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Pharma & Biopharma Manufacturing & Testing Services

White Paper

Regulatory Considerations for Implementation of Alternative Microbial Methods for Quality Control Testing

Introduction

Scientific innovations have led to the development of increasingly complex therapeutic drugs and the rate of innovation seems to be increasing. In the past few decades, biopharmaceuticals such as monoclonal antibodies and cell/gene therapies have fueled the pipelines of many companies globally. Biotherapeutic drugs have a different approval process from small molecules because the manufacture involves living cells. The process is more complex, and the use of living cells introduces variability inherent to biological systems into the manufacturing process. In addition to the complexity of the manufacturing process, raw and starting materials are complex and often less well defined, frequently made with materials of animal or human origin which have been shown to introduce microbial contaminants.

Monoclonal antibody, gene and cell therapies utilize mammalian or insect cells in the manufacturing process. These large-scale cell cultures provide a favorable environment for microorganisms to grow. A contamination event always has serious impacts on the process and product and eventually patient safety. To address this issue global regulatory guidance such as the International Council on Harmonization (ICH) Q5A[(1)], requires drug manufacturers to implement measures to prevent, remove and detect contaminants to ensure patient safety. This guidance requires manufacturers to address three areas; testing the starting materials, testing in-process samples and finally validating the steps used to inactivate or remove contaminants.

There are several guidance documents from the ICH, World Health Organization (WHO), US Food and Drug Administration (FDA), European Medicines Authority (EMA), and many national pharmacopeia which outline the required testing. The testing methods were developed based on technologies and process knowledge prevalent at the time of their issuance.

These guidance documents recognized that new technologies would emerge over time providing an improvement in the suggested methods laid out in the guidance. Language was incorporated in the guidance documents to offer the flexibility to use alternate technologies, however there has been a general resistance in the adoption of these new methods by the industry without a clear process for acceptance. Recently the use of new molecular methods has highlighted gaps in the existing testing strategy by identifying previously undetected viral contaminants in final product, the cell banks from which it was produced and intermediate manufacturing stages.

To address the viral safety of new therapeutic and vaccine modalities such as cell therapies and viral vector-based vaccines, development of sophisticated detection technologies, new manufacturing paradigms and to incorporate knowledge gained from decades of biologics development, the ICH Q5A guideline is being revised[(2)]. It is expected that sensitive and specific molecular detection technologies such as polymerase chain reaction (PCR) and high throughput/next generation sequencing (HTS/NGS) will be discussed as alternatives to *in vivo* and possibly *in vitro* cell culture based detection, viral contamination detection for starting materials and in-process testing. The inclusion of these technologies in ICH Q5A revision indicates confidence in the utility of these technologies by both the regulators and the industry for biosafety assurance of biologics. As a result of this, it is highly likely there will be widespread adoption of molecular methods for the quality control during manufacturing of biologics.

This article discusses the regulatory expectations for incorporation of alternative methods with a focus on comparability. Strategies for easier substitution of current methods are also discussed.

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Regulatory requirements for adaption of alternative methods

Developing and publishing regulatory guidance is a multi-year process. Regulations are not updated frequently and therefore often lag technological advances. Guidance is issued based on knowledge and technologies current at the time of writing and therefore, often includes language permissive to the use of alternate technologies. For example. ICH Q5A states that "Numerous assays can be used for the detection of endogenous and adventitious viruses. They should be regarded as assay protocols recommended for the present, but the list is not all-inclusive or definitive. Since the most appropriate techniques may change with scientific progress, proposals for alternative techniques, when accompanied by adequate supporting data, may be acceptable". This guidance was published more than two decades ago, and many innovative technologies have been developed since.

Regulators expect that an alternative method is demonstrated to be fit for purpose and is equivalent or better than the current method. The US FDA's 2020 guidance on CMC for gene therapy[3] states "Examples of alternative methods, which may be needed for live cells, include rapid sterility tests, rapid mycoplasma tests (including PCR-based tests), and rapid endotoxin tests. For these non-compendial tests we recommend that you qualify/validate them to ensure they are fit for their intended use". USP <1223>[4] states that "The alternative technology must be at least equivalent to the current technology in terms of performance for the intended use. Much of the technical support for equivalence may come from the peer-reviewed scientific literature or from a prior regulatory submission (e.g., a vendor submitted the Drug Master File to the FDA, or prior submission from a company on this technology), but this must be confirmed, as appropriate for the intended use."

For the biopharmaceutical industry in general, this equates to ensuring that the method has been validated according to the principles outlined in ICH Q2 (R1)[5] or other appropriate local regulation, tested in the relevant matrices and demonstrated to be equal or better through a comparability exercise. Depending on whether the method is qualitative or quantitative, parameters to be determined during validation can include accuracy, precision, specificity, quantitation limit, linearity, range, and robustness. Where possible, this testing needs to be carried out in the typical sample matrix for the assay.

The other requirement is to demonstrate that the alternate method, where possible, is equal to or better than the current method through comparability studies.

Comparability

In vitro methods

When an alternate method is a replacement of an existing *in vitro* method comparability or equivalence studies expectations are well-defined. According to the Ph. Eur. 5.1.6 [6], an adequate comparison experiment at low levels of inoculation with sufficient numbers of replicates for relevant strains of test micro-organisms is required. Alternatively, and in some cases additionally, equivalence testing can be carried out by the parallel testing of a predefined number of samples or for a predefined period of time. This parallel testing can be justified based on a risk assessment. In some instances, specific detection limits for an alternative method are identified as in the Ph. Eur 2.6.7, Mycoplasmas [7]. According to the USP, four options are available to establish the equivalence of a candidate alternative analytical method:

1. Acceptable procedures (i.e., merely meeting a minimum performance or acceptance requirement without a need to demonstrate equivalence to the compendial method)
2. Performance equivalence to the compendial method
3. Results equivalence to the compendial method
4. Decision equivalence to the compendial method.

A decision equivalence is the case of a pass/fail result obtained by the test. With this approach, the frequency of positive and negative results generated should be no worse than with the compendial method. Based on the method being implemented a suitable strategy must be employed.

Culture-based methods detect the presence of infectious units of contaminants. Molecular methods such as PCR and NGS detect the presence of contaminant nucleic acid. Presence of nucleic acid molecules do not always equate to an infectious unit capable of replication and impacting product quality. Suitable investigative tools such as confirmatory culture-based test need to be place in the event of a positive signal in a molecular test.

In vivo methods

Comparability to *in vivo* methods is more complex. As outlined in Ph. Eur. 5.2.14 [8] *in vivo* methods are inherently variable. The determination of the absence of micro-organisms *in vivo* methods is usually based on non-specific observations such as increased body temperature, change in the physical activity of the animal etc., instead of specific detection of extraneous agent genomes using molecular methods in the *in vitro* alternatives. Many of the legacy *in vivo* assays were demonstrated to be fit for purpose many decades ago, in an era when validation requirements, such as ICH Q2 (R1) guideline, were not in place.

One of the consequences associated with the inherent variability of *in vivo* assays is that their replacement by the more-consistent *in vitro* methods requiring a head-to-head assay comparison becomes challenging to perform. In addition, it is not consistent with the principles of reduce, refine or replace (3Rs) principles to conduct side-by-side comparison of the *in vivo* and alternate *in vitro* methods where the same standard stock of organisms is used in both methods to demonstrate equivalence. Globally, regulatory agencies are seeking to implement the principles of 3Rs and this is being animal use and this is also being adopted by the pharmaceutical industry. At a recent Parental Drug Association (PDA) Virus Conference, representatives from the European Directorate for the Quality of Medicines & HealthCare (EDQM) presented this view and there was agreement from regulatory and industry representatives present. When possible, industry/academia consortia could undertake these efforts for promising technologies to satisfy the needs for performance evaluation, comparability and develop standardized process. An example of such an effort is the PDA's Advanced Virus Detection Technologies Interest Group's (ADVTIG) which includes manufacturers, service providers, academics and regulators, who collaborate to share/address common challenges and experiences and, create readiness for the use of NGS to replace *in vivo* adventitious viral detection.

Strategy for adoption

Although novel microbial and viral detection methods that are rapid, have improved sensitivity, good robustness and have broad range of detection have been available for almost two decades, their adoption by the biopharmaceutical industry has been slow. There is a perception that there is an undefined risk to move away from compendial or well-established methods as it would invite regulatory scrutiny and delay approvals.

As outlined in Ph. Eur. 5.1.6, the risk level in adopting an alternative method varies depending on the technology considered, the methodology it replaces, the nature of the measurements taken (qualitative, quantitative or identification), the particular product or process attribute being evaluated, the location of the measurement in the manufacturing process chain and various other factors.

Risk analysis tools may be utilized to determine which alternative method is to be implemented, to assist in the justification of its implementation or to better understand the impact of implementation on production and/or product quality. An alternative method can be justified for use if the information obtained gives a scientifically sound measure of microbiological quality, and if the limitations of the method are not more severe than those of the currently acceptable method. Adoption of the new methods is based on improvements in parameters such as sensitivity, specificity and time-to-result which can be used in a risk-benefit analysis. This also helps in demonstration of non-inferiority of the alternate method which is a regulatory expectation.

As noted above, regulatory agencies allow the implementation of novel technologies when accompanied with the appropriate justification and information package. This has been demonstrated in approvals of cell and gene therapies using rapid sterility, PCR-based detection of mycoplasma and other novel methods for in-process and release testing. In addition, alternate methods such as next generation sequencing (NGS) have been extensively used in the development and quality control testing of vaccines against SARS-CoV2 virus (personal communication).

To incorporate alternate methods in their processes, companies should perform a risk assessment, develop a strategy, and ensure that regulatory expectations are met according to the risk identified. When feasible using the method early in development can help identify any issues, generate data and provide confidence regarding the method's suitability for the intended use in the process. When the alternate method is performed by a contract testing organization, ensuring that appropriate validation, comparability when feasible and ethical, has been performed. In addition, all the relevant data should be available to be reviewed by a regulatory body either through a drug/biologics master file (D/BMF) where feasible or through other processes. In **Table 1** below, we list a few example risk parameters with respect to the alternate method which can be used to perform a risk assessment.

Parameter	Low	Medium	High
Type of assay	Characterization	In-process	Release
Technology maturity	Compendial method	Well-established	Novel
Testing provider	Well-established testing organization, providing GMP quality control and inspected by regulatory agencies		Methods and quality systems not inspected by regulatory agencies
Validated method	Yes		No
Data package available	Yes, in a master file (DMF/BMF)		No
Regulatory maturity	Assay used in release of licensed product	Assay used in release of investigational product	Data about method not reviewed by regulatory agency
Assay comparability data package available (where feasible)	Yes		No

Table 1: Examples of risk parameters for substitution with an alternative method.

Additionally, when there are opportunities for early feedback from regulators, for example through an INTERACT meeting, include the use of the alternate technology in the discussion.

The graphic **(Figure 2)** shows some feedback points throughout the development process. All the above reduce the risk of delay in approvals.

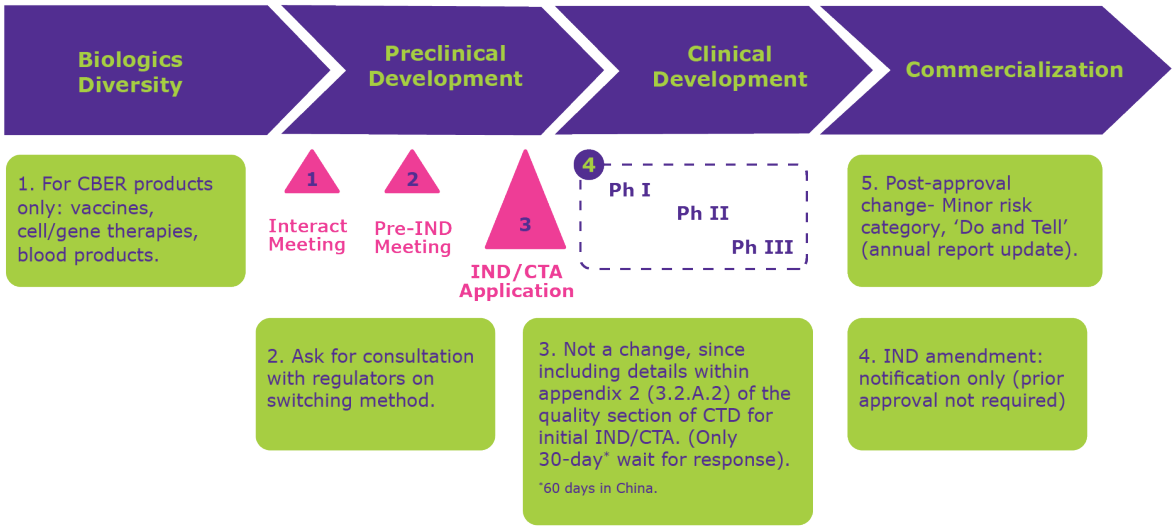

Figure 2: Opportunities for regulatory feedback on alternate methods.

Post-approval change for licensed products

Innovation and improvements to assay methodologies do not stop post-licensure and continuous improvement is expected by regulatory authorities throughout the product lifecycle. Regulators have provided extensive guidance on incorporating changes post-approval. Chemistry, manufacturing and control (CMC) changes vary from low to high potential risk with respect to product quality, safety, and efficacy. Guidance from the WHO, EMA, FDA and more recently ICH Q12[9], provide guidance for a risk-based approach and the required types of information to be communicated to them regarding the change. The change is classified with regards to the potential to have an adverse effect on quality of the drug product. The regulatory communication category, supporting information/ documentation requirements, and associated time frame for evaluation are commensurate with that potential risk.

The risk parameters for changing a method are similar to the ones outlined above **(Table 1)**. On the basis of the potential impact of the quality change (e.g. manufacturing change) on the quality attributes (i.e. identity, strength, purity, potency) of the biotherapeutic product and on their potential impact on the safety or efficacy of the product, a change should be categorized as major, moderate, minor or a 'change with no impact' quality change. A high-risk change may equate to a major change and so forth. Once the change is classified, depending on the relevant regulatory authority, the appropriate regulatory tools should be used to communicate regarding the change as outlined in **Table 3**.

Risk Level	US	EU	China	Japan	Also known as AKA
Major	Prior Approval Supplement (PAS)	Type II	Need to be approved by NMPA	Similar to Major (EU/ US)	“Tell and Wait”
Moderate	Change being effected (CBE-30 or CBE-0)	Type IB	Need to be approved by local MPA and CC to NMPA	N/A	“Tell and Do”
Minor	Annual Report	Type IA	Need to be filed to local MPA	Less strict than Moderate (EU/ US)	“Do and Tell”

Note: There is secondary (lower impact) level of “Minor” change that can be updated in the Pharmaceutical Quality System (US/EU) or the SOP (Japan). No regulatory notification is required.

Table 3: Post-approval change categories based on risk.

In addition, collaborating with the alternative technology provider (instrument manufacturer or contract testing organization) for guidance on implementation, generation of relevant data and appropriate documentation will help with affecting the change and reduce regulatory risk.

Based on risk parameters identified above here are a few examples of adventitious agent detection method changes and their potential change classification based on a typical monoclonal antibody platform process.

QC Method	Assay Type	Technology Maturity	Validation data available	Comparability data available	Regulatory Maturity	Likely change category
Blazar™ Rodent Panel replacing specific virus detection by MAP/HAP*	Characterization of master cell bank	Medium	Yes, BMF with US FDA	Yes	Medium	Minor
Substitution of broad inapparent virus detection (*in vivo*) with HTS/NGS	Characterization of master cell bank	Medium	Yes	Yes, in progress through consortia	Low	Moderate
Replace compendial sterility test with respiration-based method	Drug product release test	High	Yes	Yes	Medium	Major

*MAP/HAP – Mouse antibody production/Hamster antibody production

Table 4: Examples of technologies and likely change classification for a platform monoclonal antibody production process (shading: Rich Purple-low risk, Vibrant Green-medium risk, Vibrant Magenta-high risk)

Conclusion

The use of alternate methods to replace compendial/currently employed methods are encouraged by regulatory authorities. Regulatory requirements indicate methods should be fully validated, be comparable to established methods and demonstrated to be fit for purpose. A risk assessment prior to incorporating facilitate justification of its implementation or to better understand the impact of implementation on production and/or product quality. Comparability of *in vitro* methods to established *in vivo* methods may be required only when ethical and a meaningful result can be obtained. Meeting all regulatory expectations with respect to demonstration of non-inferiority reduces risk. Incorporation of the methods early in the development process and seeking regulatory feedback when feasible can further reduce any risk associated with using the method and implementing changes. Alternative methods can be implemented post approval by using a risk-based approach to generate appropriate information and following the regulatory communication guidelines commensurate to the risk.

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