Comparability Best Practices

Annex: Inactivated Virus Illustrative Example

1 Introduction

This document presents an illustrative example of how to utilize the comparability blank template as applied to an inactivated virus vaccine.

The content of this annex is not regulatory binding. Modification as appropriate for your organization is anticipated during the usage of the template.

1.1 Product information / Problem statement

An adjuvanted inactivated viral vaccine “INACTIVAX” is under development to address a viral threat rapidly spreading several world regions; the phase 3 clinical trial is ongoing with lots manufactured in one site. However, to increase the capacity of production in an accelerated scenario, the manufacturing process used for Phase 3 lots (Drug Substance and Drug Product) is duplicated in parallel of the clinical trial on other sites, for emergency supply. The scenario is as follows.

The objective is to perform a comparability assessment considering the reference/sending site (the site where the Phase 3 lots were produced) to the various sites including CMOs that will produce the DS and/or the DP (receiving sites) to ensure each site generates a comparable product and that related clinical/non-clinical studies are relevant to support the safety and the efficacy of commercial batches. In the example provided below, only one receiving site is considered, but the same approach would be followed if several receiving sites were considered.

1.2 Critical Quality Attributes & Related Analytical Strategy

Table 1 summarizes a guideline of (p)CQA(s), as well as the rationale for the criticality assignment. If sufficient product knowledge has been accumulated a (p)CQA in this list could become a QA.

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Table 1 (p)CQAs for INACTIVAX

Quality Attributes	**Criticality Assignment**	**DS/DP**	**Rationale**
General physicochemical characteristics *Determination of the physicochemical properties of the product*			
Appearance: Color of solution	CQA	DS, DP	Change in color suggest a change in the product which may affect efficacy and safety
Appearance: Clarity	CQA	DP	Change in clarity suggest a change in the product which may affect efficacy and safety
Appearance: Visible particles	CQA	DP	Exogenous visible particles are not acceptable
Injectable volume	CQA	DP	Variations in extractable/deliverable volume can result in incorrect dosing, impacting efficacy and safety
Pyrogenicity			
Pyrogens	CQA	DP	Absence of pyrogenicity or low pyrogenicity recommended for parenteral route of administration.
Excipients *A constituent other than the active substance, added in the formulation for a specific purpose.*			
Aluminium identity, content and antigen degree of adsorption	CQA	DP	Aluminium used as adjuvant
Content *A measure of the amount of product required to determine the dose*			
Antigen content	CQA	Inter., DS, DP	Antigen content is used instead of a true potency assay

Quality Attributes	Criticality Assignment	DS/DP	Rationale
Potency *A measure of the biological activity based on the attribute(s) of the product which is linked to the relevant biological properties*			
Antigenicity	CQA	DP	Variations in antigenicity can impact efficacy or safety
Identity			
Identity	CQA	DS, DP	Dosing with the wrong product would negatively affect efficacy and/or patient safety.
Genetic Stability			
Absence of mutant of importance	CQA	Inter.	Virus mutation is expected when viruses are grown, but mutation should remain limited and without significance on safety and potency
Product Related Substances and Impurities *Certain product variants, such as aggregates and viral protein degradation products, may have and impact on safety and efficacy*			
Structure-function characteristics	CQA	DS	Changes in structure may affect the antigenicity
Process-related Impurities			
Host Cell DNA	CQA	DS	Host cell DNA may cause safety risks and need to meet WHO expectation
Host Cell Proteins (HCPs)	QA	DS	Not considered as critical quality attributes, based on knowledge and use of the same cell substrate and banks for production of marketed vaccines.

Quality Attributes	Criticality Assignment	DS/DP	Rationale
Residual inactivating agent - *E.g.* β-propiolactone	CQA	DS	Example: beta-propiolactone: if residual remains in the product, it may cause a safety risk
Other residual compound[a]	CQA	DS	If close to toxicological limit.
No tumorigenicity	CQA	Cell bank	
No oncogenicity	CQA	Cell bank	
Virus inactivation			
Total inactivation of the virus vaccine	CQA	Inter.	Incomplete inactivation of the virus vaccine is a safety risk
Contaminants			
Bacterial endotoxin	CQA	DS, DP	Microbiological contaminants pose a safety risk
Mycoplasma	CQA	Inter.	Mycoplasma poses a safety risk
Bioburden	CQA	Inter.	Microbiological contaminants pose a safety risk
Sterility	CQA	DS, DP	Microbiological contaminants pose a safety risk
Adventitious viruses	CQA	Inter.	Viral contaminants pose a safety risk

Inter.: intermediate of the Drug Substance – DS: Drug Substance – DP: Drug Product

[a] If there is a change to the manufacturing process, the level of each residual compound needs to be assessed with respect to its toxicological limit. Based on this assessment it may be deemed a CQA.

Table 2 Analytical Testing Control Strategy

Category	CQA	Analytical Method*	Intermediate of DS	Drug Substance	Drug Product
General	Appearance - Color	Color	--	Release and Stability	Release and Stability
	Appearance - Clarity	Clarity	--	--	Release and Stability
	Appearance – Visible particles	Visible Particles	--	--	Release and Stability
	Container Closure Integrity	CCIT (e.g. Dye Ingress)	--	--	Stability
	pH	pH	In-process monitoring	Release and Stability	Release and Stability
	Volume	Extractable or Deliverable Volume	--	--	Release
Pyrogens	Bacterial Endotoxin	LAL or rFC	In-process monitoring	Release	Release
Excipients/ Adjuvants	Aluminium content	ICP-OES	--	--	Final Bulk, Filled Product release
	Antigen degree of adsorption	ELISA	--	--	Final Bulk, Filled Product release
Content	Virus Concentration	Viral Particle Concentratio	Release	--	--

Category	CQA	Analytical Method*	Intermediate of DS	Drug Substance	Drug Product
		n (plaque assay)			
	Inactivated virus concentration testing	Viral Particle Concentration (e.g. HPLC or UV)			Charac.
	Antigen content	ELISA	Release (Purified Inactivated Viral Solution)	Release and Stability	Release and Stability
Potency (Antigenicity replaced by antigen content in pandemic situation)			--		
Identity	Identity	ELISA		Release	Release
Genetic Stability	Absence of mutant of importance	High Throughput Sequencing	Characterization	--	--
Product-related substances and impurities	Structure-function characteristics (Viral particle size, viral morphology, aggregation,	SE-HPLC (UV214 nm detection)	Characterization (Purified Inactivated Virus Solution)	Characterization	--
		SE-HPLC-MALS	Characterization (Purified Inactivated Virus Solution)	Characterization	--
		SDS-PAGE	Characterization (Purified	Characterization	--

Category	CQA	Analytical Method*	Intermediate of DS	Drug Substance	Drug Product
	viral protein profile)		Inactivated Virus Solution)		
		DLS	Characterization (Purified Inactivated Virus Solution)	Characterization	--
		Nanotracking Particle Analysis	Characterization (Purified Inactivated Virus Solution)	Characterization	--
		Electron microscopy	Characterization (Purified Inactivated Virus Solution)	Characterization	--
		Western blot with specific antibodies	Characterization (Purified Inactivated Virus Solution)	Characterization	--
Process-related impurities	Host cell DNA	RT-qPCR		In-process (PPQ only) Release	--
	Host cell Protein	ELISA		In-process (PPQ only) (Release)	--
	Residual inactivating agent β-propiolactone	GC-MS		Release	
Virus inactivation	Kinetics of inactivation	Plaque assay, T=0, 1, 2, 6, 24 hours	Validation	--	--

Category	CQA	Analytical Method*	Intermediate of DS	Drug Substance	Drug Product
	Effective inactivation	Plaque assay	Release	--	--
Contaminants	Mycoplasma	Mycoplasma (culture method or NAT)	Release (on Single Harvest)	--	--
	Bioburden	Bioburden	In-process monitoring, Release on the Purified Inactivated Viral Solution	--	In process monitoring (during formulation step)
		Filter Integrity Test	--	In-process	--
	Sterility	Sterility	--	Release Stability	Release Stability
	Extraneous viruses	Test(s) according to risk assessment	Release (Single Harvest and Pooled and Clarified Harvest)	--	--

**Note: In this accelerated scenario, focus on use of method able to assess multiple CQA at a time. E.g. single ELISA test can be used to assess identity and antigenic content at the same time. In the same manner only one test can be performed for assessing single parameter in place of multiple tests e.g. for purity analysis simple test of HPLC can be used alone instead of SDS PAGE, western blot and densitometry combination whichever is most appropriate.*

2 Description of the Changes & Justification

Table 3 Changes to Drug Substance

Change ID	Description of changes	Pre-Change (Toxicology/Early phase or Late phase clinical trial)	Post-Change (Commercial/Launch)	Justification of the change
DS1	Site of manufacture	Pilot plant	Large scale facility	Increase the manufacturing capacity

Table 4 Changes to Drug Product

Change ID	Description of changes	Pre-Change (Toxicology/Early phase or Late phase clinical trial)	Post-Change (Commercial/Launch)	Justification of the Change
DP1	Site of Manufacture	Pilot Plant	Large scale facility	Increase the manufacturing capacity

3 Change Impact Assessment

See example of change impact assessment in the case study for mRNA (mRNA Annex).

4 Comparability Strategy

<u>Scenario</u>: the manufacturing process used for Phase 3 lots (DS and DP), is transferred to one or multiple sites in order to increase the production capacity in a pandemic situation.

The objective is to perform a comparability assessment considering the reference/sending site (the site where the Phase 3 lots were produced) to the various sites including CMOs that will produce the DS and/or the DP (receiving sites) to ensure each site generates a comparable product and that related clinical/non-clinical studies are relevant to support the safety and the efficacy of commercial batches.

The Phase 3 and PPQ batches from the reference site are the reference in term of product comparability for all the PPQ validation batches. Each receiving site must be comparable to the reference site.

Several success criteria could be used to verify the product comparability:

- Specification tests and IPC: conformity to the acceptance criteria, and comparability of relevant tests.
- Characterization tests: characterization tests are selected for comparability based on their potential of detecting relevant differences that might result from the manufacturing process change.
- Comparison of Stability profiles based on ICH stability studies using the kinetic modelling.

Baseline reference values will be established using a minimum of 3 lots from the reference site and comparing against a minimum 2 lots for each DS and DP receiving sites.

Drug Substance and Drug Product specifications are provided in **Table 5** and **Table 7**. Tests are performed for conformity to the specification for release.

Table 6 describes the characterization tests performed at DS stage to assess potential impact on potency, purity and safety. These tests are complementary to release tests performed at this stage.

Table 5 Drug Substance Specification and In Process Control

Category	**CQA/Test**	**Step**	**Acceptance Criteria**
General	Appearance (color)/visual	DS	Colorless, clear liquid with possible presence of endogenous particles
	pH	DS	6.6 – 7.4
Content	Virus concentration/plaque assay	SH	≥ 5.0 log10 CCID50/mL
		CVH	≥ 6.7 log10 CCID50/mL
	Antigen content	PIV	≥ 140 ETU/mL
		DS	≥ 70 ETU/mL
Pyrogens	Bacterial endotoxin/ LAL or rFC	DS	< 0.5 EU/µ
Identity	Identity / ELISA	DS	Positive
Process-related impurities	Host-cell DNA/RT-qPCR	DS	For calculation, ≤ 10 ng/dose in final DP
	Residual β-propiolactone / GC-MS	DS	≤ limit of detection
Virus inactivation	Effective inactivation/virus titer by plaque assay	IV	Absence of pfu after inactivation

Category	CQA/Test	Step	Acceptance Criteria
Contaminants	Bacterial and fungal contaminant/Ph. Eur.	SH	No growth
		PIV	No growth
		DS	No growth
	Mycoplasma	SH	Absence of Mycoplasma
	Extraneous viruses / tests according to risk assessment	SH	No extraneous virus detected

SH: single harvest – CVH: concentrated viral harvest – IV: inactivated viral solution – PIV: purified inactivated viral solution – DS: Drug Substance – DP: Drug Product

Table 6 Drug Substance Complementary Characterisation Tests

Category	CQA	Analytical Method
Genetic stability	RNA sequence integrity, presence of mutation	High Throughput Sequencing
Product-related substances and impurities	Structure-function characteristics (viral particle size, viral morphology, aggregation, viral protein profile)	SE-HPLC (UV214 nm detection)
		SE-HPLC-MALS
		SDS-PAGE
		DLS
		Nanoparticle analysis
		Electron microscopy
		Western blot with specific antibodies
Content	Viral Particle Concentration	HPLC or UV
Process-related impurities	HCP profile	ELISA

Table 7 Drug Product Specification and In Process Control

Category	CQA	Acceptance Criteria
General	Appearance – Color, clarity, physical state	White to off white suspension
	Appearance – Visible particles	Absence of visible particles
	pH	7.0 – 8.0
	Osmolality	270 – 330 mOsmol/kg
	Volume	≥ 0.5 mL
Excipients	Aluminium content	0.5 mg Al^{3+}/dose
Potency	Potency	≥ 35 ETU/dose (0.5 mL)
	Antigen degree of adsorption	≥ 80%
Identity	Identity	Positive
Pyrogens	Bacterial Endotoxin	≤ 10 EU/dose
Contaminants	Sterility	No growth / Pass for sterility

5 Results and Analysis

The results on the intermediates of the Drug Substance, the Drug Substance and the Drug Product are provided in the comparability report (not provided here).

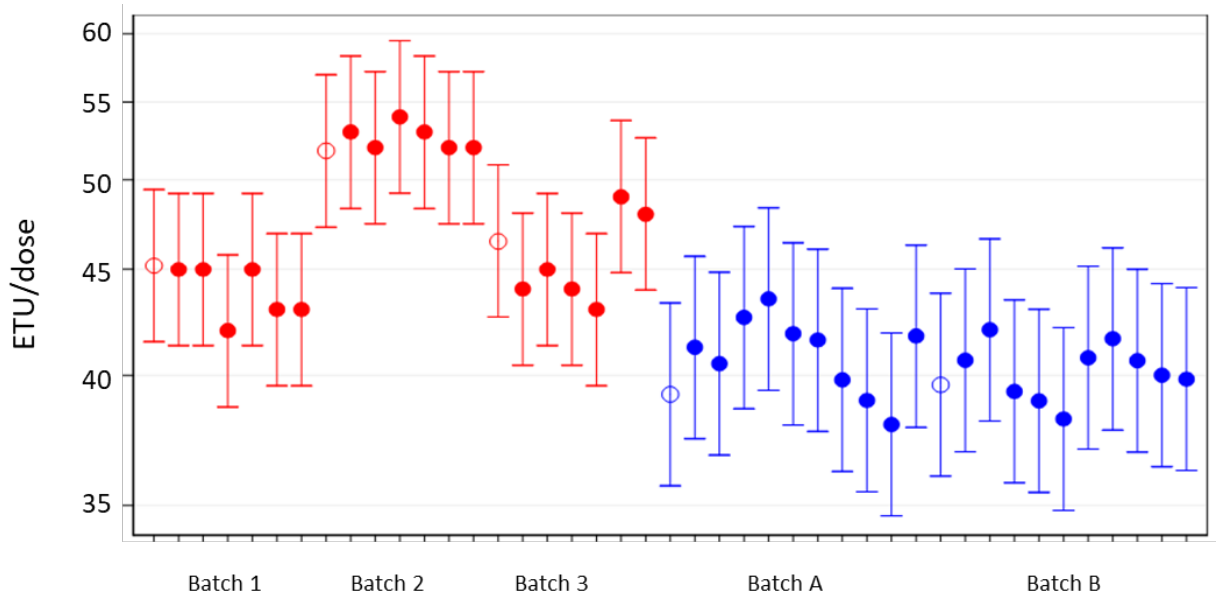
Beside the global review and comparison of the results for release tests, the most important quality criteria for an inactivated viral vaccine in a comparability exercise to be discussed are:

- The genetic sequence integrity and the absence of mutations of significance
- The total inactivation of the vaccine virus, to be controlled by validation and release test
- The integrity of the antigenic proteins (e.g. the Spike protein for a Covid-19 vaccine)
- The potency/antigen content, at release and during shelf-life

When moving from one site to another one, a detailed assessment of the differences and associated site adaptation actions is done in the frame of the process transfer activities and documented accordingly.

The test results on the CQA are compared side by side, and if differences are observed, they are discussed further. One example of comparison of results is given below for potency.

DP Potency results are presented in the figure below with in red the reference site results and in blue the receiving site results. The acceptance criterion lower limit based on clinical data was set at ≥ 35 ETU/dose by ELISA. The first lot in red (Batch 1) was tested in clinical trial.



ETU: ELISA Test Unit

To get more data on each lot, the validation data obtained to validate the formulation homogeneity were used. Therefore, in red we have the results of three lots: with the empty circle representing the release test result, and the full circles representing the results obtained during process validation (with test performed at the beginning, middle and end of the filing step). The same approach was taken for the receiving site.

The figure confirms that there is no statistical difference between the sending site and the receiving site batches as Confidence Intervals (CI (95%)) of the method do overlap, which meets the Comparability criteria.

For the sending site, the results of the second lot show a slightly higher trend for Potency. A thorough assessment has been conducted to justify that this difference was due to an underestimated antigen content of the DS batch at the time of formulation, leading to an over formulated batch. This variability does not constitute a trend, nor does it negate the comparability claim. Despite the over formulation, the Potency results meet the specification for Drug product and exceeds the level observed in the clinical trial batch.

6 Conclusion

Based on the reported analytical comparability, it was concluded that change of manufacturing site did not have a significant impact on product quality. Due to the heavily accelerated scenario, the following risk- based approaches were considered:

- Use of potency test results from the formulation homogeneity validation package in addition to release results to increase the data to analyze when limited number of batches are available.

- Analysis of the potency data with the antigen content data used during formulation to explain results that at first sight might be considered not comparable.
- Adjustment of the formulation target of the receiving site to be in the same potency range compared to the clinical lot used in phase 3.

Continuing discussion with relevant Authorities was ensured to confirm suitability of the proposed approaches.

7 References

European Public Assessment Report (EMA/627695/2022)– COVID-19 Vaccine (inactivated, adjuvanted) Valneva (23 June 2022) (link)