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Strategy for Viral Safety Risk Reassessment with Changes in the Manufacturing Process of Recombinant Biotechnology Products

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In the manufacturing of biotechnology drug products, the viral safety control strategy is a critical pharmaceutical quality management framework that contains three key elements: prevention, testing, and clearance. The prevention strategy involves rigorous screening for any adventitious virus contamination in the raw materials, reagents, and endogenous/adventitious virus contamination in the cell banks. The main objects of the testing strategy are critical process intermediate, such as the end of production cell (EOPC), limit of *in vitro* cell age (LIVCA), and unprocessed bulk (UPB). The clearance strategy often involves demonstrations of the effectiveness and robustness of a manufacturing process to inactivate or remove any endogenous/adventitious viruses through viral clearance (VC) validations [1].

Changes in a manufacturing process may affect the viral safety attributes of a product, which would draw significant attention from regulatory agents and lead to a complete repetition of previous relevant studies; however, with the accumulation of both industrial and regulatory experiences, and more thorough understandings of viral safety-related processes under a life-cycle management framework defined by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q12 [2], we propose a more flexible approach under certain circumstances, defined as a "reassessment approach" in this work, to address changes in the processes. The purpose of the reassessment approach is to avoid the unnecessary, laborious, and duplicative studies that have traditionally been conducted by many pharmaceutical companies and regulatory agencies.

Currently, an increasing number of pharmaceutical companies apply comparative data analysis as an alternative to studies for a change that occurs in either the clinical trial or the post-approval stage; however, there is no specific guidance document describing the requirements, and the results vary substantially among different pharmaceutical companies, especially regarding the scopes of validation/qualification data required and the comprehensiveness of the reassessment. Although there have been many relevant studies, most of them focus on a few technical details rather than an overall reassessment strategy, and the conclusions are sometimes contradictory. On the other hand, most of the guidance documents are limited only to the performance validation of the VC process associated with manufacturing process changes. Which situations require a comprehensive reassessment across the entire manufacturing process to manage the viral safety risk post-change and how this reassessment can be implemented are still ambiguous, and both questions will be addressed and elaborated in detail in this work.

1. Three key dimensions to consider for a "reassessment approach

The reassessment approach can be divided into two levels depending on whether the change has a potential adverse impact on the viral safety attributes of the products manufactured by the post-change processes: in level 1, when the change does not affect the viral safety attributes of the product, a thorough evaluation of the conducted studies should be carried out, and the establishment of representativeness between the studies and post-change processes should be justified; in level 2, when a manufacturing process change has the potential to cause an adverse impact on the viral safety attributes of a product, a complete or limited (but rationalized) repetition of the studies is necessary and may include cell substrate testing, critical process intermediate testing, and VC validation to evaluate process performance, as shown in Fig. 1.

In Fig. 1, the three dimensions of the reassessment approach are described in detail. The first dimension is the evaluation of any change to the viral safety profile of a cell substrate, including considerations of the differences in susceptibility to adventitious viruses of different types of cell substrates, the characteristics of endogenous viruses or virus-like particles, the virus load titer, and the model virus selected for process validation. All the studies related to viral safety should be repeated if the cell substrate for manufacturing is changed, such as a change from Chinese hamster ovary (CHO) cells to NS0 cells, *Spodoptera frugiperda*-9 (SF-9) cells, or an engineered cell substrate modified by introductions of viral vectors. The second dimension of the reassessment concerns the testing of critical process intermediate, including the EOPC/LIVCA and the UPB. In particular, reassessment of the EOPC/LIVCA is determined by whether its generation is prolonged or there are new risks of virus contamination, such as the use of animal origin raw materials; the UPB needs to be retested for all types of changes

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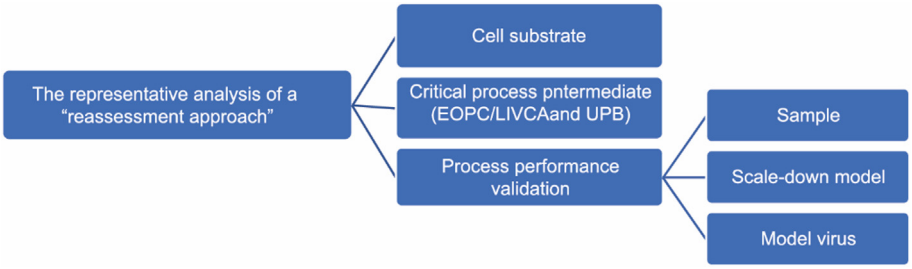

Fig. 1. Three dimensions of a "reassessment approach" to evaluate the viral safety profile of a manufacturing process change.

in the upstream culture process, which is consistent with the current regulatory practice that adventitious virus testing should be routinely applied to each batch of UPB. The third dimension is a representativeness analysis of the performance validation of the virus removal and inactivation process. Considering the complexity of this topic, the details are described in a separate section below.

2. Representativeness of process performance validation

2.1. Importance of sample representativeness

Ensuring the representativeness of the samples by careful selection, control, and comprehensive analysis is essential to the accuracy and reliability of validation experiment results yet easily neglected. Two key factors greatly affect the representativeness of the sample, namely, the physicochemical characteristics of the active drug substance, such as the isoelectric point, glycosylation profile, and major impurity composition; and the process intermediate characteristics, such as the concentration of target protein, the buffer matrix, and the total titers of retrovirus-like particles (RVLPs). Process intermediate characterization studies may suffice to ensure representativeness only for processes with high robustness, including low-pH incubation and nanofiltration, but for processes that exhibit high inherent variability, such as chromatography, comprehensive comparability analyses of both factors should be carried out.

For a low-pH incubation process (pH at or above 3.70), virus inactivation performance can be affected by other factors, such as the protein concentrations and type of acid titrant used [3]; thus, a validation study should be conducted with a new acid titrant or the post-change protein concentration (if beyond the 30%–300% range) when the incubation pH is above 3.70. If the representativeness of all levels of process intermediate remains unchanged but the quantity of RVLPs in the EOPC/LIVCA/UPB changes due to process alterations in the upstream culture stage or other possible reasons, the safety margin of the entire process should be recalculated based on the capacity of the overall virus clearance process being validated. When changes that are unlikely to affect the process intermediate occur, such as a complete duplication of the original manufacturing line, the replacement of centrifuge equipment, or the replacement of a low pH inactivation tank, only performance qualifications of the post-change equipment may be necessary.

For process changes that lead to a change in the levels of related substances and impurities, the lifetime of purification process consumables such as chromatographic resins may be affected, and the original resin lifetime validation results may not apply to the new process; thus, a revalidation may be necessary at a smaller scale or a full scale. It is well accepted that reuse of Protein-A resin may not lead to a decrease in its virus removal capability; thus, revalidation may not be required for Protein-A under this circumstance [4]. However, variations in the levels of related substances and impurities can affect the fouling of filters and decrease the virus removal capability of nanofiltration due to flow decay; it is highly recommended to carry out comparability studies on related substances and impurities of the process intermediate pre-change and post-change [5]. The sponsor can assess whether potential differences will trigger a new virus removal process validation based on prior knowledge.

2.2. Representativeness of the scale-down validation model

For representativeness of the scale-down validation model, the key point is to ensure that the validated established conditions (ECs) of both material attributes and critical process parameters (CPPs) can bracket the proposed manufacturing process. For a conventional recombinant biotechnology product, Table 1 [3,6–14] shows a summary of the worst-case conditions representative ECs of the manufacturing process.

For example, in a nanofiltration process, the pressure that leads to virus leakage can be the upper pressure limit for virus filters of some suppliers or the lower pressure limit for filters of other suppliers; in addition, the possible impact of process interruption or pause should also be accounted for in a scientific experimental design. The ECs associated with viral safety for chromatography can be more intricate; for instance, for anion exchange (AEX) chromatography, under flow-through mode, a higher conductivity tends to result in a lower log reduction value (LRV), whereas under binding-elution mode, the opposite is true. In addition, it is important to evaluate the relative residence time under different working modes, which is mainly dependent on the column height and sample flow rate.

As shown in Table 1, the various working modes involved in the purification process steps can considerably impact the worst-case conditions for virus clearance validation studies, which also highlights the significance of developing validation study protocols based on distinct features of the individual manufacturing process of the product.

2.3. Representativeness of model virus

ICH Q5A (R1) has provided sufficient guidance for the selection of model viruses based on both process characteristics and virus property considerations [1]. Recently, we have observed a change in the host cell substrate of an increasing number of sponsors for commercial reasons. Given these situations, it is necessary to consider the potential risk of endogenous and exogenous viruses in host cells; for example, for SF-9 cells, it is recommended to use baculovirus as a model virus, while for CHO cells, murine leukemia virus should be added.

Table 1
Worst-case conditions for common VC process steps.

Process steps	Parameter	Worst-case condition[a]
Low pH virus inactivation [3,6,7]	Inactivation pH	Highest pH
	Inactivation temperature	Lowest temperature
	Inactivation time	Shortest inactivation time
	Buffer matrix	Determined based on process development[b]
Flow-through mode chromatography [8–11]	Relative residence time	Shortest residence time[c]
	Load capacity	Highest load capacity
	Sample	Dependent on the mechanism of the resin[d]
	Collection	Widest collection criteria
Binding-elution mode chromatography [8,11–13]	Relative residence time	Longest residence time[c]
	Binding and elution buffer	Dependent on the mechanism of the resin[e]
	Collection	Widest collection criteria
Viral filtration [5,14][f]	Volumetric throughput	Maximum volumetric throughput of product intermediate loaded and the buffer used for flushing filters
	Pressure/ flow	Determined according to the challenge experiments performed by suppliers
	Others	Challenge study on frequency and time of process interruption

[a] The worst-case conditions of the process parameters are all based on the premise that the process parameters are within the range determined.
[b] It is recommended to keep the buffer matrix consistent, especially when the inactivation pH is equal to or greater than 3.70.
[c] The factors that affect the relative residence time of the material in the chromatography process include column height and flow rate.
[d] The items that may need to be analyzed include the protein concentration, pH, and conductivity of the sample. For example, for anion exchange chromatography, higher pH, and conductivity are the worst-case conditions. However, for strong anion complex chromatography, pH has less influence, and conductivity has a wider operating range.
[e] The items that may need to be analyzed include the loading sample (protein concentration, pH, conductivity) and elution buffer (wash volume, wash buffer pH, conductivity, and temperature). For example, for hydrophobic chromatography, the worst-case condition is a higher protein concentration. For cation exchange (CEX) chromatography, higher conductivity, and pH in the elution buffer are the worst-case conditions.
[f] The integrity requirements of the filter should be met after use.

3. Overall representative analysis in a reassessment

The three key dimensions discussed above should be systematically evaluated for an overall representative analysis in a reassessment approach: the representativeness of the cell substrate, the critical process intermediate, and the scale-down validation model. Three scenarios are possible after the reassessment: if all three key dimensions remain fully representative after the changes, no further action is needed except for the comprehensive comparative data analysis mentioned above; if the three key dimensions are only partially representative of the post-change process, a further evaluation is needed to determine whether the experimental study should be reconducted; and if the conclusion is nonrepresentative for the post-change process or the supporting data from the study are not sufficient for the reassessment, the experimental study must be fully reconducted.

In particular, for the second scenario, it is essential to apply the subsequent general principles for further evaluation. First, the evaluation should be carried out on a case-by-case basis. Second, it should be risk-based, accounting for both internal and external prior knowledge, comprising well-researched literature and pertinent guidelines.

Moreover, it is advisable for the sponsor that the manufacturing process be established prior to the pivotal clinical trials to avoid further preclinical or clinical comparability studies, and to minimize uncertainties associated with the representativeness of samples, scale-down models, or model viruses.

4. Conclusion

Changes occur frequently during all phases of drug development and can be complicated; it is difficult to find an example of a process that remains unchanged during the entire life cycle, and changes are particularly evident for monoclonal antibodies and fusion proteins. Balancing the risks and benefits of product improvement and ensuring safety has always been a practical issue for industry and regulatory authorities. This work provides a more flexible "reassessment approach" framework, which is especially favored under the trend of continuous and iterative advancements in the manufacturing technology and raw material. Instead of conducting repetitive studies for every single change, a risk- and science-based instructive framework can be used to assess the necessity and scope of a new study.

Compliance with ethics guidelines

Wenbo Sai, Dongchen Jia, Hao Chen, and Wei Wei declare that they have no conflict of interest or financial conflicts to disclose.

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