of primary antibody for each batch that is intended for release in Australia. This should be supplied as one consignment. The corresponding qualification certificates should also be provided that states the comparator strain and expiry.

d. Supply 3 batches (or as previously approved by the TGA; see Section 4.2.1.2.1.3 Process validation / process consistency) of Monovalent Bulk (5 mL) for each strain included in the vaccine. These should be from recent batches and should be provided along with the associated protocol or Certificate of Analysis that states the target mean range for the bulk.

e. For each batch that you intend to release in Australia supply the batch release protocol; the protocol need only be provided with the first consignment for release.

f. For all consignments for release complete the TGA 'Request for Release.' Copies of the 'Request for Release' template are available on request from vaccines@health.gov.au.

Where there are excursions outside of the approved storage conditions the submission number under which such excursions have been previously approved should be quoted. Refer to the TGA guidance Stability testing for prescription medicines (Specifically, 14.4 Biological medicines: specific requirements). Where prior approval of temperature excursions has not been approved then additional data may be requested to support the release of the product into the market.

g. For each batch that is intended for release in Australia provide 20 samples in final packaging for 0.2 mL formulations from the first consignment received in Australia. Note that delivery of samples to TGA can only be accepted between the hours of 08:30 and 15:30 on normal business days.

h. For each subsequent consignment of a batch into Australia i.e. only differ in shipping details, additional samples are not required by the TGA. However, the TGA may request 10 additional samples of further consignments if they are associated with a quality issue, such as if the samples experience temperature deviations.

i. The supply of pre-consignment samples in the same quantities as stated in part g. is optional. The provision of pre-consignment samples may expedite the release process by reducing the optimisation time for the assay of the drug product. However, pre- consignment samples are not usually accepted as replacements for any of the other samples requested above.

j. If the manufacturing batch has been released in Europe or United Kingdom a copy of the EU OCABR certificate (or equivalent from the UK) must be provided.

When an OCABR certificate has been provided for batches supplied in Australia, supply of samples, batch details and evidence of the maintenance of adequate shipping conditions for the batch are still required

Appendix 2: Seasonal verification of the Single Radial Immunodiffusion – Advice on the annual update submissions for seasonal influenza vaccines

The following components have been identified as comprising the verification of the SRID as outlined in the EMA guidelines. Further guidance includes Note for Guidance on Validation of Analytical Procedures: Text and Methodology ICH topic Q2(R2) (EMA/CHMP/ICH/82072/2006) notably for Specificity, Accuracy, Precision, Linearity and Robustness.

Serum qualification

The study should demonstrate that the chosen serum is able to form clear measurable zones with the homologous antigen and that it forms a linear dose response over the intended range at which the reference antigen/drug substance/drug product will be used in the assay. The serum dilution chosen should ideally not form zones with non-homologous antigens included in the product. Ideally, the study report should include the standard initial dilution of reference antigen used for the assay of drug substance and drug product and the optimised antiserum dilution.

When a serum is replaced in the absence of any effective change to the reference antigen or the product then, it is recommended that a bridging study be completed that contains the above data for the serum along with a suitable comparison of assays on the drug product that directly compare the replaced and replacement serum. Ideally, the data should also compare the two sera in assays for the drug substance. It should be noted that bridging studies themselves are unlikely to provide sufficient information on the verification of the assay when there have been strain changes and that the remainder of this following procedure should also be followed.

Identity/specificity

Identity and specificity can generally be inferred from the data garnered from the Serum Qualification and Accuracy studies. Ideally, data should be provided for both reference antigens and drug substance (monovalent bulk/monovalent pooled harvest) to show that the antiserum used for a given strain is able to identify the homologous antigen alone

Accuracy

The study should ascertain the ability of the selected reference antigen and serum to quantitate the homologous drug substance when measured in suitable matrices. For example, an appropriate dilution of the drug substance spiked into vaccine diluent and into vaccine diluent + non-homologous drug substance(s). The optimal study design would comprise this experiment performed in triplicate for the drug substance at the intended target concentration for the assay of the drug product and at ± 20% of this target concentration.

Intermediate precision

Intermediate precision should ideally be determined for the assay of drug substance and drug product. Data from the study outlined under accuracy can provide appropriate data if a suitable design is chosen. As a minimum, data should be provided on the drug product.

Repeatability

Repeatability should ideally be determined for the drug substance and drug. As a minimum, data should be provided on the drug product.

Linearity

As a minimum, the study should demonstrate that for a fixed set of dilutions of the reference antigen (at the optimised antigen and serum dilutions) that the expected and observed values recovered from the assay of the drug product across the validated range of the assay has a linear relationship. Ideally similar data should be provided for the drug substance.

Robustness

Assay parameters that may be strain dependent and therefore affected by strain changes should be investigated. Such parameters may include, but are not limited to, stability of the reference antigen/product in the presence of Zwittergent and zone reading methodology. As a minimum, any parameters studied should be investigated with respect to the assay of the drug product.

Changes in reagents

There are often instances when there has only been a change of either anti-serum or reference reagents for the assay of a given strain. In such instance it is advisable that an appropriate bridging study be performed to show the previously verified and proposed reagents are effectively equivalent. It is sufficient to show that there is no significant difference in the results obtained in assays with the previously qualified and replacement antisera.

Appendix 3: Control of contamination with extraneous agents

A risk assessment should be conducted in compliance to applicable standards (e.g. pharmacopoeia monographs etc.) and provided in Section 3.2.A.2 of the quality module.

The risk assessment should include, but not be limited to, what control measures would be appropriate to ensure that the final vaccine product is free from extraneous agents. The risk assessment should:

1. Identify the points of manufacture where extraneous agents that may be harmful to humans have the potential to enter the manufacturing chain. For example, when human or animal origin materials are used.
2. Identify the extraneous agents that may be harmful to humans and have the potential to enter the manufacturing chain.
3. Where possible, estimate the potential input load of these extraneous agents (with clear explanations of the assumptions and methodology used and reference to supporting evidence).

If an estimation is not possible, provide a justification for why an estimation is not required to determine the acceptability of the residual risk from extraneous agents in the final product.

Summarise the control measures used to mitigate the risk of extraneous agent contamination. The control measures may include, but are not limited to:

Qualification of each raw material of human or animal origin.

Note: The criteria used to qualify each material to ensure that the potential input load is as low as practicable may vary (depending on applicable standards and guidelines) and should be justified. Examples of qualification are donor/animal selection, viral testing, BSE/TSE risk assessment, vaccination and disease surveillance programs.

In-process testing for extraneous agents to further control the potential input load of agents identified in 2) and conducted in accordance with applicable standards and guidelines (e.g. Pharmacopoeia).

Validation of the capacity of the manufacturing process to clear or inactivate the extraneous agents identified in 2) and compare the clearance or inactivation capacity with the potential input loads of these extraneous agents identified in 3), in accordance with existing guidance on virus validation studies.

Discussion on the contribution of each control measure and justify the acceptability of the residual risk from extraneous agents in the final product.

Any changes to the assumptions and parameters used in the risk assessment (e.g. new/emerging viruses potentially present in the clinical specimen/isolates or chickens, addition/removal of human or animal materials from the manufacturing chain, etc) should be incorporated in an updated risk assessment. The updated risk assessment should justify the continued acceptability of the residual risk in the final product as a result of the changes. The factors that lead to an update of the risk assessment should be clearly identified and the frequency by which the risk assessment is updated should be justified.

Any changes to the risk assessment that relate to seasonal strain updates may be submitted as part of an annual strain update (ASU) Category 3 application. All other changes that lead to an update of the risk assessment should be submitted as a Category 3 application.

For further clarification, please email the Blood Biologicals and Infectious Disease Unit (BBIDU) in the Biological Sciences Section (BSS) of the TGA at infectiousdiseasesafety@health.gov.au.

Version history

Version	Description of change	Author	Effective date
V1.0	Original publication	Laboratories Branch	August 2017
V2.0	Update to reflect current batch release requirements and control of contamination with extraneous agents	Laboratories Branch	September 2023
V3.0	Update to include Live attenuated influenza vaccines (LAIV) and 'Annual update' application for a seasonal LAIV sections. Update to batch release requirements relating to further consignments. Minor updates to improve clarity and consistency.	Laboratories Branch	August 2026

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