Australian Government
Department of Health and Aged Care
Therapeutic Goods Administration

Seasonal influenza vaccines – quality module

Explanatory document for the EMA guideline on influenza vaccine (EMA/CHMP/BWP/310834/2012) adopted by TGA on 1 November 2014

Version 2.0, September 2023

TGA Health Safety Regulation

Contents

1. Introduction

The TGA adopted the EMA Guideline on Influenza Vaccines – Quality Module (EMA/CHMP/BWP/310834/2012) on 1 November 2014. 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.

2. 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
- 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 seasonal updates (ASU)
- does **not** contain explanation on pre-pandemic, pandemic and live attenuated influenza vaccines, covered under sections, 4.1.2, 4.1.3 and 4.2 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, if required. Send requests to vaccines@tga.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.

3. Legal basis and relevant guidelines

You should also refer to other relevant European and ICH guidelines, European Pharmacopoeia Monographs and Chapters, WHO TRS for the Recommendations for the production and control of influenza vaccine (inactivated) and to 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. Wherever possible, tables should be used to summarise the available data for a given process/parameter across all strains for which data are available. Further details of the summary 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 shown on WHO Influenza vaccine website to be suitable vaccine strains for the relevant SH season.

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 then the following applies:

> "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. The Eur. Ph. 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."

On the basis of 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 batches of monovalent pooled harvest (MPH) produced from all working seed lots.

Testing for extraneous agents: Please refer to Appendix 4

4.1.1.1.3. Substrate for vaccine virus manufacture

Testing for extraneous agents: Please refer to Appendix 4

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 and 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 the result of the splitting process should also be detailed/quantitated if this is used as part of any risk assessment.

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 it is expected that the method by which the test is quantitated be adequately described in the validation of analytical methods. Similarly it is also expected that for any other analytical methods the method by which the test is quantitated is to be adequately described in the validation of analytical methods.
- 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 allow relevant specification to be established and may support the scientific evaluation of comparability after product or process changes have been introduced.

As indicated, there may be strain-specific characteristics and product-specific characteristics and it is therefore not expected that all characteristics will apply to all strains, subtypes or products. Furthermore, as indicated in the EMA guidelines "Marketing authorisation holders should take account of scientific and technical progress" and so, as new data become available it is possible that characterisation studies will vary through 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 p9).
- 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 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 are determined not to apply for a particular substance, then the submission would include a justification/evidence used to determine this.

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."

It is expected that the original submission will address all of the above categories for the drug product and provide a primary characterisation. If certain characteristics are determined not to apply for a particular product, then it is expected that justification/evidence used to determine this, will 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 for the drug substance and product should be viewed as the baseline from which to determine parameters to be assessed as part of the ASU. It is expected that the characterisation protocol developed for the drug substance will be applied for process changes and maybe as part of the stability program. It is advisable that the baseline should be used to determine which parameters are included as part of initial characterisation studies on the drug product. Further characterisation studies on the drug product should also be considered when there is a process change for the drug substance/product and also as part of the stability program for the drug product.

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 especially the case when the parameter in question is expected to change as a function of time i.e. the relative amounts and size of aggregates and the concentration of potentially unstable impurities.

It is advisable that a characterisation protocol summarising the suite of studies undertaken for the drug substance and drug product as part of the ASU be provided. It is expected that this protocol will be applied to the first three batches of drug substance produced from each new strain. It should also be considered, that dependent on the characteristics chosen, the application of the protocol (or parts thereof) may be appropriate for changes in the manufacturing process.

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. For instance, the interaction between B viruses in quadrivalent vaccines is well known, so suitable mechanisms for assessing the impact of this interaction should be identified for future use in the annual verification of the assay.

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

4.1.1.1.10 Stability / Shelf life

Guidance for the studies to be undertaken to support storage times and shelf-life is detailed in the EMA guideline. 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 of 2-8°C and applies particularly to excursions during transportation. 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 8°C and below 2°C. These should be submitted as

variations under s9D(3) and be reflected in an updated CPD.

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

4.1.1.2. 'Annual update' application for a seasonal vaccine

4.1.1.2.1.2. Vaccine seed lots

Updated risk assessment- Please refer to Appendix 4

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. A justification for the overage used for each strain should be provided that takes account of the known/predicted stability of the drug substance/drug product and any 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 3 batches for each new strain.

Batch analysis results for the first 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 batches of drug substance from new vaccine strains are assessed with the Characterisation protocol indicated in section 4.1.1.1.6. . Ideally these three 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 4.1.1.1.6 above.

4.1.1.2.1.6. Vaccine standardisation

The EMA guidelines indicate that verification of the SRID test should be undertaken when the analytical procedures are potentially impacted by strain changes. For new strains there is obviously a need for a full verification as outlined in the EMA guidelines. (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 emphasis in the verification process should be on ensuring that there have been no biases introduced into the assay by inclusion of new strains. In the case of B strains, the interaction between the two lineages is well understood and known to bias the assay. To compensate for this interaction it is a common solution to use a bivalent reference rather than a monovalent reference. In this instance assurance should be provided that the bivalent reference and the interacting drug substances behave in the same manner in the assay. An example of a study design to provide such an assurance is detailed in Appendix 3.

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 guideline indicates that both real-time (at recommended storage temperature) and accelerated data are provided for the drug substance (Monovalent Pooled Harvest - MPH). These data support the shelf-life of the MPH and may also inform a vaccine quality database. Towards the latter end manufacturers are 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 in final reports the company present the stability data for HA content both as absolute amounts of HA and also as % losses against the HA content at the start of the study. 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, reference to the report from which the data are drawn should also be included as part of the summary table along with the reagents used during the testing. The report should also indicate the reagents used for the stability testing of HA content and note if the same reagents were not used throughout 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.

Appendix 1: Procedural and sampling requirements for Annual Seasonal 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 Seasonal 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 reflecting 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 time-frames.

It is preferable 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@tga.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@tga.gov.au

Sampling requirements

The current OCABR guidelines for Influenza Vaccine have been adopted by TGA (http://www.tga.gov.au/industry/pm-euguidelines-adopted-biological.htm) with the following notation:

> "Sponsors should note that for each of the vaccines listed below, Section 2 of the EDQM OCABR guideline (which refers to mandatory testing) is NOT adopted in Australia, however TGA reserves the discretionary right to take samples and test."

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 for inactivated influenza vaccines to assist in the batch release process:

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@tga.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, 10 samples of 0.5 mL formulations should be provided. For 0.25 mL formulations, 20 of any subsequent consignment should be provided. All samples should be clearly labelled with a unique identifier or set of identifiers, e.g., Delivery Number or Airway Bill Number in conjunction with the arrival date/port.

h. For any 'reworking' of samples, provide 20 samples from the first re-working operation and then 10 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 Official Control Authority Batch Release (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 is provided under each of the headings below to indicate the data/information that is to be included in the dossier for the Annual Strain Update (ASU).

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. For the two B strains in QIVs there is an expectation that neither serum will be demonstrated to be specific and so a quantitative estimate of the interactions between the references and drug substances as outlined in Appendix 3 should be followed.

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.

In the case of quadrivalent vaccines where there is a known interaction between the B virus components, this is compensated for by the use of a bivalent reference in which the references for the two B virus strains are mixed to generate a single reference at the desired concentration. In this case it should be demonstrated that the relative HA content of the bivalent reference relative to the monovalent references is the same as the relative HA content of bivalent drug substance against monovalent drug substance. An example of how this may be achieved is provided in Appendix 3. Further details/clarifications on study design can be obtained/discussed via email on vaccines@tga.gov.au.

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 product in accordance with the Note for Guidance on Validation of Analytical Procedures: Text and Methodology (ICH topic Q2(R1) (CPMP/ICH/381/95). 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: Quadrivalent SRID – An example of qualification of a bivalent reference for the SRID assay of B virus antigen Content

A second B virus strain has recently been incorporated into trivalent seasonal influenza vaccines (TIV) to produce a quadrivalent vaccine (QIV). The addition of the second B strain has been accompanied by several observations that estimates of the HA content for the two B viruses can be anything up to 20% greater than that predicted from the formulation estimate. Subsequent studies have indicated an interaction between the two B virus strains in the SRID assay that leads to the overestimate of the HA content of a strain when assayed against its homologous, monovalent reference. The assay model proposed to compensate for this overestimate is to mix the two reference antigens to produce a "bivalent reference" against which the two strains are estimated in the assay. At the current time the two B strains included in QIV are from either the Victoria (Vic) or Yamagata (Yam) lineages and so the composition of the QIV can be designated as:

H1N1, H3N2, B(Vic), B(Yam).

To carry out the SRID for the QIV, four antigen references are therefore required which we shall designate:

Ref_{H1N1}, Ref_{H3N2}, Ref $_{B(Vic)}$ and Ref $_{B(Yam)}$

Also required are 4 antisera that are homologous for each of the four vaccines strains. We shall designate these:

AS_{H1N1}, AS_{H3N2}, $AS_{B(Vic)}$ and $AS_{B(Yam)}$

The 4 monovalent pooled harvests that are used to formulate the vaccine are also critical in helping to characterise the SRID for the QIV and we shall designate these:

MPH_{H1N1}, MPH_{H3N2}, $MPH_{B(Vic)}$ and $MPH_{B(Yam)}$

The assay model for the SRID of QIV makes a certain set of assumptions based around the estimate of the HA content of any given sample that contains both B strains. In the first instance

1. The HA content of B(Vic) in a sample containing B(Vic) and B(Yam) when assayed in the SRID against Ref $_{B(Vic)}$ using the serum $AS_{B(Vic)}$ will give a HA content of V+v. 'V' is the HA content of Ref $_{B(Vic)}$ when assayed against itself (i.e. would be within accepted limits of the assigned HA content for Ref $_{B(Vic)}$) and 'v' is the additional HA content attributable to the interaction between the two strains.

2. Likewise, in the reciprocal reaction the HA content of B(Yam) in the sample when assayed in the SRID against Ref $_{B(Yam)}$ using the serum Ref $_{B(Yam)}$ will give a HA content of Y+y where 'Y' is the HA content of Ref $_{B(Yam)}$ and 'y' is the additional HA content attributable to the interaction between the two strains.

3. v and y need not be the same proportion of HA content as the sera are not necessarily expected to behave in exactly the same way. Likewise, different sera produced against B(Vic) may produce different estimates of v and different sera produced against B(Yam) may produce different estimates of y.

However, for the model to be valid - and of use in the estimates of HA content for a QIV - then for any given serum, estimates of v and y obtained from the two antigen references (Ref $_{B(Vic)}$ and Ref $_{B(Yam)}$) must be the same as the estimate of v and y from the vaccine. As the values for v and y cannot be derived directly from the QIV sample there is a need to demonstrate that the interaction between the two B viruses in the vaccine is the same as that between the references.

To do this, it is necessary to use the MPHs used to formulate the QIV. There are probably several designs for the assays needed to ascertain that the above case fits the expectation of the model. Below we give an example of an assay with four samples – in this example the assay is to be carried out using $AS_{B(Vic)}$. For each sample the concentration of each component is adjusted to 30ug/ml and the assay carried out under normal assay conditions. More importantly the samples of the references and the MPHs should all be drawn from a single pool i.e. the same pool of reference - $Ref_{B(Vic)}$ is used to make samples 1 and 2 and the same pool of $MPH_{B(Vic)}$ used to make samples 3 and 4. Clearly there is some efficiency to be gained by carrying out the reciprocal test on $Ref_{B(Yam)}$ in parallel so that the same samples may be used across the two tests.

Table 1: Four sample assay

Number	Sample	Expected HA content
1	$Ref_{B(Vic)}$	NA
2	$Ref_{B(Vic)} + Ref_{B(Yam)}$	V + v
3	$MPH_{B(Vic)}$	M
4	$MPH_{B(Vic)} + MPH_{B(Yam)}$	M +m

In the 'Four Sample Assay', for the assay to be a suitable surrogate for the assay of the QIV then the expectation would be that v and m were equivalent. Experience from this approach to date has indicated that differences of less than 10% between v and m are unlikely to have a significant impact on the assay of the final product. For differences of 10% or greater argument will need to be provided for how the HA content of the final product will be standardised and controlled.

Appendix 4: Control of contamination with extraneous agents

A risk assessment should be conducted in compliance to applicable standards (e.g. pharmacopoeial monographs, etc).

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:

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.

Identify the extraneous agents that may be harmful to humans and have the potential to enter the manufacturing chain.

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, if available) and should be justified. Examples of qualification are donor/animal selection, viral testing, vaccination, 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 the extraneous agents identified in 2) and compare the clearance capacity with the potential input loads identified in 3); and

Discuss 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 seasonal 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

Therapeutic Goods Administration

PO Box 100 Woden ACT 2606 Australia
Email: info@tga.gov.au Phone: 1800 020 653 Fax: 02 6203 1605
https://www.tga.gov.au

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