CEPI

# Comparability Template

# Annex: mRNA illustrative example

## 1 Introduction

This document presents an illustrative example of how to utilize the comparability template as applied to an mRNA platform technology.

A hypothetical scenario of mRNA manufacturing was developed in response to an emergency outbreak. This scenario could be applicable to rapid scale-out activities, leading to application for emergency use authorization within 100 days of recognition of a pandemic pathogen and readiness for commercial license authorization in parallel.

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

A non-replicating mRNA vaccine is manufactured through the following unit operations : (1) pDNA production from transformed *E. Coli* cell banks; (2) pDNA linearization; (3) IVT reaction – co-capping and polyA tailing in a single IVT reaction; (4) mRNA purification; (5) formulation; and (6) fill and finish.

During an outbreak situation, two clinical batches are manufactured within 30 - 40 days since a pathogen is recognized. At the same time, IND/IMPD is authored and aligned with regulators for expedited approval to enter the clinics within a few days. In next 50 days, sufficient clinical evidence is collected for emergency use authorization (EUA). In parallel, scale-out through technology transfers are underway to increase supply of the vaccine candidate post EUA. Three batches are manufactured at an intended commercial scale post technology transfer. Comparability protocol is prepared as soon as technology transfer decision is made using the template. In-process measurement/controls, characterization and stability of released DS and DP batches are executed following indications of this best practices document.
Comparability criteria are pre-defined by leveraging the data of two clinical batches manufactured for FIH clinical trial and prior knowledge of other mRNA vaccines and vaccine candidates that are manufactured by a highly similar platform process. Utilization of platform data needs to be justified by proven safety and efficacy and well-characterized physicochemical properties of previous vaccine candidates. Consultation

with NRAs is critical for this approach, especially when there is insufficient manufacturing experience of the specific vaccine candidate in emergency situations.
Comparability analyses are documented on the template and could be used as a source document for regulatory dossier preparation.

## 1.2 Critical Quality Attributes & Related Analytical Strategy

**Table 1** summarizes CQA examples, including potential CQAs (pCQA), of non-replicating mRNA and the rationale for the criticality assignment. This list has been developed based on extensive literature reviews (see for instance 1,2) and landscape analysis. The list might not be considered comprehensive and each developer could have different assignment of CQAs based on its own institutional knowledge and experience. The testing strategies, described in **Table 2**, could be differentiated further to bulk drug product (mRNA-LNP bulk) and drug product if appropriate.

**Table 1 CQAs for non-replicating mRNA vaccine**[3]

| Quality Attributes | Criticality | DS/DP | Rationale |
|---|---|---|---|
| **Physicochemical characteristics** | | | |
| RNA content | CQA | DS/DP | Content is critical for efficacy and safety |
| RNA integrity | CQA | DS/DP | Critical on vaccine efficacy |
| 5' Capping efficiency | CQA | DS | 5' capping is required for RNA translation. 5' capping efficiency correlates with stability |
| PolyA tail length | CQA | DS | The length of PolyA tail impacts on durability of immunity |
| PolyA tail distribution | pCQA | DS | |
| RNA higher order structure4 | CQA | DS | Regulate protein expression |
| RNA Encapsulation Efficiency | CQA | DP | Critical on vaccine efficacy |

[1] COVID-19 Vaccine Quality Attributes Toolkits (usp.org)
[2] post-ecbs-who-regulatory-considerations-document-for-mrna-vaccines---final-version---29-nov-2021_tz.pdf
[3] Quality by Design for enabling RNA platform production processes. Trends in Biotechnology, 40: 1213 – 1228, 2022
[4] mRNA structure regulates protein expression through changes in functional half-life, PNAS, 116:24075-24083, 2019

| Quality Attributes | Criticality | DS/DP | Rationale |
|---|---|---|---|
| LNP Size | CQA | DP | Critical on vaccine efficacy and safety |
| LNP Polydispersity | CQA | DP | Indicator of process control and manufacturing consistency |
| LNP surface charge5 | CQA | DP | In vivo delivery efficiency |
| pH | CQA | DS/DP | Critical on vaccine stability, safety and efficacy |
| Osmolality | CQA | DP | Critical on vaccine safety |
| Formulation | | | |
| Lipid identity | CQA | DP | Lipid components are critical for efficacy |
| Lipid ratio | CQA | DP | A ratio of lipid contents is critical for efficacy |
| **Biological function** | | | |
| Potency | CQA | DP | Indicator of vaccine efficacy |
| Identity | | | |
| Identity | CQA | DS/DP | Critical on vaccine efficacy and safety |
| **Product-Related Impurities** | | | |
| Residual dsRNA content | CQA | DS | dsRNA are highly immunogenic impurities, which can hamper RNA translation. |
| Residual oligonucleotide | QA | DS | Indication for process optimization |
| **Process-related Impurities** | | | |

[5] Ionization and structural properties of mRNA lipid nanoparticles influence expression in intramuscular and intravascular administration, Communications Biology, 4:956, 2021

| Quality Attributes | Criticality | DS/DP | Rationale |
|---|---|---|---|
| Residual DNA content | CQA | DS | Regulatory requirement for product safety (EP, USP). |
| Residual protein content | CQA | DS | Regulatory requirement for product safety (EP, USP). |
| Residual solvents (ethanol) | QA | DP | Regulatory requirement for product safety (EP, USP). |
| **Safety** | | | |
| Bacterial Endotoxin | CQA | DS/DP | Regulatory requirement for product safety (EP, USP). |
| Bioburden | CQA | DS | Regulatory requirement for product safety (EP, USP). |
| Sterility | CQA | DP | Product standard for parenteral administration (EP, USP). |
| Appearance | | | |
| Color | QA | DS/DP | Compendial evaluation |
| Visible particles | CQA | DS/DP | Regulatory requirement for product safety (EP, USP). |
| Sub-visible particles | CQA | DP | Regulatory requirement for product safety (EP, USP). |

**Table 2 Analytical Testing Control Strategy**

| Category | CQA | Analytical Method | Drug Substance (DS) | Drug Product (DP) |
|---|---|---|---|---|
| **Physiochemical Characteristics** | 5' Capping efficiency | IP-RP-HPLC | Release / Characterization | N/A |

| Category | CQA | Analytical Method | Drug Substance (DS) | Drug Product (DP) |
|---|---|---|---|---|
| | PolyA tail length | IP-RP-HPLC | Characterization | N/A |
| | PolyA tail distribution | IP-RP-HPLC | Characterization | N/A |
| | RNA integrity | cGE/IP-RP-HPLC | Release/Characterization / Stability/IP | Release/Stability /IP |
| | RNA Encapsulation Efficiency | Ribogreen fluorescence assay | N/A | Release/Stability /IP |
| | RNA higher order structure | CD | Characterization | N/A |
| | LNP Size | DLS | N/A | Release/Stability /IP |
| | LNP Polydispersity | DLS | N/A | Release/Stability /IP |
| | LNP Charge | ELS | N/A | Characterization |
| | Osmolality | Ph.Eur. 2.3.3 | N/A | Release/ /IP |
| | pH | Ph.Eur. 2.2.35 | Release/Stability/IP | Release/Stability /IP |
| **Formulation** | Lipid identity | HPLC-CAD | N/A | Release |
| | Lipid ratio | HPLC-CAD | N/A | Release/Stability |
| **Content** | RNA content | DS: UV<br>DP: Ribogreen fluorescence assay | Release/Stability/IP | Release/Stability /IP |

| Category | CQA | Analytical Method | Drug Substance (DS) | Drug Product (DP) |
|---|---|---|---|---|
| **Biological function** | Potency | In-vitro translation | Characterization | Release/Stability |
| **Identity** | Identity | RT-PCR | Release | Release |
| | Primary structure | NGS | Characterization | Characterization |
| **Product-related impurities** | Residual dsRNA content | ELISA | Release | N/A |
| **Process-related impurities** | Residual oligo nucleotides | RP-HPLC | Release | N/A |
| | Residual DNA content | qPCR | Release | N/A |
| | Residual protein content | Nano-orange | Release | N/A |
| **Safety** | Bacterial Endotoxin | Ph.Eur.2.6.14 | Release | Release |
| | Bioburden | Ph.Eur. 2.6.12 | Release/Stability | Release/Stability |
| | Sterility | Ph.Eur.5.1.6 | N/A | Release/Stability |
| **Appearance** | Color | Ph.Eur. 2.2.2 | Release/Stability | Release/Stability |
| | Visible particles | Ph.Eur. 2.9.20 | N/A | Release/Stability |
| | Sub-visible particles | Ph.Eur. 2.9.19 | N/A | Release/Stability |

**IP: meaning either In-Process Controls or In-Process Measurements*

## 2. Description of the Changes & Justification

The hypothetical changes and justification, which could occur during manufacturing technology transfer of non-replicating mRNA DS and DP, are presented in **Table 3** and **Table 4**, respectively.

**Table 3 Changes to Drug Substance**

| **Change ID** | **Description of changes** | **Pre-Change** (Emergency Use Authorization) | **Post-Change** (Commercial scale) | **Justification of the change** |
|---|---|---|---|---|
| DS1 | Site of manufacture | Site 1 | Site 2 | Increase production capacity for commercialization |
| DS2 | IVT scale-up* | Scale 1 | Scale 2 | Increase production capacity |
| DS3 | T7 Polymerase | Supplier 1 | Supplier 2 | Alternative supplier introduced to secure supply chain of key reagents and to increase quality grade of raw material. |
| DS4 | Prolong holding time of product intermediate | </= x hours | </= y hours | Stability data available allowing for prolonging the holding time |

**IVT scale-up includes both IVT and downstream process.*

**Table 4 Changes to Drug Product**

| **Change ID** | **Description of changes** | **Pre-Change** (Emergency Use Authorization) | **Post-Change** (Full commercial batch) | **Justification of the Change** |
|---|---|---|---|---|
| DP1 | Site of Manufacture | Site 1 | Site 2 | Tech transfer to increase production. |
| DP2 | Mixing flow rate | Flow rate 1 | Flow rate 2 | Site 1 and 2 use different equipment for formulation. Flow rate was optimized for site 2 for comparability. |
| DP3 | Number of mixers | N | 5xN | Increase DP throughput |
| DP4 | Vial Type | Single-dose vial | Multi-dose vial | Commercialization readiness |

## 2.1 Change Impact Assessment

Each change, as given in **Table 3** and **Table 4**, is subjected to risk assessment to determine its impact on CQAs and pCQAs. For a demonstration purpose, one change (scale-up of IVT reaction) is chosen and evaluated using a tool described in **Figure 1**. The assessment and justification presented in this example is solely hypothetical, not representative of any mRNA projects in development.

Firstly, a risk class (I, II or III) of change is determined based on the scoring of severity and probability (low, medium or high). Then, the risk classes are subjected to the following assessment with detectability (low, medium or high) to determine the final risk priority (low, medium or high).

The outcome of impact assessment of IVT scale-up onto CQAs is summarized in **Table 5**. Assuming that this vaccine candidate is manufactured based on mRNA platform process and analytical methods, the risk priorities for listed CQAs could be justified by including accumulated science and data from previous development. Scientifically sound assessment and justification would be essential to develop robust comparability strategies (Section 3).

IVT is a well-controlled unit operation but, in this hypothetical case, there is a new reagent (T7 RNA polymerase) introduced at the tech transfer receiving site. It implies that the performance data of the new reagent is insufficient and, therefore, the risks of RNA integrity, polyA tail and residual dsRNA are rated as

medium. Potency assay is dependent on the product quality and is presumed to be variable and, therefore, it is rated as high-risk priority.

Source: Figure M3.5, GAMP 5: A Risk-Based Approach to Compliant GxP Computerized Systems, © Copyright ISPE 2008. All rights reserved. www.ISPE.org.

**Figure 1. A risk-based approach**

**Table 5 Drug Substance – Change Impact Assessment on CQA[1]**

| Change ID | CQA / Attributes impacted | Risk Score on safety and efficacy | | | | Justification |
|---|---|---|---|---|---|---|
| | | Severity[2] | Probability | Detect-ability | Risk Priority[2] | |
| DS2 (IVT Scale-up) | RNA integrity | High | Medium | High | Medium | High impact on mRNA efficacy and stability. Process optimized for scale-up but there is insufficient experience with a new polymerase. |
| | Capping efficiency | High | Low | High | Low | High impact on mRNA potency and stability. Capping reagent has demonstrated robust capping efficiency under various conditions, evaluated by internal development and the reagent manufacturer. Characterization only applied to selected batches. |
| | Poly A tail length/ distribution | High | Medium | High | Medium | High impact on mRNA potency and stability. Process optimized for scale-up but there is insufficient experience with a new polymerase. Characterization only applied to selected batch. |
| | Residual dsRNA | High | Medium | High | Medium | Development data support <1% of dsRNA under worst-case IVT conditions across mRNA platform development. There is insufficient |

| Change ID | CQA / Attributes impacted | Risk Score on safety and efficacy | | | | Justification |
|---|---|---|---|---|---|---|
| | | Severity[2] | Probability | Detect-ability | Risk Priority[2] | |
| | | | | | | experience with a new polymerase. |
| | Potency | High | Medium | Medium | High | mRNA integrity is a relevant CQA impacting potency. Potency assay is variable. |
| | Process-related impurity | High | Low | High | Low | Process-related impurity is mainly dependent on starting and raw material quality and purification process. The pDNA manufacturing was not changed. The downstream process is robust to remove excessive impurity. |

1. Color coding under severity and probability indicates the risk classes I, II or III, determined according to Figure 1. Color coding under detectability and risk priority indicates the risk priorities I, II or III, determined using the risk classes and detectability as shown in **Figure 1**.
2. Individual organization will use its own risk assessment tool and, therefore, the structure of Table 5 could be updated per need. In this case, the table heading of "Impact" and "Risk Score" are changed to Severity and Risk Priority to align with the tool we chose for this exercise.

## 3. Comparability Strategy

The comparability strategy is developed based on the risk priority presented in Section 2. Prioritization of analytical testing to address high risk CQAs would be necessary to respond to an emergency situation and an urgent need to acquire regulator's approval.

**Table 5** indicates the risk priority of potency is rated high but it is possible that the potency assay for specific vaccine candidate could still be in development. It is proposed to prioritize mRNA physicochemical assays including mRNA integrity, polyA tail length/distribution, and dsRNA, which are rated medium risks. Additionally, mRNA primary structure is characterized to strengthen comparability. It is published that the primary structure including modified nucleoside determines mRNA higher order structure and regulates

protein expression and half-life[6]. The strategy to prioritize physicochemical properties could be justified with platform knowledge and experience, supporting strong causation between these mRNA quality attributes and potency.

The analytical comparability of mRNA DS will be addressed in four categories:

(1) DS in-process measurements – mRNA integrity, content and pH are classified as in-process (IP) control or measurement for the DS manufacturing (**Table *2***). IVT intermediates post affinity chromatography and prior to final filtration are subjected to the IP tests. The results from pre-change and post-change batches will be compared side-by-side.
(2) DS/DP release – DS and DP release results will be compared between pre-change and post-change batches for all quality attributes classified as release in **Table *2***.
(3) DS characterization – Characterization tests will be prioritized for mRNA integrity, polyA tail length/distribution and primary structure.
    a. mRNA integrity – In addition to cGE method used for release test, RP-HPLC will be added as an orthogonal characterization method for DS comparability.
    b. polyA tail length – characterization test using IP-RP-HPLC will be prioritized.
    c. Primary structure – One batch of each pre- and post- change will be subjected to mRNA sequencing.
(4) DS/DP accelerated stability – mRNA DS and DP batches subjected to accelerated stability conditions will be compared for the prioritized CQAs. In addition, stability-indicating compendial tests (such as appearance and pH) will be conducted to evaluate platform-specific qualities.

Appropriate comparability criteria should be pre-defined as part of strategy. Methodologies of developing comparability criteria need to be chosen carefully and justified in consideration of product knowledge, number of batches available and analytical method variabilities.

For a demonstration purpose, comparability strategies are further described in **Table 6** –**Table 7**-**Table 8** and

[6] PNAS | November 26, 2019 | vol. 116 | no. 48 | 24075–24083

***Table 9***. In this scenario, there are only two batches of vaccine candidate manufactured prior to technology transfer. The results from the analytical test package (release, stability, product and process characterization data, when applicable) could be compared side-by-side. It was not feasible to develop statistically-sound comparability assessment with only two batches.

Platform experience could provide comparability benchmark for some CQAs. In this emergency scenario, we propose to apply the tiered approach for the comparability criteria: (1) vaccine-specific criteria based on the release specifications, if applied, and (2) platform-based criteria based on statistics derived from the platform data of various clinical and commercial batches. This strategy would allow to assess where the analytical results of the product attributes stand with respect to historical data. Additionally, clinical evidence in correlation with analytical data could provide confidence in platform robustness and vaccine safety. The vaccine-specific criteria will be further refined as the later phase of development continues and more experience is gained from future campaign.

Comparability of in-process controls is assessed per the control limits. In-process measurements are compared for trending only. The platform experience of IPMs is leveraged to evaluate performance of the platform process. The batches subjected to comparability assessment are described in **Table *6***.

**Table 6 Comparability strategy – Batch Selection**

| **Process** | **Batch Number** | **In-process** | **Release** | **Characterization** | **Stability (accelerated)** |
|---|---|---|---|---|---|
| Pre-change – EUA | Lot a | X | X | X | X |
| Pre-change – EUA | Lot b | X | X | X | X |
| Post-Change – phase III | Lot c | X | X | X | X |
| Post-Change – phase III | Lot d | X | X | | |
| Post-Change – phase III | Lot e | X | X | X | X |

**Table 7 Comparability strategy – In-process Controls/ Measurement for DS**

| Change ID | Affected Step | Affected Attributes | Analytical Method | Comparability Acceptance Criteria | Justification |
|---|---|---|---|---|---|
| DS2 | IVT | mRNA integrity | cGE | Trending only | Routine IPM for information only |
| | | Content | UV | | |
| | | pH | Ph.Eur. 2.2.3 | In-process control limits | In-process control (IPC). The limits must be met. |
| | mRNA purification | mRNA integrity | cGE | Trending only | Routine IPM for information only |
| | | Content | UV | | |
| | | pH | Ph.Eur. 2.2.3 | In-process control limits | In-process control (IPC). The limits must be met. |

**Table 8 Comparability strategy – DS Release***

| Quality Attributes | Analytical Method | Comparability Acceptance Criteria | Justification |
|---|---|---|---|
| Content | UV | Specification | Specification only as it is antigen-specific limits |
| Identity | RT-PCR | | |
| Color | Ph.Eur. 2.2.2 | | Qualitative assessment. |
| pH | Ph.Eur. 2.2.3 | Specification / Platform driven statistics | Suitable for IM and antigen stability |
| RNA integrity | cGE | Specification / Platform driven statistics | Antigen-specific and platform-dependent attribute |
| 5' Capping efficiency | IP-RP-HPLC | | |
| RNA integrity | cGE | | |
| Residual dsRNA content | RP-HPLC | | |
| Residual oligonucleotides | RP-HPLC | Specification / Platform driven statistics | Platform process dependent attribute |
| Residual DNA content | qPCR | | |

| Quality Attributes | Analytical Method | Comparability Acceptance Criteria | Justification |
|---|---|---|---|
| Residual protein content | Nano-orange | | |

* Table 8 heading is changed to fit for purpose of DS release.

**Table 9 Comparability strategy – Characterization (DS)**

| Quality Attributes | Analytical Method | Comparability Acceptance Criteria | Justification |
|---|---|---|---|
| mRNA integrity | RP-HPLC | Trending only | No specification for characterization. Insufficient platform data available. |
| PolyA tail length / distribution | IP-RP-HPLC | Trending only | No specification for characterization. In line with platform data. |
| Primary structure | NGS | Conform sequence | Qualitative assessment |
| Higher order structure | CD | Conform spectra | Qualitative assessment |
| Potency | *In-vitro* translation* | In development | Antigen-specific assay in development. |

* Potency assay will be utilized as release assay upon its development and qualification.

**Table 10 Comparability strategy – Accelerated Stability (DS)**

| Temp / Humidity | Quality Attributes | Analytical Method | Comparability Acceptance Criteria | Justification |
|---|---|---|---|---|
| 4°C & 25°C / 60% RH | RNA integrity | cGE | Degradation trending | Degradation trending is antigen-specific. If model regression analysis is feasible, the fitted models could be compared. |
| | RNA content | UV | | |
| | pH | Ph.Eur. 2.2.3 | | |
| | Color | Ph.Eur. 2.2.2 | | |

## 4. Results and Analysis

For demonstration purpose, comparability analyses of mRNA integrity are illustrated in **Figure 2**-**Figure 3** and **Figure 4** for the in-process, DS release and characterization.

Product X is the vaccine candidate in development for emergency response and there is a limited number of batches to assess pre- and post-change comparability. Platform manufacturing data of other products in clinics are leveraged to provide additional information to the analytical comparability demonstrated with product X, and understand where the newly generated batches are positioned with respect to previously manufactured batches. Platform data encompass variations of products, minor modification of process

parameters, manufacturing scales and sites and, therefore, it requires careful selection of appropriate statistical tool. Antigen-specific attributes, such as potency, are not suitable for platform approach for comparability.

In-process measurement of integrity is compared for trending purpose (***Figure 2***) and it supports consistent performance of IVT reaction between pre- and post-changes. Pre- and post-change integrity results meet the release specification (***Figure 3***). Additional assessment against platform-derived criteria supports that integrity of pre- and post-changes are within the historical ranges from other products with the established safety profile.

Characterization for integrity is performed with an orthogonal analytical method (***Figure 4***). There is no specification defined for characterization. In this example, the characterization method of integrity appears more precise than the release assay and, therefore, the difference between pre- and post-changes of product X seems more pronounced. However, the integrity ranges observed with product X were still within the historical range, which did not yield any meaningful impacts on safety and efficacy of other vaccine candidates.

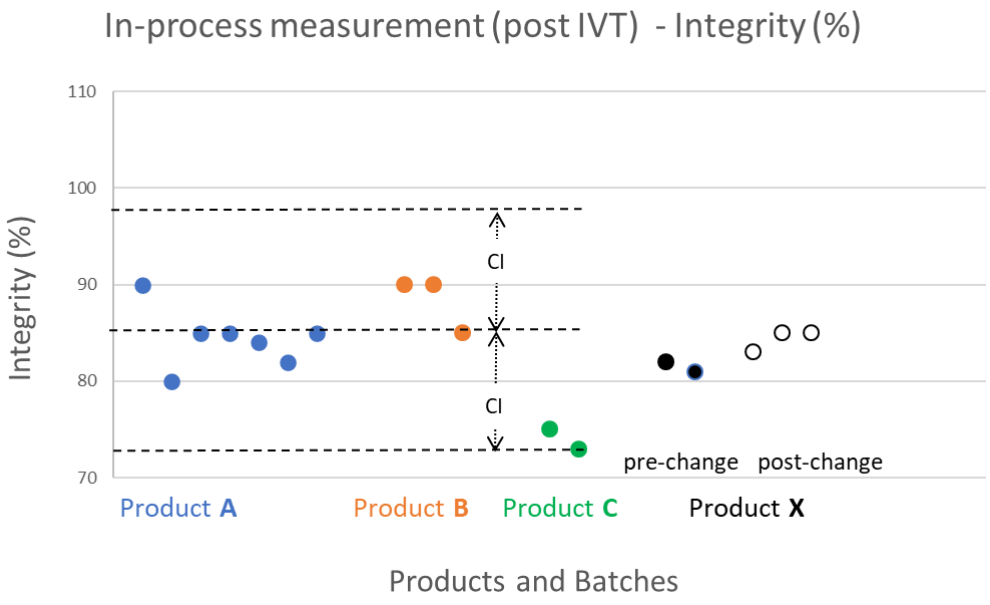


**Figure 2. Integrity of post IVT of platform products A to X. In-process measurements are for trending only but mean with confidence interval of product A to C is included for guidance.**

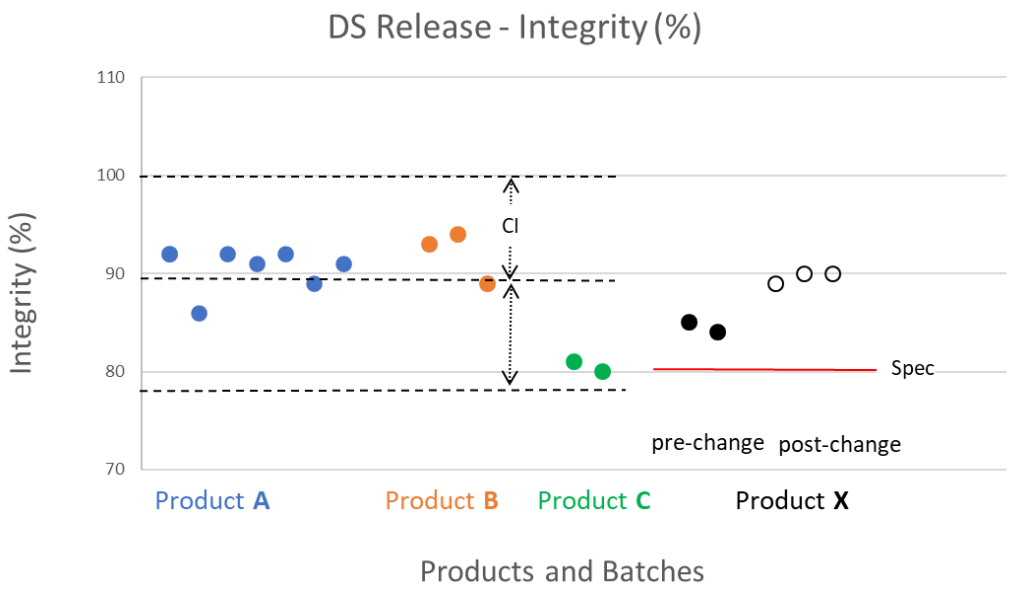


**Figure 3. Integrity of DS release of platform products A to X. Specification for product X and mean with confidence interval of product A to C are included as comparability acceptance criteria.**

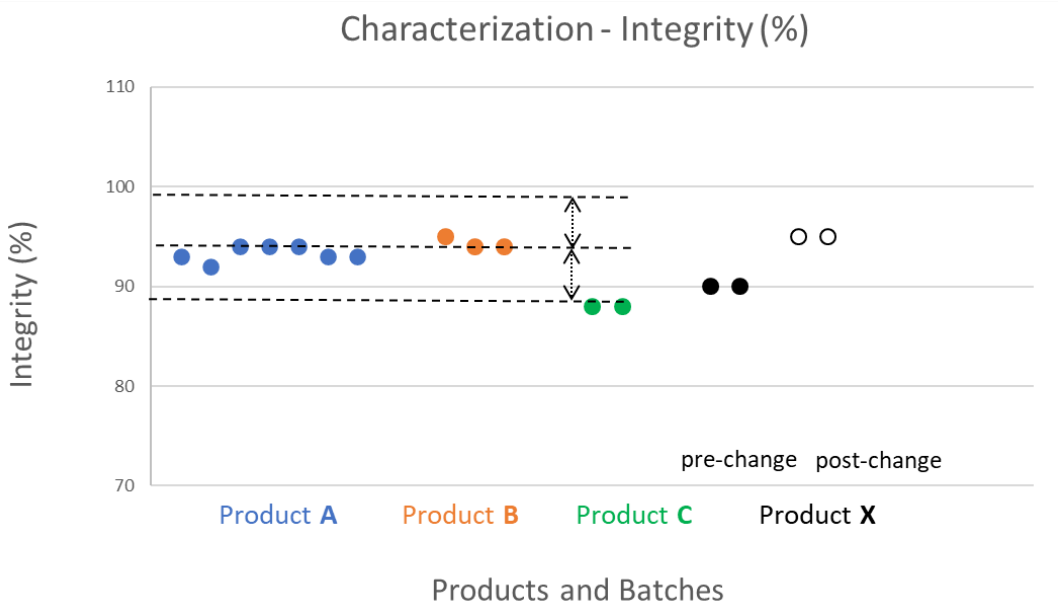


**Figure 4. Integrity of DS characterization of platform products A to X. Mean with confidence interval of product A to C are compared with those of product X.**

## 5. Conclusion

In this hypothetical scenario, it can be concluded that the changes caused by technology transfer do not have significant impact on integrity. In the real-world situation, all of CQAs would be subjected to comparability exercise by leveraging both vaccine- and platform-specific knowledge. In emergency, it is probable that certain analytical methods are still early in development (e.g., potency assay for DS characterization and DP release) and the strategy to onboard such assays during the development needs to be discussed with regulators.

## 6. References

References are included in the footnotes.