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Government of India
Directorate General of Health Services
Central Drugs Standard Control Organization
(Biological Division)

FDA Bhawan, Kotla Road,
New Delhi 110002
Date: 06 MAY 2025

NOTICE

Subject: Inviting comments on Revised Guidelines on Similar Biologics- Regulatory requirements for Marketing Authorization in India, 2025 drafted by CDSCO

In view of advances in scientific knowledge and experience, it was decided to revise the existing guidelines in line with recent international guidelines. To facilitate this process, a Committee comprising of technical subject experts, representatives from NIB, DBT and representatives from Industries involved in manufacturing of similar biologics was constituted. The committee meetings were convened to discuss the revisions in the guidelines.

The Draft Guidelines is now being placed in the public domain for inviting comments/suggestions from concerned stakeholders. This window of opportunity will close within 30 days of publishing the draft guidelines on CDSCO website, and, once finalized, there will be minimal scope for change in this document. Therefore, all interested stakeholders are requested to provide comments/suggestions within the window of 30 days, at biological@cdsco.nic.in in word document as per the annexed format.

The suggestions/comments received on the above email address within the 30 days shall be taken into consideration for finalisation of the draft Guidance document.

Stakeholder's Comments format

Name and Designation:
Firm Name:

S.No.	Page No.	Line No.	Section/Sub-section/Heading	Current text	Proposed text	Explanation/Reference

Dr. Rajeev Singh Raghuvanshi
Drugs Controller General (India)

To: All Stakeholders through CDSCO website

Encl: Copy of Draft CDSCO Guidelines on Similar Biologics- Regulatory Requirements for Marketing Authorization in India, 2025

DRAFT

GUIDELINES ON SIMILAR BIOLOGICS

Regulatory Requirements for Marketing Authorization in India, 2025

Central Drugs Standard Control Organization
Ministry of Health & Family Welfare
Government of India

Department of Biotechnology
Ministry of Science & Technology,
Government of India

Document Name: **GUIDELINES ON SIMILAR BIOLOGICS**	
Effective From Year: 2025	Validity: Till Further Revision

Contents

Message

Foreword

Preface

The Guidelines on Similar Biologic-Regulatory Requirements for Marketing Authorization in India was published in the year 2012 by CDSCO in collaboration with Department of Biotechnology (DBT) to address the regulatory pathway for Similar Biologics in India. The Guidelines was then revised in the year 2016 with more focus on scientific principles and stepwise approach to be applied during the demonstration of similarity between a similar biological product and its reference biological product. Keeping in view the advances in scientific knowledge and experience, it was decided to update the existing guidelines in line with recent international guidelines. A Committee was constituted for the same including technical subject experts, representatives from NIB, DBT and representatives from Industries involved in manufacturing of similar biologics. The committee meetings were convened to discuss the revisions in the guidelines.

In view of committee recommendations, the present Guideline document, 2025 was framed which represents the outcome of the revision process and replaces GUIDELINES ON SIMILAR BIOLOGICS: Regulatory Requirements for Marketing Authorization in India, 2016. This guideline considers the current scientific evidence and scientific updates from the International Guidelines majorly WHO TRS 1043: Guidelines for evaluation of biosimilars. Since, major countries are moving for waiver of non-clinical studies for similar biologics, the current revision principally focus on strengthened orthogonal analytical tools and in vitro studies to establish similarity between the similar biologic product and Reference Biological Product.

The salient features of the revision include-

a. Introduction of scientific considerations and key principles for licensing of similar biologics.
b. Sections of quality, and nonclinical and clinical evaluation are updated to make them more consistent with current international practices and to provide more clarity and flexibility.
c. Revised pathway for approval of similar biologics in India
d. Specific topics addressed in the revision include but are not limited to: –
    - Next generation analytical methodologies introduced for establishing analytical similarity
    - Use of reference standards and development of in-house reference standards elaborated
    - Elaborative list of in vitro studies included
    - New guidance on determining the need for in vivo animal studies and on the implementation of the 3Rs principles (“Replace, Reduce, Refine”) to minimize the use of animals in testing

- Statistical intervals for establishment of similarity ranges to provide clarity and focus on statistical consideration in calculation of sample size for clinical studies.

List of Acronyms

ADA	Anti-Drug Response
ADCC	Antibody-Dependent Cellular Cytotoxicity
ADCP	Antibody-Dependent Cellular Cytotoxicity
BP	British Pharmacopoeia
CDSCO	Central Drugs Standard Control Organization
CDC	Complement Dependent Cytotoxicity
CRS	Chemical Reference Standards
CQA	Critical Quality Attributes
DBT	Department of Biotechnology
DCGI	Drug Controller General of India
EMA	European Medicines Agency
EP	European Pharmacopoeia
FC	Fragment Crystallizable
GEAC	Genetic Engineering Appraisal Committee
GMP	Good Manufacturing Practice
IBSC	Institutional Biosafety Committees
ICH	International Council of Harmonisation
IRS	In-house reference Standards
IU	International Units
JP	Japanese Pharmacopoeia
LMO	Living Modified Organism
MA	Market Authorization
mAbs	Monoclonal Antibodies
MoHFW	Ministry of Health & Family Welfare
NDCT	New Drugs and Clinical Trial Rules 2019
NIBSC	National Institute for Biological Standards and Control
NIST	National Institute of Standards and Technology
PD	Pharmacodynamic
PK	Pharmacokinetic
PSUR	Periodic Safety Update Reports
QA	Quality Attribute
RBP	Reference Biological Product
RCGM	Review Committee on Genetic Manipulation
SBP	Similar Biological Product
TNF	Tumour Necrosis Factor
USFDA	United States Food and Drug Administration
USP	United States Pharmacopeia
WHO	World Health Organization

Guidelines on Similar Biologics

Regulatory Requirements for Marketing Authorization in India

1. Introduction

Biotherapeutic products have a proven track record in treating numerous life-threatening and chronic diseases. As patents and data protection periods for many of these products expire, a new wave of products has emerged that are designed to be highly "similar" to the licensed "originator" products. These similar products can partly rely the safety and efficacy data of the originator products, based on a thorough head-to-head comparison demonstrating high similarity.

CDSCO is the national regulatory authority in India that evaluates safety, efficacy, and quality of drugs in the country. The “Guidelines on Similar Biologics” prepared by Central Drugs Standard Control Organization (CDSCO) and the Department of Biotechnology (DBT) lay down the regulatory pathway for a Similar Biologic claiming to be Similar to an already authorized Reference Biologic.

As per NDCT Rules 2019, “Similar Biologic” means a biological product which is similar in terms of quality, safety and efficacy to Reference Biological Product (RBP) licensed or approved in India, or any innovator product approved in International Council of Harmonisation (ICH) member countries. The term “Similar biologic” is being widely used by many Drug regulatory agencies such as United States Food and Drug Administration (USFDA), European Medicines Agency (EMA), WHO etc. Both the terms “Similar Biologics” and “Biosimilar” essentially refers to the same terminology and can be used interchangeably.

Presently, several organizations are actively engaged in manufacturing and marketing similar biologics in India. In the past, these Similar Biologics were approved by RCGM and Central Drugs Standard Control Organization (CDSCO) using an abbreviated version of the pathway applicable to new drugs on a case-by-case basis.

These guidelines are for the guidance of all stakeholders and are not meant to substitute or rephrase the Rules made under Drugs and Cosmetics Act, 1940 or any other relevant Acts and are subject to being in conformity with the Drugs and Cosmetics Act and Rules as may be amended from time to time.

2. Background

CDSCO in collaboration with Department of Biotechnology (DBT) published the first guidelines titled as “Guidelines on Similar Biologic- Regulatory Requirements for Marketing Authorization in India” in 2012 to address the regulatory pathway regarding manufacturing process and quality aspects for Similar Biologics. The said guidelines also address the pre-market regulatory requirements including comparability exercise for quality, preclinical and clinical studies and post market regulatory requirements for similar biologics.

Keeping it at par with latest regulatory requirements and to provide more clarity, the guidelines were revised in the year 2016 with more focus on scientific principles and stepwise approach to be applied during the demonstration of similarity between a similar biological product and its reference biological product. It was however viewed as a “living” document that would be further revised in line with advances in scientific knowledge and experience.

It was decided that a review of existing guidelines should be undertaken of current scientific evidence and international guidelines including Guidelines on Evaluation of Similar Biologics WHO Technical Report Series, No. 1043, 2022 (Replacement of Annex 2 of WHO Technical Report Series, No. 977). This revised guideline would provide an opportunity to evaluate new developments and identify areas where the current guidance could be more flexible without compromising its basic principles and allow for the provision of additional explanation of the possibility of tailoring the amount of data needed for regulatory approval.

3. Purpose & Scope

The objective of this document is to provide guidance to applicants to enable them to understand and comply with the regulatory requirements for market authorization of Similar Biologics in India.

These guidelines apply to Similar Biologics that contain well characterized proteins as their active substance, derived through modern biotechnological methods such as use of recombinant DNA technology. The demonstration of similarity depends upon detailed and comprehensive product characterization, preclinical and clinical studies carried out in comparison with a Reference Biological Product.

Similar Biologics can only be developed against the Reference Biological Product that has been approved using a complete data package in India. In case the RBP is not authorized in India, it should have been approved / licensed and marketed in an ICH (The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) country namely USA, UK, Japan, Australia, Canada and EU.

Any product can be considered as a similar biologic, only if it is proven to be similar using totality of the evidence concept requiring that sufficient structural, functional, nonclinical, and clinical data is acquired in stepwise manner to demonstrate that there are no clinically meaningful differences between the similar biological product (SBP) and the reference biological product (RBP) in terms of the safety, purity, and potency of the product.

The reference biological product (RBP) is central to the licensing of a similar biological product, and the choice of a suitable RBP is fundamental for a similar biologic development. The RBP should have been marketed for a suitable duration, have a significant volume of marketed use in the relevant country or area, and have a long established history of good safety and efficacy.

These guidelines are applicable for similar biologics to be developed in India or imported into the country for marketing authorization. Detailed regulatory pathway for approval of Similar Biologics is given in **Annexure I** and **Annexure IA.**

4. Applicable Regulations and Guidelines

The similar biologics are regulated as per the Drugs and Cosmetics Act, 1940, the Drugs Rules, 1945 (as amended from time to time), New Drugs and Clinical Trial Rules 2019 (NDCT) and Rules for the manufacture, use, import, export and storage of hazardous microorganisms/

genetically engineered organisms or cells, 1989 (Rules, 1989) notified under the Environment (Protection) Act, 1986. Various applicable guidelines are as follows:

- Guidelines for generating preclinical and clinical data for rDNA vaccines, diagnostics and other Biologicals, 1999.
- CDSCO guidance for industry, 2024
    - Submission of Clinical Trial Application for Evaluating Safety and Efficacy
    - Requirement for permission of New Drug Approval.
    - Preparation of Quality Information for Drug Submission for New Drug Approval: Biotechnological/Biological Products
- Post approval changes in biological products: Quality, Safety and Efficacy Documents, 2024
- Regulation and Guidelines for Recombinant DNA Research and Biocontainment, 2017
- Guidelines and Handbook for Institutional Biosafety Committees (IBSCs), 2020.

5. Competent Authorities

The competent authorities involved in the approval process are as follows:

Institutional Biosafety Committee (IBSC)

IBSC is required to be constituted by any person including research institutions handling hazardous microorganisms and/ or genetically engineered organisms. IBSC is responsible for ensuring biosafety on-site and is also assigned with the responsibility to review and authorize firm for exchange of aforesaid organisms for the purpose of research.

Review Committee on Genetic Manipulation (RCGM)

RCGM is functioning under the Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India. In the context of Similar Biologics, RCGM is responsible for authorizing the conduct of research and development involving Risk Group 3 and 4 organisms and exchange of genetically engineered cell banks for the purpose of research and development .

Central Drugs Standard Control Organization (CDSCO)

CDSCO, headed by the Drug Controller General of India (DCGI) is the apex regulatory body under Ministry of Health & Family Welfare (MoHFW), Government of India, which is responsible for the approval of New Drugs, Clinical Trials in the country, laying down the standards for Drugs, control over the quality of Imported Drugs, coordination of the activities of State Drug Control Organizations and providing expert advice with a view of bring about the uniformity in the enforcement of the Drugs and Cosmetics Act.

In the context of Similar Biologics, CDSCO is responsible for clinical trial approval (also grants permission for import of drugs for clinical trial) and permission for import and manufacturing for sale or for distribution.

6. Scientific Considerations and Concept for Licensing Similar Biologics

The regulatory framework for licensing generic medicines is well-established in many countries. Typically, demonstrating structural similarity and bioequivalence between a generic medicine and its RBP is sufficient to infer therapeutic equivalence. However, this approach is not applicable to the licensing of similar biologics, as biological products are generally large and complex proteins that are more difficult to characterize and manufacture than small molecules.

The first step in developing a similar biologic should be the characterization and evaluation of the quality attributes of the RBP. This is followed by a comparability exercise using sensitive, orthogonal analytical methods and assays to demonstrate structural, functional, and clinical similarity. Comprehensive characterization and comparison at the quality and nonclinical (in vitro) levels serve as the basis for establishing comparability, with a tailored confirmatory clinical data package required for licensing. If relevant differences between the similar biologic and the RBP are identified, the underlying causes should be explored. Unless these differences can be explained and justified in terms of their lack of clinical impact, additional data, such as on safety, may be needed.

In addition to quality and nonclinical (in vitro) data, clinical data are typically required for any similar biologic. The type and extent of such data needed will depend on factors such as the specific product or product class, the level of characterization achievable through advanced analytical methods, observed or potential differences between the similar biologic and the reference biological product (RBP), and clinical experience with the RBP.

Manufacturers must demonstrate a thorough understanding of their product, ensure consistent and reliable manufacturing processes, and provide a comprehensive quality dossier that includes detailed product characterization. The dose and route of administration for the similar biologic must be same as that of RBP. Clinical studies must be conducted using the final formulation of the similar biologic derived from the final process; otherwise, additional evidence is needed to confirm that the marketed product matches the one used in clinical trials.

In case more than one indication is approved for the RBP, the similar biologic also qualifies for all the indications only if it is justified and if meets the conditions set forth in the section “Extrapolation of Efficacy and Safety Data to other Indications”. Justification for extrapolation of indication shall be based on comparability in quality, preclinical and clinical studies, available literature data and whether or not the same mechanism of action is involved in specific indications.

7. Key Principles for the Licensing of Similar Biologics

- Characterization of the quality attributes of the RBP should be the first step in guiding the development of the similar biologic. The subsequent comparability exercise should demonstrate structural, functional and clinical similarity.
- Demonstration of similarity of a similar biologic to an RBP in terms of structural and functional aspects is a prerequisite for establishing comparability, with a tailored clinical data package required as needed.
- Comparative clinical trial, assessment of pharmacokinetic (PK) and pharmacodynamic (PD) parameters (if available), and immunogenicity in human subjects, will typically be a core part of the clinical comparability assessment, unless scientifically justified.
- The decision to license a similar biologic should be based on evaluation of the whole data package generated during the overall comparability exercise.
- If relevant differences between the proposed similar biologic and the RBP are found at the structural, functional or clinical level, the product is unlikely to qualify as a similar biologic.
- If comparability exercises are not performed as outlined in this document, then the final product should not be referred to as a similar biologic.
- The authorization process of generic medicines does not apply for similar biologics.
- As with other biological products, similar biologics require effective regulatory oversight pre- and post-approval in order to manage the potential risks they pose and to maximize their benefits.

8. Reference Biological Product (RBP)

Comprehensive information on the reference biological product (RBP) provides the basis for establishing the quality, safety and efficacy profile against which the similar biologic will be compared. The RBP has to be used in all the comparability exercises with respect to quality, preclinical and clinical considerations.

The choice of RBP is therefore critically important in the evaluation of a similar biologic. The following factors should be considered for selection of the RBP.

- The RBP should be licensed / approved in India or ICH countries and should be the innovator's product. The RBP should be licensed based on a full safety, efficacy and quality data. Therefore, another similar biologic cannot be considered as a choice for RBP.
- In case the RBP is not marketed in India, the RBP should have been licensed in any ICH countries. The RBP can be imported for developing the similar biologic for quality, pre-clinical and clinical comparability.
- The same RBP should be used throughout the studies supporting the safety, efficacy and quality of the product (i.e. in the development Programme for the similar biologic).
- The dose and route of administration of the similar biologic should be the same as that of the RBP. However, the strength e.g. fills volume, pharmaceutical form, formulation, excipients and presentation (for example, use of a different medical device or number of syringes in a pack) of the similar biologic might differ from the RBP, if justified.
- Packaging configuration can be decided by the manufacturer if justified.

- The acceptance of an innovator product as a RBP for evaluation of similar biologic does not imply approval for its use in India.

Note: ICH countries in this context include USA, UK, Japan, Australia, Canada and EU.

9. Quality

The comparison showing molecular similarity between the similar biologic and the RBP provides the essential rationale for predicting that the clinical safety and efficacy profiles of the RBP apply to the similar biologic. Therefore, a high degree of analytical and functional similarity between the similar biologic and the RBP is the basis for developing a similar biologic.

Development of a similar biologic involves the thorough characterization of multiple RBP batches in order to obtain an understanding of the overall quality profile as well as range of variability of the RBP batches on the market. Based on the knowledge gained from the RBP characterization studies, as well as available in-house and public information, the manufacturing process of the similar biologic is developed to produce a product that is highly similar to the RBP in all clinically relevant quality attributes (that is, attributes that may impact clinical performance).

The manufacturer of the similar biologic should additionally carry out a comprehensive and comparative state-of-the-art physicochemical and biological characterization of the similar biologic and the RBP and document the results in the submitted marketing authorization application.

9.1 Reference standards

Biological reference materials which serve as reference sources of defined biological activity expressed in internationally agreed units. International units (IU) are assigned to such standards or other reference materials to allow the assessment of ‘biologicals’ in a consistent manner. The Reference Standard is usually assigned an estimated potency value after a multi-centre collaborative study. These standards are considered to be the ‘gold standard’ against which regional, national and international laboratories and manufacturers calibrate their own working standards. Typically, it is established by a public agency (e.g. WHO), Government (e.g. Indian Pharmacopeia Commission, National Institute of Standards and Technology (NIST), National Institute for Biological Standards and Control (NIBSC), or compendia (e.g., Indian Pharmacopoeia, United States Pharmacopeia (USP), Ph. Eur.), and is officially recognized as standard by individual regulatory authorities. There are also other types of external reference standards such as the Chemical Reference Standards (CRS), which are higher in concentration as compared to biological reference.

In the absence of established Reference Standards, the development of in-house reference standards derived from the manufacturer’s own manufacturing process should be established. Extensive characterization of in-house standards is performed through comprehensive analytical testing to confirm identity, potency, purity, impurity profiles etc. While RBP may be

used for establishing bio similarity/comparability, relying on reference standards or in-house standards guarantees authenticity, consistency, and alignment with the manufacturer's production process, which is vital for maintaining the production integrity and consistent quality of the product.

9.2 Manufacturing process

The manufacturing process of the similar biologic should be developed based on a comprehensive understanding of the RBP gained through detailed characterization studies of a sufficient number of RBP batches.

The similar biologics manufacturer should develop the manufacturing process to yield a comparable quality product in terms of identity, purity and potency to the RBP. The manufacturing process for similar biologics should be validated and demonstrated to be highly consistent and robust.

The manufacturer must demonstrate the consistency and robustness of the manufacturing process by implementing state-of-the-art quality control and assurance procedures, in-process controls and process validation. The similar biologic manufacturing process should meet the same standards required for originator products, including manufacture under current good manufacturing practices.

As for any biological product, if process changes are introduced during the development of a similar biologic, then the impact of the changes should be assessed through a comparability exercise. Although many of the same principles are followed, the assessment of manufacturing process changes should be addressed separately from the comparability exercise performed to demonstrate similar biological activity with the RBP. It is, however, strongly recommended that the pivotal data used to demonstrate similarity are generated using similar biologic batches manufactured using the commercial manufacturing process and therefore representing the quality profile of the batches to be commercialized.

Although the similar biologic does not need to be expressed in the same type of host cell as that used for the RBP, it is recommended that a similar host cell type is used (for example, Escherichia coli, Chinese hamster ovary cells, etc.). This will reduce the potential for critical changes in the quality attributes of the protein, or in post-translational modifications, product-related impurities or the process-related impurity profile, that could potentially affect clinical outcomes and immunogenicity. If a different host cell is used (for example to avoid unwanted and potentially immunogenic glycan structures present in the RBP) then changes introduced in terms of product-related substances, as well as product- and process-related impurities, need to be carefully considered.

The manufacturing process used can significantly affect the structure of the drug substance and thereby impact upon the potency of the product. For example, in the case of mAbs, when deciding upon the expression system to employ, manufacturers should be guided by the

potential for both enzymatic and non-enzymatic modifications, such as incomplete disulfide bond formation, formation of aggregates, glycosylation, N-terminal pyroglutamine cyclization, C-terminal lysine processing, deamidation, isomerization and oxidation, modification of the N-terminal amino acids by maleuric acid, and amidation of the C-terminal amino acid.

The data requirements for review of manufacturing process at developmental stage includes a complete description of the manufacturing process from development and characterization of cell banks, stability of clone, cell culture/fermentation, harvest, excipients, formulation, purification, primary packaging interactions (if different from RBP), etc. and the consequences on product characteristics as indicated below:

9.2.1 Molecular Biology Considerations

The details regarding host cell cultures (including viral clearance), vectors, gene sequences, promoters etc. used in the production of similar biologics should be provided with appropriate drawings/figures. The detail of post-translational modifications (glycosylation, oxidation, deamidation, phosphorylation etc.), if any should be explained.

9.2.2 Upstream Process Development

- Upstream process should be described in detail including media components used for cell growth.
- At least three batches of reproducible fermentation data at pilot scale (batch size adequate to give enough purified product to generate preclinical/developmental data).
- Upstream process should be well controlled and monitored.
- Details of upstream process kinetics data from consistency batches indicating cell growth, product formation, pH, temperature, dissolved oxygen, major nutrient consumption pattern and agitation rate.
- Concentration to be defined in terms of product/ liter, yield and volumetric productivity.
- Data to verify that the specific protein yield (amount of protein per unit cell mass) remains constant for all upstream batches.
- Demonstrate that the overall productivity is reproducible and scalable.

9.2.3 Downstream Process Development

- Detail description of the methods followed for the cell harvesting and extraction of the protein.
- Steps involved in purification of protein.
- Batch size for protein purification.
- Description of each unit operation step during purification and recovery of protein along with quantitative recovery of product at each stage.

- Consistency of recovery in three consecutive batches of purification from three independent batches of cell culture/fermentation. Describe post translational variation, if any.
- Details of removal of impurities like product related variants & impurities, and host cell & process related impurities considered to pose a risk of Immunogenicity (EMA 2017)
- Virus clearance validation studies should be part of Marketing Authorization application.

For clinical trial application, additional requirements are applicable as per CDSCO guidelines. A well-defined manufacturing process with its associated process controls assures that an acceptable product is produced on consistent basis in accordance with Good Manufacturing Practice (GMP). Data for submission should include:

- Detailed description of the drug substance and drug product processes
- Critical Quality Attributes (CQA) of the product
- Manufacturing process controls
- Critical process parameters
- Stability data
- Comparability of product manufactured at intended commercial scale against RBP
- Data from consistency batches and/ or process validation batches at commercial scale as applicable.

9.3 Analytical considerations

Thorough characterization of both the RBP and the similar biologic should be carried out using state-of-the-art chemical, biochemical, biophysical and biological analytical techniques. The goal of the comparability investigation is to be as comprehensive as possible in order to minimize the possibility of undetected differences between the RBP and the similar biologic that may affect safety and clinical activity.

Details should be provided on primary and higher-order structure, post translational modifications (including, but not limited to, glycoforms), biological activity, purity, impurities, product-related (active) substances (variants) and immunochemical properties, where relevant.

The methods should be scientifically sound and demonstrated to be of appropriate sensitivity and specificity for their intended use. The analytical methods should be chosen for establishing product comparability as per the critical quality attributes of the product. For certain attributes (e.g. product aggregation) it is customary to use multiple, orthogonal methods for characterization. Extensive state of the art analytical methods should be applied to detect even “slight differences” in all relevant quality attributes. Indian Pharmacopoeia or equivalent like USP / European Pharmacopoeia (EP)/ British Pharmacopoeia (BP) / Japanese Pharmacopoeia (JP) / etc. monograph should be followed, if available. However, if advanced

analytical methods superior to Pharmacopoeia are used, those methods can be employed based on method validation with suitable justification.

The analytical limitations of each technique (for example, limit of detection or resolving power) should be considered when determining the similarity of a similar biologic to its RBP.

Representative raw data should be provided for analytical methods (for example, high-quality reproductions of gels and chromatograms) in addition to tabular data summarizing the complete dataset and showing the results of all release and characterization analyses carried out on the similar biologic and the RBP. Graphical presentation of datasets comparing similar biologic and RBP analytical data should also be produced where possible. The results should be accompanied by sufficient interpretation and discussion of the findings.

The measurement of quality attributes in characterization should entail the use of appropriately qualified assays, which are reproducible and reliable. The methods used to measure quality attributes for batch release, stability studies and in- process controls should be validated in accordance with ICH guidelines (ICHQ2, Q5C, Q6B), as appropriate. The characterization studies should include samples of the applicant 's r-DNA derived product, RBP as control, known positive standard and negative control, wherever relevant. A complete description of the analytical techniques employed for release and characterization of the product, along with method validation or qualification data (as appropriate), should be provided in the dossier.

Due to the unavailability of drug substance for the RBP, the similar biologic manufacturer will usually be using a commercial drug product for the similarity exercise. The commercial drug product will, by definition, be in the final dosage form containing the drug substance(s) formulated with excipients. It should be verified that these excipients do not interfere with the analytical methods used and thus have no impact on test results. If the drug substance in the RBP needs to be purified from a formulated reference drug product in order to be suitable for characterization then studies must be carried out to demonstrate that product heterogeneity and relevant attributes of the active moiety are not affected by the isolation process. The approach used for isolating the drug substance of the RBP and comparing it with the similar biologic should be justified and demonstrated (with accompanying data) to be appropriate for the intended purpose.

Physicochemical and Biological characterization methods (Quality Attributes) to be used for r-DNA derived products are given in **Annexure II**. It may be noted that this Annexure is suggestive but not limited to the specified method and the requirements may vary on case by case.

9.3.1 Product Characterization

Characterization studies for similar biologics include physicochemical properties, biological activity, immunological properties, functional assays, purity (process and product-related impurities etc.), strength and content. Principles outlined in the ICH Q6B guideline should be followed.

i. **Structural and Physicochemical Properties:** The analysis of physicochemical characteristic should include determination of primary and higher order structure (secondary/tertiary/quaternary) and product variants of the drug substance and the product along with other significant physicochemical properties.

The amino acid sequence of a similar biologic should be confirmed to be the same as that of its RBP. It is, however, further recommended that manufacturers should pay special attention to any sequence variants present in the similar biologic. Although an identical primary sequence between the similar biologic and the RBP is expected, low-level sequence variants may occur due to transcription and translation errors, especially through amino acid misincorporation during high-level expression, and should be identified if present. The presence of such variants could be acceptable if properly described and controlled to a reasonable level. An assessment of the potential clinical impact of such variants would also need to be considered.

An inherent degree of structural heterogeneity occurs in proteins as a result of biosynthesis processes. These include C-terminal processing, N-terminal pyroglutamation, deamidation, oxidation, isomerization, fragmentation, disulfide bond mismatch and free sulfhydryl groups, N-linked and O-linked oligosaccharide, glycation and aggregation. The structural heterogeneity present in the similar biologic should be evaluated relative to the RBP. Experimentally determined disulfide bonding patterns should be compared to the predicted structure based on well-established structural data on the molecule. In cases, where post translational modifications are taking place, these modifications need to be identified and quantified. In case any significant differences are found, these should be scientifically justified and critically examined in preclinical studies and clinical trials.

ii. **Biological Activity:** Biological activity is the specific ability or capacity of the product to achieve a defined biological effect. It serves multiple purposes in the assessment of product quality and is required for characterization and for batch analysis. Ideally, the biological assay used will reflect the understood mechanism of action of the drug substance of the RBP and will thus serve as a link to clinical activity. A biological assay is a quality measure of the activity of the drug substance and can be used to determine whether a product variant is active (that is, a product-related substance) or inactive (and therefore defined as an impurity). Biological assays can also be used to confirm that small differences observed in the higher-order structure of a molecule have no influence on its biological activity. Thus, the use of relevant biological assay(s) of appropriate precision, accuracy and sensitivity provides an important means of confirming that there is no significant functional difference between the similar biologic and the RBP.

For a product with multiple biological activities, manufacturers should perform, as part of product characterization, a set of relevant functional assays designed to evaluate the range of activities of the product. For example, certain proteins possess multiple functional domains that express enzymatic and receptor-binding activities. In such situations, manufacturers should evaluate and compare all relevant functional activities of the similar biologic and the RBP.

Potency is the measure of the biological activity. The potency assay should be used together with an in-house qualified reference material that is representative of the similar biologic material. The use of the international standards for determining potency depends on the prevailing practice for the product. Where appropriate, international or national standards and reference reagents should be used to determine product potency and to express results in International Units (IU) – for other products, a suitable in-house reference material should be used. In-house reference materials should be quantitatively calibrated against either an international or national standard or reference reagent, where available and appropriate.

Depending on the purpose of the method (batch release assay or characterization), the functional assays used may or may not be fully validated, but they must be scientifically sound and produce consistent and reliable results. The available information on these assays (including extent of validation, assessed parameters and available validation data) should be confirmed before they are applied to the testing and establishing of biosimilarity between a similar biologic and its RBP. It should be noted that many biological assays may have relatively high variability that might preclude detection of small but significant differences between the similar biologic and RBP. Therefore, it is recommended that assays are developed that are more precise and can detect changes in the intended biological activities of the product to be evaluated with adequate accuracy. Such assays can include target-binding assays (which are usually less variable) in addition to cell-based assays. Adopting automated laboratory equipment to help minimize manual operations, applying good analytical practices and appropriate control sampling, and using critical reagents calibrated against WHO or national reference standards where available (for example, tumour necrosis factor alpha (TNF-α) for potency assays for anti-TNF products) may help to reduce the variability of biological assays. For a given method variability, the number of RBP batches tested should be high enough to allow for a reliable assessment of similarity.

Biological assays should be validated against an international or national reference standard, where available and appropriate. If no such standards are available, an internal reference standard must be established as per the ICH guidelines. If the methods of bioassay(s) are documented in the specification, test(s) can be conducted accordingly

iii. **Immunological Properties:** The manufacturing process of similar biologics is known to affect the level of process related impurities and post translational modifications of the product. These characteristics may affect the immunogenicity of the product. Hence evaluation by characterization (antibody or antibody-derived product); comparison to reference biologic with respect to specificity, affinity, binding strength and Fc function; and evaluation by animal studies if required should be performed. When immunochemical properties are part of the activity attributed to the product (for example, antibodies or antibody-based products) analytical tests should be performed to characterize these properties and used in the comparative studies.

For mAbs, the specificity, affinity and binding kinetics of the product to relevant fragment crystallizable (Fc) receptors (for example, neonatal Fc receptor, complement component 1q (C1q) and Fcγ receptors) should be compared using suitable methods such as surface

plasmon resonance and biolayer interferometry. In addition, appropriate assays should be used to provide information on Fc mediated functions – for example, antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and complement dependent cytotoxicity (CDC), where relevant.

The correlation between Fc-mediated effector functions, Fcγ receptor or C1q binding and physicochemical characteristics (for example, glycan pattern) should be considered and, whenever possible, established. Such analyses will facilitate the interpretation of subtle differences between the similar biologic and the RBP and inform prediction of their clinical impact.

iv. **Purity and Impurities:** Characterization of a similar biologic requires evaluation of the following using orthogonal and state-of-the-art technologies:

- Product related variants (e.g., glycoforms, isomers, aggregated, oxidized or deamidated product)
- Process related impurities (residual media components, resin leachates etc., Host cell related impurities (e.g., host cell protein, host cell DNA etc.

Product-related substances and impurities, such as those caused by protein degradation, oxidation, deamidation, aggregation or potential post translational modification of the protein, should be compared for the similar biologic and RBP. If comparison reveals differences in product-related substances and impurities between the similar biologic and RBP, the impact of the differences on the clinical performance of the drug product (including its biological activity) should be evaluated.

Specifically, if the manufacturing process used to produce the proposed similar biologic introduces different impurities or higher levels of impurities than those present in the RBP then additional functional assays to evaluate the impact of the differences may be necessary. To obtain sufficient information of the product-related substances and impurities it is recommended that comparative stability studies under accelerated and/or stress conditions are conducted Process-related impurities such as host cell proteins, host cell DNA, cell culture residues and downstream processing residues may be quantitatively and/or qualitatively different between the similar biologic and RBP due to the different manufacturing processes used for their drug products. Nevertheless, process related impurities should be kept to a minimum through the use of state-of-the-art manufacturing technologies. The risk related to any newly identified impurities in the similar biologic should be evaluated.

Differences observed in the purity and impurity profiles of the similar biologic relative to the RBP should be evaluated to assess their potential impact on safety and efficacy. Where the similar biologic exhibits different impurities, those impurities should be identified and characterized when possible. Depending on type and amount of the impurity, conduct of preclinical and/or clinical studies can help to confirm that there is no adverse impact on safety and efficacy of the similar biologic.

9.3.2 Quantity

In general, a similar biologic is expected to have the same concentration or strength e.g. fill volume of the drug substance as the RBP. However, concentration deviations not affecting the posology might be permissible, if justified. The quantity of the similar biologic drug substance should be expressed using the same measurement system as that used for the RBP (that is, mass units or units of activity). A description with appropriate justification should also be included to describe how the quantity was calculated (including, for example, the selection of the extinction coefficient).

9.4 Comparative analytical assessment

9.4.1 Considerations for the RBP and the similar biologic

The number of RBP batches needed for the comparative analytical assessment will be influenced by the criticality of the quality attribute(s) under investigation and the approach chosen for demonstrating similarity. The manufacturer of the similar biologic should include an appropriate and scientifically supportable number of batches of the RBP in the comparability assessment. In order to characterize independent RBP batches, it is recommended that the RBP batches are sourced over an extended time period.

These batches should also include the RBP batches used in the clinical comparison studies of the similar biologic. In general, adequate number of RBP batches will provide a better estimate of the true batch-to-batch variability of the RBP and allow for a more robust statistical comparison with the similar biologic. Random sampling of RBP batches is desirable but may be difficult to achieve in practice depending on the availability of such batches. However, the sourcing of RBP batches should be carefully managed to generate a sample that captures the inherent variability of the RBP (for example, collected over a sufficient timeframe with the aim of covering different manufacturing campaigns).

The RBP batches should be transported and stored under the recommended conditions and tested within their approved shelf-life. Any exception to this would have to be fully substantiated with experimental data. The shelf-life of the RBP at time of characterization should be considered and it is expected that RBP batches of different ages will be included in the similarity assessment. The similar biologic batches included in the comparability assessment should be manufactured using the intended commercial manufacturing process and should preferably originate from different drug substance batches. Generally, each value for an attribute being assessed for a similar biologic should be contributed by an independent batch.

For example, a single drug product batch produced from a single drug substance batch would be considered to be an independent batch while different drug product batches produced from the same drug substance batch cannot be considered to be independent. In addition, small- or pilot-scale batches can be included if comparability between the small- and commercial scale batches has been properly demonstrated.

Usually all commercial-scale batches produced – including process performance qualification batches and batches applied in the clinical trial(s) – should be included in the similarity assessment. As with the RBP, the exact number of similar biologic batches required will be

influenced by several factors, such as the criticality of the quality attribute(s) under investigation and the approach applied for similarity evaluation. In general, the risk of a false-positive conclusion on similarity will decrease with increasing number of batches. A robust manufacturing control system and demonstrated batch-to-batch consistency of the similar biologic are prerequisites for a successful similarity assessment.

9.4.2 Considerations for similarity assessment

The quality comparison between Similar Biologic and Reference Biological Product is essential. The applicant should submit a full quality dossier as per CDSCO guidance for industry, 2024 including the results of comparability exercise for the similar biologic with the RBP before the applicant proposes to take the similar biologic to clinical development. All manufactured batches (including developmental and clinical batches) used in the similarity assessment should be presented at the time of MA application.

Three consecutive standardized batches which have been used to demonstrate consistency of the manufacturing process should be used.

The quality comparison between the similar biologic and the reference biologic should be governed by Quality Attributes (QA), which employ state-of-the-art high resolution analytical techniques and methods that are sensitive enough to detect the possibilities of changes to the product.

Quality attributes are those quality attributes which have direct impact on the clinical safety or efficacy. QAs must be controlled within limits that need to be established based on the Reference Biologic.

The most frequently used approach for similarity assessment relies on demonstrating that the quality attributes of the similar biologic batches lie within the predetermined similarity ranges established based on characterization data from multiple batches of the RBP. Other approaches (such as equivalence testing of means) can also be used for similarity assessment.

Each statistical approach has, however, specific strengths and weaknesses which should be appropriately discussed in the submission and considered in the similarity conclusion. In order to mitigate the risks inherent in employing statistical tests on limited samples (false-positive and false-negative conclusions), a comprehensive control strategy must be established for the similar biologic to ensure consistent manufacturing.

i. Statistical intervals for the establishment of similarity ranges

Where possible, quantitative similarity ranges should be established for the similar biologic comparability exercise. As the allowable differences in quality attributes between the similar biologic and the RBP are usually difficult to establish based on clinical considerations alone, the batch-to-batch variability of the RBP is typically used to further inform acceptable differences in quality attributes. The established similarity range should therefore tightly reflect the quality profile of the marketed RBP batches. The ranges should normally not be wider than

the batch-to-batch variability present in the RBP unless it can be determined which differences would be acceptable (for example, less impurities is usually acceptable). Wide similarity ranges based on inappropriate use of statistical methods should not be used.

Different statistical intervals can be used to establish similarity ranges. Commonly used approaches include mean ± x SD, the min-max range and tolerance intervals:

The most commonly applied approach for establishing similarity ranges is the x-sigma interval, that is, mean ± x SD of the RBP batch data. The multiplier used (x) should be scientifically justified and could be linked to the criticality of the quality attribute tested, with a smaller multiplier applied for high criticality quality attributes.

- A conservative approach would be used to establish the similarity ranges directly based on the min-max quality attribute data obtained from the characterization studies of RBP batches. Such similarity ranges could be viewed as clinically qualified (since the RBP batches are on the market and taken by patients). However, compared to other approaches the min-max approach is often associated with high risk of a false-negative conclusion (that is, a high risk of concluding non-similarity even though the underlying data distributions for the RBP and similar biologic would support a similarity claim).
- Similarity ranges based on tolerance intervals would usually require a high number of RBP batches for establishing meaningful ranges. With a limited number of RBP batches characterized and/or inappropriate parameterization, the tolerance interval approach can result in an estimated range that is much wider than the actual minmax quality attribute ranges of the RBP. The risk of a false-positive conclusion of similarity (that is, the risk of concluding similarity where the underlying data distributions do not support such a claim) may therefore be unreasonably high when the similarity ranges are based on inappropriately applied tolerance intervals.

The most frequently applied overall similarity criteria require that a certain percentage of the similar biologic batches (usually between 90% and 100%) fall within the similarity range. This figure should be determined prior to the initiation of the similarity assessment.

ii. Analytical similarity evaluation

It is up to the manufacturer to justify the relevance of the established similarity ranges and criteria. Ideally, the data analyses should be robust and should as far as possibly minimize the risk of a false-positive conclusion. Although decreasing the risk of a false-positive conclusion is of primary importance from a patient and regulatory point of view, the risk of a false-negative conclusion also needs to be managed by the manufacturer and should be thoroughly considered during the planning of the similarity exercise.

Differences between the Similar Biologic and the RBP should be evaluated for their potential impact on safety and efficacy of the Similar Biologic and additional characterization studies may be necessary.

Some minor differences between the RBP and the similar biologic are expected. Nevertheless, any quality attributes not fulfilling the established similarity criteria should be considered as a potential signal for non-similarity and should be assessed for possible impact on clinical safety and efficacy.

Confirmed differences in low criticality quality attributes also need to be adequately considered, but in the case of such differences reference to available information (which could, for example, originate from scientific publications) is usually sufficient.

Lower impurity levels in the similar biologic (for example, of aggregates) or differences in quality attributes present at very low levels in both the RBP and the similar biologic would in most cases be predicted to have no clinical relevance, and could therefore be accepted without further assessment.

For differences in quality attributes with higher criticality, functional assays to thoroughly address their possible clinical impact are generally expected. Where there are confirmed differences in the most critical quality attributes it will be more challenging to justify the conclusion that the product is a true similar biologic. For example, if differences are found in quality attributes that alter the PK of the product and thereby change the dosing scheme then the product cannot be considered to be a similar biologic.

9.5 Specifications

Specifications of Similar Biologics (for drug substance and drug product) are established around quality attributes (QAs) with the intent of ensuring consistency in product quality and comparability to Reference Biologic according to relevant guideline (ICH Q6B). Methods used for setting specifications may or may not be the same as the analytical methods used for product characterization and for establishing product comparability. Acceptance limits should be set based on Reference Biological product data and candidate similar biologic data including data from developmental or clinical batches, which must be in line with international norms.

Furthermore, a similar biologic should show the same level of compliance with a pharmacopeial monograph as that required for the RBP – however, compliance with a pharmacopeial monograph is not sufficient to establish biosimilarity.

Reference to the analytical methods used and acceptance limits for each test parameter of the similar biologic should be provided and justified. All analytical methods referenced in the specification should be validated and the corresponding validation documented. Specifications for a similar biologic may not be the same as for the RBP since the manufacturing processes will be different, and different analytical procedures and laboratories will be used for the assays. Nonetheless, the specifications should capture and control important known product quality attributes.

The setting of specifications should be based on: (a) the manufacturer's experience with the similar biologic (for example, with regard to its manufacturing history, assay capability and the

quality profile of batches used for establishing similarity); (b) the experimental results obtained by testing and comparing the similar biologic and RBP; and (c) attributes with potential impact on product performance. The manufacturer should take into consideration that the limits set for a given specification should not, unless properly justified, be significantly wider than the range of variability of the RBP over the shelf-life of the product.

For release specifications, Indian Pharmacopoeia Monograph should be followed, if available as per the provisions of Drugs and Cosmetics Act and Rules made thereunder.

9.6 Stability

The shelf-life and storage condition of drug substance and drug product should be assigned based on real-time stability studies. Stability studies on drug substance and drug product should be carried out using containers and conditions that are representative of the actual storage containers and conditions, according to relevant guidelines (e.g. ICH Q1 A(R2), ICH Q5C, WHO TRS 822 and WHO TRS 953). Side-by side accelerated and stressed stability studies comparing the Similar Biologic to the Reference Biologic will be of value in determining the Similarity of the products by showing comparable degradation profiles. Stability studies should be carried out to show which release and characterization methods are stability-indicating for the product.

Stability studies should be summarized in an appropriate format (such as tables) and should include results from accelerated degradation studies and studies under various stress conditions (for example, high temperature, oxidation, freeze-thaw, light exposure, humidity and mechanical agitation).

10. Data Requirements for Preclinical Studies

This section addresses the pharmaco-toxicological assessment of the similar biologic. It is important to note that in order to design an appropriate nonclinical study programme a clear understanding of the characteristics of the RBP is required. The nature and complexity of the RBP will have an impact on the extent of the nonclinical studies needed to confirm similarity. In addition, any differences observed between the similar biologic and RBP in the physicochemical and biological analyses will also guide the planning of the nonclinical studies. Other factors that need to be taken into consideration include the mechanism(s) of action of the drug substance (for example, the receptor(s) involved) in all authorized indications of the RBP, and the pathogenic mechanisms involved in the disorders included in the therapeutic indications.

A stepwise approach should be applied during nonclinical development to evaluate the similarity of the similar biologic and its selected RBP. At first, in vitro studies should be conducted and then a decision made on whether or not additional in vivo animal studies are required.

The following approach to nonclinical evaluation may be considered and should be tailored on a case-by-case basis to the similar biologic concerned. In all cases, the approach chosen should be scientifically justified in the application dossier.

10.1 In vitro studies

In order to assess any relevant difference in pharmaco-toxicological activity between the similar biologic and chosen RBP, data from a number of comparative in vitro studies – some of which may already be available from the quality-related assays – should be provided. In light of this data overlap, it is suggested that the in vitro nonclinical studies related to characterization of the biological activity of the similar biologic be addressed alongside the related quality data in the corresponding quality module. Any other nonclinical in vitro studies should then be addressed in the relevant nonclinical modules of the dossier where they should be reviewed and discussed from the point of view of potential impact on the efficacy and safety of the similar biologic.

Since experience has shown that in vitro assays are in general more specific and sensitive than in vivo studies in animals for detecting differences between the similar biologic and RBP, the use of in vitro assays is of paramount importance in the nonclinical similar biologic comparability exercise.

For such in vitro studies, the following general principles apply:

- Typically, a battery of interaction studies addressing the primary binding events should be performed, along with cell-based or isolated-tissue-based functional assays (see below) in order to assess if any (clinically) relevant differences in reactivity exist between the similar biologic and RBP and, if so, to determine the likely causative factor(s).
- Together, these assays should cover the whole spectrum of pharmaco-toxicological aspects with potential clinical relevance for the RBP and for the product class. In the dossier, the manufacturer should discuss to what degree the in vitro assays used can be considered representative/predictive of the clinical situation according to current scientific knowledge.
- The studies should be comparative and designed to be sufficiently sensitive, specific and discriminatory to allow for the detection of (clinically) relevant differences in pharmaco-toxicological activity between the similar biologic and RBP – or, conversely, to provide evidence that any observed differences in quality attributes are not clinically relevant.
- The studies should compare the concentration–activity/binding relationship of the similar biologic and the RBP at the pharmacological target(s), covering a concentration range within which potential differences are most accurately detectable (that is, the ascending part of the concentration–activity/binding curve).
- A sufficient number of RBP batches and similar biologic batches (preferably representative of the material intended for commercial use) should be evaluated. Assay and batch-to-batch variability will affect the number of batches needed. The number tested should be sufficient to draw meaningful conclusions on the variability of a given parameter for both the similar biologic and the RBP and on the similarity of both products.
- Where available, international reference standards can be used to support assay characterization, calibration and performance. When no such reference standard exists, an inhouse reference material should be established.

The nonclinical in vitro programme for similar biologics should usually include relevant assays for the following:

Binding studies- Evaluation of the primary binding events – that is, binding of the similar biologic to cell membrane receptors or to other membrane-bound or soluble targets that are known/assumed to be involved in the pharmaco-toxicological effects of the RBP in the clinically approved indications – for example, for immunoglobulin G (IgG)-based mAbs, antigen-binding fragment (Fab)-associated binding to the antigen and Fc-associated binding to representative isoforms of the relevant Fc receptors and to C1q .

Functional studies/determination of biological activities- Studies should evaluate signal transduction and/or functional activity/viability of cells or isolated tissues known to be of relevance for the pharmaco-toxicological effects of the RBP. Together these assays should broadly cover all the known mechanisms of action of the RP in the clinically authorized indications – for example, for IgG-based mAbs directed against membrane-bound antigens, evaluation of Fab-associated functions and of Fc-associated functions such as ADCC, ADCP and CDC

Such assays are often technically demanding and the experimental approach chosen should be appropriately justified by the manufacturer.

10.2 Determination of the need for in vivo animal studies

On the basis of the totality of quality and nonclinical in vitro data available and the extent to which there is residual uncertainty about the similarity of a similar biologic and its RBP, it is at the discretion of Licensing Authority to waive or not to waive a requirement for additional nonclinical in vivo animal studies. The decision of Licensing Authority on whether or not to require such studies should take into account the following:

- If the quality comparability exercise and the nonclinical in vitro studies have shown high similarity and the level of residual uncertainty is considered acceptable to move to the clinical phase of the similarity exercise then an additional in vivo animal study is not considered necessary.

- If a need is identified to reduce remaining uncertainties concerning the similarity (including drug safety) of a similar biologic and its RBP before the initiation of clinical evaluations then additional in vivo animal studies may be considered, if a relevant animal model is available – however this should only occur: (a) when it is expected that such studies would provide relevant additional information; and (b) if the needed additional information cannot be obtained using an alternative approach that does not involve in vivo animal studies. In this respect, the factors to be considered could include: – qualitative and/or quantitative differences in potentially or known relevant quality attributes between the similar biologic and its RBP (for example, qualitative and/or quantitative differences in the post-translational glycosylation of proteins); and – relevant differences in formulation (for example, use of excipients in the similar biologic not widely used in medicinal products).

- On the basis of regulatory experience gained to date in marketing authorization applications for similar biologics, the need for additional in vivo animal studies would be expected to represent a rare scenario.

- If the quality and nonclinical in vitro comparability exercises indicate relevant differences between the similar biologic and the RBP (thus making it unlikely that similarity would eventually be established), then standalone development to support a full marketing authorization application should be considered.

Animal toxicity studies waiver for a similar biologic product may be considered if the following conditions/criteria are met:

1. Candidate similar biologic is expressed in an established expression system.
2. The amino acid sequence of the similar biologics is identical to that of the RBP.
3. The strength, route of administration, human dose, and indications proposed for similar biologics are the same as the RBP.
4. Applicant should use appropriate analytical methodologies with adequate sensitivity and specificity to detect and characterize differences between the proposed similar biologic and the RBP.
5. For all the product-related variants, identification and determination of the relative levels of these variants should be included in the comparative analytical characterization studies.
6. For all the product-related impurities, applicants should characterize, identify and quantify product-related impurities (as defined in ICHQ6B) in the proposed similar biologic and the RBP, to the extent feasible. Further, if the manufacturing process used to produce the proposed similar biologic introduces different impurities or higher levels of impurities than those present in the RBP, additional pharmacological/ toxicological studies may be necessary.
7. Applicant to refer the Annexure II for the list of all the “potential” Quality Attributes (QA). Further, based on the potential impact on the mechanism of action and function of the product, the applicant to identify the other QAs.
8. Acceptance limits should be set based on Reference Biological product data and accordingly sufficient number of batches of RBP to be used (Minimum of n=3). Further, for the quantitative data analysis, statistical methods such as Min-Max approach is the most recommended for establishing the similarity acceptance criteria because a very large number of RBP batches would not be required to establish meaningful intervals. For the similar biologic data, falling beyond the Min-Max range, if not supported by other orthogonal techniques, then additional pharmacological/ toxicological studies may be necessary. Further, the applicants may propose other methods of data analysis, including equivalence testing. The data generated using qualitative methods, which is not amenable to statistical evaluation, may be analyzed by visual comparison of the data for similarity.
9. To the extent possible, RBP batches to be selected with a range of expiration dates spread across the product`s shelf-life to provide a representation of the data from different time points for obtaining marketing authorization.

10. Applicant to conduct analytical similarity with state-of-the-art techniques as per tests mentioned in **Annexure II.** For example, secondary structure analysis can be performed either by FAR UV CD or FTIR, as applicable. Applicant to submit the summary sheet of the generated CMC data.

In case, the proposed dosage form and formulation of a similar biologic is different from the Reference biologics, the applicant needs to provide the rationale for this difference.

Toxicity waiver for a similar biologic product may not be granted in any of the following scenarios:

1. If there are differences that cannot be ruled out as having no safety impact.
2. When a novel excipient is being used for the first time for biological products specific to the claimed route of administration.
3. If the applicant plans to do a clinical study using a route of administration that is not tested/approved by regulatory authorities for the Reference biologics.
4. If the planned human dose of the drug is higher than approved for the Reference biologics.

If the toxicity study is requested by the Licensing Authority, the applicant shall refer to relevant application requirement which is detailed in **Annexure IV.**

10.3 In vivo studies

10.3.1 General aspects to be considered

The 3Rs principles for animal experiments (Replace, Reduce, Refine) should always be followed to minimize the use of animals in testing in accordance to New Drugs and Clinical Trial Rules 2019.

To address the residual uncertainties, the use of relevant/suitable animal species and/or of specific animal models (for example, transgenic animals or transplant models) may be considered.

Animal models are often not sensitive enough to detect small differences. If a relevant and sufficiently sensitive in vivo animal model cannot be identified, the manufacturer may choose to proceed directly to clinical studies, taking into account strict principles to mitigate any potential risk.

The effects of RBPs are often species specific. In accordance with ICH S6(R1) and the WHO Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology, in vivo studies should be performed only in relevant species – that is, species which are known to be pharmacologically and/or toxicologically responsive to the RBP.

The duration of the study/studies should be justified, taking into consideration the PK behaviour of the RBP, the time to onset of formation of anti-drug antibodies (ADAs) in the test species and the clinical use of the RBP.

10.3.2 Specific aspects

PK and/or PD studies

In cases where such studies are considered necessary, the PK and/or PD of the similar biologic and the RBP should be compared quantitatively, when the model allows, using a dose–response assessment that includes the intended exposure in humans.

The studies may include animal models of disease to evaluate functional effects on disease-related PD markers or efficacy measures.

Safety studies

Where in vivo safety studies are deemed necessary, a flexible approach that follows the 3R principles to maximize the readout of relevant data and minimize the use of animals in testing should always be followed. If appropriately justified, a repeated dose toxicity study with refined design – for example, using just one dose level of similar biologic and RBP, and/or just one gender and/or no recovery animals, and/or only in-life safety evaluations such as clinical signs, body weight and vital functions – may be considered. Depending on the chosen end-points, it may not be necessary to sacrifice the animals at the end of the study.

Repeated dose toxicity studies in non-human primates are not recommended and nor are toxicity studies in non-relevant species (for example, to assess unspecific toxicity due to impurities).

Immunogenicity studies

Qualitative or quantitative difference(s) in product-related variants (for example, in glycosylation patterns, charge, aggregates, and impurities such as host-cell proteins) may have an effect on immunogenic potential and on the potential to cause hypersensitivity. Antibody response to the Similar Biologic should be compared to that generated by the reference Biologic in suitable animal model. The test serum samples should be tested for reaction to host cell proteins. For evaluating immune toxicity of the Similar Biologic under study, the results of local tolerance (part of repeat dose or standalone test) should be analyzed with the observations regarding immunogenicity in sub-chronic study. Therefore, the immunogenicity testing should be included as part of the sub-chronic repeated-dose study while developing the protocols.

The other parameters for evaluating immune toxicity include immune complexes in targeted tissues may be considered while evaluating histopathology observations, etc.

Local tolerance studies

Studies on local tolerance are usually not required. However, if excipients are introduced for which there is little or no experience with the intended clinical route of application, local tolerance may need to be evaluated. If other in vivo animal studies are to be conducted, the evaluation of local tolerance may be integrated into the design of those studies.

Other studies

In general, safety pharmacology and reproductive and development toxicity studies – as well as genotoxicity and carcinogenicity studies– are not warranted during the nonclinical testing of similar biologics.

11. Data Requirements for Clinical Trial Application

The applicant has to submit application for conduct of clinical trial as per the CDSCO guidance for Industry, 2024. The quality data submitted should indicate that there are no differences in Quality Attributes (QAs), and all quality attributes are well controlled in order to allow the initiation of clinical evaluation.

Clinical studies play an important role in validating similarity by confirming that there are no clinically significant differences between the proposed similar biologic and the RBP. These studies should be designed to demonstrate confirmatory evidence of similar clinical performance of the similar biologic and RBP and therefore need to use sensitive testing strategies that are sufficiently sensitive to detect any clinically relevant differences between the similar biologic and the RBP.

Clinical data should be generated using the similar biologic produced from the final manufacturing process, representing the product intended for marketing authorization. Any deviation from this recommendation needs to be justified and additional data may be required. For manufacturing process changes, the appropriate guidelines should be followed. Ideally, reference biologic product (RBP) from a single marketing authorization holder should be used as the comparator throughout quality and clinical comparability studies, to ensure consistency in data and conclusions.

If relevant differences between the similar biologic and the reference biological product (RBP) are identified at any stage of development, these differences must be thoroughly investigated and justified. If a justification cannot be provided, the product may not meet the criteria for a similar biologic, and a standalone licensing application should be considered.

For clinical evaluation, a comparative bioequivalence study assessing pharmacokinetic (PK) and/or pharmacodynamic (PD) similarity is generally required. An adequately powered comparative efficacy and safety trial will not be necessary if sufficient evidence of similarity can be drawn from other parts of the comparability exercise. The need for a comparative clinical efficacy and safety trial for the proposed similar biologic (and type of trial if required) will be influenced by factors such as:

- the ability to thoroughly characterize the similar biologic;
- the availability of suitable sensitive, orthogonal assays for robust analytical and functional characterization; the extent of analytical and functional similarity with the reference biological product (RBP);
- the existence of a relevant pharmacodynamic (PD) marker;
- the degree of understanding of the biological product's mechanisms of action across different indications, and the extent to which these can be explored in binding and functional in vitro assays, the contribution of each mechanism of action to the observed clinical effect is not relevant as long as it can be measured.
- understanding of any potential unwanted immunogenicity concerns, such as ADA incidence, ADA response magnitude, levels of neutralizing antibodies, and antibodies against endogenous substances (e.g., erythropoietin, coagulation factors); and clinical concerns related to the similar biologic's impurity profile or nature of excipients.

Current examples of biologics that can be well-characterized and have established mechanisms of action include, but are not limited to, teriparatide, insulin, G-CSF, and somatropin. Current data also suggest that more complex products, such as monoclonal antibodies, can be effectively characterized with advanced analytical methods, as structure–function relationships are well-defined and measurable through sensitive, orthogonal functional assays.

11.1 Pharmacokinetic (PK) Studies

The clinical comparability assessment should typically include a comparative pharmacokinetic (PK) study if the drug can be measured in blood, along with pharmacodynamic (PD) marker measurements (if available) and immunogenicity data.

The PK study should be designed to confirm similar PK profiles between the similar biologic and reference biological product (RBP). When the RBP and its proposed similar biologic have more than one route of administration (most commonly intravenous and subcutaneous) then carrying out the study/studies using the non-intravenous route of administration is preferred as this is usually the more immunogenic route and will provide more meaningful information for the comparability exercise.

The omission of a PK study of other approved routes of administration needs to be justified for approval of all available options – for example, in cases when the molecule has an absorption constant that is much lower than the elimination constant (flip flop kinetics).

The study should have an adequate sample size, considering PK variability in the population studied, statistical rationale (i.e. statistically justified) and comparability limits should be defined and justified prior to conducting the study and consideration should be given to whether a cross-over or parallel group design would be the most adequate. If existing population PK or PK-PD models for the RBP are available in the literature, modeling and simulation may be used to refine the study design, such as by determining the appropriate dose and selecting the most sensitive population to detect PK differences, as well as optimizing sample size. When ethically

acceptable, PK studies should be performed in healthy volunteers with a standardized population regarding factors that may influence PK variability (e.g., ethnicity, body weight, and gender). If safety or tolerability concerns make PK studies in healthy volunteers unsuitable, PK study should be a part of Efficacy and safety study in patients

The preferred design is a randomized, two-period, two-sequence, single dose cross-over PK study using a dose within the therapeutic range at which the ability to detect differences is sufficient to observe meaningful differences. A cross-over design eliminates inter-subject variability, thus reduces the sample size required to demonstrate PK equivalence between the similar biologic and RBP. The treatment periods should be separated by a wash out phase that is sufficiently long to ensure that drug concentrations are below the lower limit of bioanalytical quantification in all subjects at the beginning of the second period – that is, at least 5 times the terminal half-life.

If a cross-over design is unsuitable (e.g., for biologics with long half-lives or those associated with immunogenicity impacting PK), a parallel group design should be used. In parallel group studies, attention should be given to maintaining balance between groups to prevent factors such as ethnicity, body weight, and gender from affecting PK results.

A multiple-dose study in patients is acceptable as a pivotal PK study if a single-dose study cannot be conducted in healthy volunteers due to risks or tolerability reasons or if a single-dose study is not feasible in patients.

Multiple-dose studies may also be allowed in rare cases where limitations in the sensitivity of analytical methods prevent precise measurement of plasma or serum concentrations after a single dose. However, since a multiple-dose study is less sensitive to differences in Cmax compared to a single-dose study, this approach should be justified with valid reasoning.

PK comparisons between the similar biologic and the reference biological product (RBP) should consider not only the rate and extent of absorption but also include a descriptive analysis of elimination characteristics, such as clearance and/or elimination half-life, as these may differ between the two products. Both linear (nonspecific) and nonlinear (target-mediated) clearance should be evaluated through partial areas under the curve (pAUCs)."

Acceptance criteria for the demonstration of PK similarity between the similar biologic and the RBP must be predefined and appropriately justified. It should be noted that the criteria used in standard clinical PK comparability studies (bioequivalence studies) may not necessarily be applicable to all biotherapeutic products. However, the traditional 80–125% equivalence range will in most cases be sufficiently conservative to establish similar PK profiles Correction for protein content may be acceptable on a case-by-case basis if pre-specified and adequately justified, with the assay results for the similar biologic and RBP being included in the protocol. If adjustments for covariates are intended for parallel group studies (for example, in the case of adalimumab, stratification for body weight and gender), they should be predefined in the statistical analysis plan rather than being included in post hoc analyses.

Additional PK studies, such as interaction studies with commonly co-administered drugs or studies in special populations (e.g., children, elderly, or patients with renal or hepatic impairment), are not required for a similar biologic.

Particular attention should be given to the chosen analytical method's ability to track the protein over time in a complex biological matrix with other proteins. The method should be optimized to offer satisfactory specificity, sensitivity, and quantification accuracy, and the same assay should measure serum concentrations of both the similar biologic and RBP. A single PK assay (using the same binding reagents and a single analytical standard, typically a similar biologic) may be used to assess similar biologic and RBP concentrations, provided that bioanalytical comparability is verified with supporting data.

In cases where measurable endogenous protein affects the concentration-time profile of the administered exogenous protein, manufacturers should describe and justify their method to account for this (e.g., using baseline correction).

Establishing PK similarity may be challenging or impractical for certain substances (e.g., heparin fractions that cannot be measured in blood), specific administration routes (e.g., intraocular injections of aflibercept or ranibizumab), or products with high PK variability (e.g., romiplostim). In such cases, clinical similarity should be demonstrated through pharmacodynamics (PD), immunogenicity, or other clinical parameters.

11.2 Pharmacodynamic Studies

It is preferable to investigate PD parameters alongside comparative PK studies. However, when conducting PK studies is not feasible, PD markers may become more critical. For instance, with heparins, where serum concentrations are unmeasurable, similarity should be established based on key PD endpoints, specifically anti-FXa and anti-FIIa activity.

PD effects should be evaluated in an appropriate population, using doses within the steep portion of the dose-response curve to improve the likelihood of identifying any differences between the similar biologic and the reference biologic. PD markers should be selected on the basis of their clinical relevance.

11.3 Confirmatory PK and/or PD studies

If an adequately powered comparative efficacy trial is not necessary, comparative PK and/or PD studies may be sufficient for establishing confirmative evidence of the similar clinical performance of a similar biologic and its RBP, provided that:

- the acceptance ranges for confirmatory PK and/or PD end-points are predefined and appropriately justified;
- the PD biomarker reflects the mechanism of action of the biological product;
- the PD biomarker is sensitive to potential differences between the proposed similar biologic and the RBP; and
- the PD biomarker assay is validated.

The applicant should consider the option of using additional PD measures (usually as secondary end-points) to assess the comparability of the PD properties of the RBP and proposed similar biologic. Furthermore, even if relevant PD measures are not available, sensitive PD end-points may be assessed if such assessment may help to reduce residual uncertainty about similar biosimilarity.

An example of acceptable confirmatory PK/PD studies would be the use of euglycaemic clamp studies to compare the efficacy of two insulins. In addition, absolute neutrophil count and CD34+ cell count are the relevant PD markers for assessing the activity of G-CSF and could be used in PK/PD studies in healthy volunteers to demonstrate the similar efficacy of two medicinal products containing G-CSF.

The study population and dosage should represent a test system that is known to be sensitive in detecting potential differences between a similar biologic and the RBP. In the case of insulin, for example, the study population should consist of non-obese healthy volunteers or patients with type 1 diabetes rather than insulin-resistant obese patients with type 2 diabetes. Otherwise, it may be necessary to investigate more than one dose to demonstrate that the test system is discriminatory.

The acceptance ranges for confirmatory PK and/or PD parameters (that is, for primary end-points) should be predefined and appropriately justified. If PD comparison is not essential for a conclusion of similar biosimilarity but the results are still expected to reasonably support similar biosimilarity then a purely descriptive analysis of the PD results may be justified. This may be the case for biological substances that have been extensively characterized and for which similar biosimilarity can already be concluded from the analytical, functional and PK comparisons. If appropriately designed and performed, such PK/PD studies are usually more sensitive in detecting potential differences in efficacy than trials using hard clinical end-points.

However, PD markers may also be used as end-points in clinical efficacy studies in patients.

Examples of appropriate markers include haemoglobin for measuring the efficacy of an epoetin, and lactate dehydrogenase (which is a sensitive biochemical marker of intravascular haemolysis) for evaluating the efficacy of a complex drug such as eculizumab. For denosumab, investigation of bone formation and resorption markers as part of the PK study may be useful or possibly sufficient. This would involve measurement of bone mineral density and bone turnover markers such as serum C-terminal telopeptide of type 1 collagen (CTX-1) and procollagen type 1 N-terminal propeptide (P1NP) after denosumab administration.

In certain cases (for example, when analytical similarity of the active ingredient in the similar biologic and the RBP can be demonstrated to such a degree that clinical differences can be excluded) a comparative PK study may provide sufficient clinical evidence to support similar biosimilarity. However, a risk assessment (including for example, the impurity profile) should be conducted to determine the need for additional safety/immunogenicity data on the similar biologic.

11.4 Efficacy studies

A comparative efficacy trial may not be necessary if sufficient evidence of biosimilarity can be inferred from other parts of the comparability exercise. A comparative clinical trial, if necessary, should confirm that the clinical performance of the similar biologic and the RBP is comparable. Demonstration of comparable potency, PK and/or PD profiles provide the basis for use of the RBP posology in the comparative clinical trial. If a comparative clinical trial of the similar biologic and RBP is deemed necessary then it is expected that it will be an adequately powered, randomized and controlled clinical trial performed in a patient population that allows for sensitive measurement of the intended clinical parameters.

In principle, equivalence trial designs (requiring lower and upper comparability margins) are preferred for comparing the efficacy and safety of the similar biologic and RBP. Non-inferiority designs (requiring only one margin) or trials with asymmetrical margins may be considered if appropriately justified. Regardless of which design is selected in a particular case, the comparability margin(s) must be pre-specified and justified on the basis of clinical relevance – that is, the selected margin should represent the largest difference in efficacy that would not matter in clinical practice. Treatment differences within this margin would therefore be acceptable as they would have no clinical relevance.

Similar efficacy implies that similar treatment effects can be achieved when using the same posology, and the same dosage(s) and treatment schedule should be used in clinical trials comparing the similar biologic and RBP. In this regard, equivalence trials are again preferable to ensure that the similar biologic is not clinically less or more effective than the RBP when used at the same dosage(s).

A non-inferiority design could be acceptable, if justified by the applicant, for example:

- for biological products with high efficacy (for example, a response rate of over 90%), making it difficult to set an upper margin; or
- in the presence of a wide safety margin.

When using asymmetrical margins, the narrower limit should rule out inferior efficacy and the broader limit should rule out superior efficacy. The use of asymmetrical margins should be fully justified by the sponsor of the proposed similar biologic. Factors that would allow for the use of such margins in a clinical trial include:

- if the dose used in the clinical study is near the plateau of the dose– response curve; and
- there is little likelihood of dose-related adverse effects (for example, toxicity).

Careful consideration should be given to the design of the comparative study/studies, including the choice of primary efficacy end-point(s). Studies should be conducted using a clinically relevant and sensitive end-point within a homogenous population that responds well to the pharmacological effects of the biological product of interest to show that there are no clinically meaningful differences between the similar biologic and RBP. Clinical outcomes, surrogate

outcomes (PD markers) or a combination of both can be used as primary end-points in similar biologic trials. The same study end-points used to establish the efficacy of the RBP may be used because a large body of historical data would generally be available in the public domain for setting the comparability margin(s) and calculating the sample size. However, the primary end-point could be different from the original study end-point for the RBP if it is well justified and relevant data are available to support its use as a sensitive end-point and its suitability for the determination of the comparability margin(s). A relevant PD end-point can be used as the primary end-point – for example, when it is a known surrogate of efficacy or when it can be linked to the mechanism of action of the product. The primary or secondary end-points can also be analyzed at different time points compared to those used in clinical trials with the RBP if these are considered to be more sensitive in capturing the pharmacological action(s) of the biological product – for example, adalimumab efficacy could be measured by responses at week 12 or 16 in addition to week 24.

The sample size and duration of the comparative clinical study should both be adequate to allow for the detection of clinically meaningful differences between the similar biologic and RBP. When a comparative clinical trial is determined to be necessary then adequate scientific justification for the choice of study design, study population, study end-point(s), estimated effect size for the RP and comparability margin(s) should be provided and may be discussed with regulators in order to obtain agreement at least in principle prior to trial initiation.

11.5 Safety

Safety data should be collected throughout clinical development, including from PK/PD studies and clinical efficacy trials, when conducted. Key factors informing the data needed to characterize the similar biologic's safety profile include: (a) the type, frequency, and severity of adverse events compared to the RBP; (b) whether these events result from enhanced pharmacological effects; (c) the level of analytical and functional similarity between the similar biologic and RBP; and (d) any novel impurities or excipients present in the similar biologic.

If the clinical program for the similar biologic is limited to confirmatory PK/PD studies, a clear justification and risk assessment are required to evaluate the need for additional safety data. For example, in the case of insulin, hypoglycemia—an effect of its pharmacological action—is the primary safety concern. Highly similar physicochemical properties and PK/PD profiles between the similar biologic and RBP could sufficiently ensure a comparable hypoglycemia risk, potentially eliminating the need for further safety data. Similar cases include teriparatide, filgrastim, or somatropin. Emerging data also suggest that more complex products, such as mAbs, may be characterized effectively and could fit into this category.

If the similar biologic contains impurities not found in the RBP (e.g., due to the use of a novel expression system), additional safety data may be required, or scientific justification should be provided to explain why such data are unnecessary. Manufacturers should consult with regulators when proposing a clinical program that relies exclusively on PK/PD studies.

As for all medicinal products, further monitoring of the safety of the similar biologic will be necessary in the post-marketing phase.

11.6 Immunogenicity

Immunogenicity should be evaluated as part of the clinical development of the similar biologic in comparison to the RBP, unless the manufacturer provides a scientific justification for not including human immunogenicity data. This justification should be based on the extent of physicochemical similarity between the similar biologic and RBP, as well as a comprehensive risk assessment of potential immunogenicity and its known clinical consequences for the RBP. While published data can help assess the immunogenicity risk of the RBP and guide the immunogenicity strategy, it is typically insufficient on its own to support similar biologic approval. The goal of the immunogenicity programme is to exclude an unacceptable/marked increase in the immunogenicity of the similar biologic when compared with the immunogenicity of the RBP and to generate descriptive data in support of similar biologic approval and its clinical use. If conducted, the immunogenicity study report should include data on antibody incidence, magnitude of ADA response and neutralization ability, whether antibodies are transient or persistent, and their impact on PK and clinical correlates.

The marketing authorization application should include a comprehensive immunogenicity summary, which should cover a risk assessment and, if applicable, the results of testing using appropriately validated assays. It should also provide details on the clinical study duration, sampling schedules, dosing regimen, and the clinical immunogenicity assessment.

Immunogenicity studies should be specifically designed for each product and require a multidisciplinary approach that considers both quality and clinical factors. The risk assessment should include:

- Information on the immunogenicity of the RBP, such as the nature, frequency, and clinical significance of the immune response.
- Evaluation of quality aspects, including the complexity of the drug substance, glycosylation status, expression system, product and process-related impurities, and aggregates.
- Consideration of excipients, the container closure system, product stability, route of administration, and dosing regimen.
- Consideration of patient- and disease-related factors, such as immune status (immune-competent or compromised) and any concurrent immunomodulatory treatments.

Focusing on differences in product-related factors, such as impurities from novel expression systems or new excipients, is essential in the immunogenicity risk assessment of a similar biologic. It is also important to consider the type of product, as the risk is higher for products with an endogenous non-redundant counterpart (e.g., epoetin). In these cases, particular attention should be given to the potential for an immune response to adversely affect the endogenous protein and its unique biological function, leading to serious side effects. Real-time testing for neutralizing ADAs is recommended for high-risk products like epoetins, enzyme replacement therapies, and coagulation factors. On the other hand, for well-characterized biologics, such as insulin, somatropin, filgrastim, and teriparatide, where extensive literature and clinical experience show that immunogenicity does not impact safety or efficacy, immunogenicity studies may not be required, provided the similar biologic is highly similar to the reference biologic and the risk assessment indicates a low risk. This approach may also

apply to other products, including monoclonal antibodies (mAbs). In such cases, manufacturers should engage with regulatory authorities and provide a valid scientific justification for not conducting a safety or immunogenicity study.

1.6.1 Immunogenicity testing

A comprehensive, multi-tiered approach that includes screening and confirmatory immunoassays to detect binding ADAs, followed by assays to assess ADA magnitude and neutralization potential, is typically required. Any deviation from this approach must be justified. Information on the current assays, their formats, benefits, limitations, and result interpretations has been thoroughly reviewed. The manufacturer must justify the antibody-testing strategy and the selection of assays. Special attention should be paid to choosing appropriate controls for assay validation and determining cut-off points to differentiate antibody-positive from antibody-negative samples. Consideration should also be given to potential interference from matrix components, such as the pharmacological target or residual drug in the sample. To minimize such interference, corrective measures should be taken. For example, drug interference, often seen in samples from patients treated with monoclonal antibodies, can be managed by allowing time for drug clearance before sampling or incorporating steps to dissociate immune complexes or remove the drug. Care must be taken to ensure these measures do not interfere with ADA detection or affect patient treatment.

When required, comparative immunogenicity testing should use the same assay format and sampling schedule. In new drug development, antibody testing typically uses the therapeutic administered to the patient. However, in the similar biologic context, developing screening assays with comparable sensitivity for both the similar biologic and reference biological product (RBP) within the same study is challenging. As such, relative immunogenicity is often assessed using a single assay that uses the similar biologic's drug substance as the antigen for both patient groups. This approach ensures the detection of all antibodies against the similar biologic. The manufacturer must demonstrate the suitability of the methods used and provide data showing that the methods detect ADAs to both the RBP and similar biologic similarly.

Neutralization assays, which reflect the product's mechanism of action, are typically based on the product's potency assay. Non-cell ligand-based assays are appropriate when the therapeutic binds to a soluble ligand and inhibits its biological action. For high-risk products (e.g., those with non-redundant endogenous counterparts) and those where effector functions are crucial, functional cell-based bioassays are recommended. If necessary, guidance on the need for a neutralization assay and the appropriate assay format (cell-based, ligand-based, or enzyme activity-based) can be sought from regulatory authorities.

Additional characterization of antibodies, such as isotype determination, should be performed if clinically relevant or in specific circumstances (e.g., the occurrence of anaphylaxis or the use of certain assay formats), considering the immunogenicity profile of the reference biologic (RBP). For instance, if the RBP does not trigger an IgE response, it is unlikely that the similar biologic

will do so if the same expression system is used. Patient samples should be stored under suitable conditions to allow for retesting in cases where issues arise with the original assay

1.6.2 Clinical evaluation

Clinical evaluation can impact the pharmacokinetics (PK), pharmacodynamics (PD), safety, and/or efficacy of the administered product. The immunogenic risk of a biological product is influenced by the incidence of ADAs in the treated population and the extent of any adverse clinical effects, which in turn affects the benefit-risk profile of the therapy.

If human immunogenicity data are necessary, they should be generated in a comparative manner throughout the clinical program. The preferred patient population for immunogenicity studies is typically the one most likely to mount an immune response. For instance, if epoetin is approved for treating both renal anemia and chemotherapy-induced anemia, it is recommended to select patients with renal anemia. Comparative PK and/or PD studies should also collect immunogenicity data, regardless of the population being studied (e.g., healthy volunteers or patients). A PK/PD crossover design can be used for immunogenicity testing, but if the exposure time before switching is insufficient to gather enough immunogenicity data, the sponsor must ensure a sufficient number of patients are treated without crossover—either by extending the crossover study with two parallel treatment arms or by proposing a separate immunogenicity study.

If ADAs are known to influence the pharmacokinetics (PK) of the reference biologic (RBP), assessments of ADA rates and kinetics should be conducted, along with an analysis of their impact on PK through pre-specified subgroup comparisons of ADA-negative and ADA-positive subjects.

The duration of the observation period for immunogenicity testing should be based on the expected time for antibody development and must be justified by the manufacturer. Sampling during immunogenicity testing should include baseline samples (taken before treatment) to detect pre-existing antibodies, as well as samples during treatment and, in some cases, post-treatment, especially if ADAs persist or are undetectable at earlier time points (due to the product's immunosuppressive effects or technical issues like drug interference). The sampling schedule should align with PK evaluations, as well as safety and efficacy assessments, to understand how antibodies may affect clinical outcomes.

Significant differences in immunogenicity between the similar biologic and reference biologic (RBP) would require further investigation to identify the underlying cause. Data and a clear justification must be provided to support any claim that the observed difference is not clinically relevant. The clinical impact of ADAs on pharmacokinetics (PK), efficacy, and/or safety should be analyzed through a stratified comparison of ADA-negative and ADA-positive subjects.

If there is a potential for the development of neutralizing antibodies against critical endogenous factors (e.g., after epoetin administration), clinical studies in patients will be required.

As with the RBP, the similar biologic must undergo thorough post-marketing surveillance, including the monitoring of any serious adverse events related to immunogenicity.

11.7 Waiver of safety and efficacy study

The confirmatory clinical safety and efficacy study can be waived if all the below mentioned conditions are met:

i. Structural and functional comparability of Similar Biologic and Reference Biologic can be characterized to a high degree of confidence by physicochemical and in vitro techniques.

ii. The Similar Biologic is comparable to Reference Biologic in all preclinical evaluations conducted.

iii. PK / PD study has demonstrated comparability of PD markers validated for clinical outcome and has preferentially been done in an in-patient setting with safety measurement (including meaningful immunogenicity assessment) for adequate period justified by the applicant and efficacy/PD measurements.

iv. A comprehensive post-marketing risk management plan has been presented that will gather additional safety data with a specific emphasis on gathering immunogenicity data.

The confirmatory clinical safety and efficacy study cannot be waived especially for large molecular weight biologics like Monoclonal antibodies if validated PD marker is not available.

In case, the safety and efficacy study is waived all the indications approved for reference product may be granted based on comparable quality, non-clinical as well as convincing PK/PD data.

Wherever the phase III trial is waived, the immunogenicity should have been gathered in the PK/PD study and will also need to be generated during post- approval Phase IV study.

The confirmatory clinical safety and efficacy study cannot be waived if there is no reliable PD marker validated for clinical outcome. For a product which is found Similar in pre-clinical, in-vitro characterization having established PK methods and a PD marker that is surrogate of efficacy, the residual risk is significantly reduced in the Phase I study if equivalence is demonstrated for both PK and PD. In such cases clinical trials may be waived.

11.8 Extrapolation of Efficacy and Safety Data to Other Indications

Extrapolation of the safety and efficacy data of a particular clinical indication (for which clinical studies has been done) of a Similar Biologic to other clinical indications may be possible if following conditions are met:

- Similarity with respect to quality has been proven to Reference Biologic.
- Similarity with respect to non-clinical assessment has been proven to Reference Biologic.
- Clinical safety and efficacy is proven in one indication which covers the most sensitive population.
- Mechanism of action is same for other clinical indications.
- Involved receptor(s) are same for other clinical indications.

- Immunogenicity of the product in patient population
- PK and biodistribution of the product in patient population.

For example, authorization of all indications may be obtained based on highly comparable functional data – for example, for similar biologics of mAbs such as infliximab and adalimumab if they show fully comparable activity (including ADCC, CDC, reverse signaling and apoptosis) both in terms of binding to soluble TNF and membranous TNF. However, new indications not mentioned by innovator needs to be covered by separate application.

12. Data Requirements for Market Authorization Application

The applicant should submit application for market authorization as per CDSCO guidance document for Industry, 2024. For cases where commercial manufacturing is performed either at a different scale and/or with a different process as compared to that used for manufacturing phase III clinical trial batches, then information on comparability of quality needs to be additionally submitted with appropriate justification and will be dealt with on a case-to-case basis. Data from all manufactured batches (including developmental and clinical batches) used in the similarity assessment should be submitted at the time of MA application.

13. Risk management plan (RMP)

The RMP for a similar biologic candidate should reflect that of the RBP in terms of safety concerns, additional pharmacovigilance activities and additional risk minimisation. If there are additional safety concerns for the similar biologic candidate these are unlikely to be due to the active molecule but rather factors such as excipient or device that are different from the RP. These should be included in the RMP.

Where ongoing additional pharmacovigilance activities are required for the RBP (for example, participation in ongoing disease registries), these should also apply to the similar biologic candidate. Where possible, this would be through collaboration or participation in those studies or registries already in place for the RBP , or otherwise in other existing disease studies or registries. This will enable collection of real-world information to support characterization of risks and signal detection of potential safety signals related to the RBP and its biosimilars.

Any additional risk minimisation measures that continue to be required for the RBP should also be implemented for the similar biologic candidate, for example educational materials for healthcare professionals and patients or patient alertcards.

14. Post-Market Data for Similar Biologics

It is important to establish a formal Risk Management Plan to monitor and detect both known inherent safety concerns and potential unknown safety signals that may arise from the Similar Biologic since authorization is based on a reduced preclinical and clinical data package. If there are any remaining uncertainties regarding the similar biologic – due for example to the use of a

novel excipient or device – then these should be included in the pharmacovigilance plan and followed up post-marketing. The risk management plan should consist of the following:

14.1 Pharmacovigilance Plan

The clinical studies done on similar biologics prior to market authorization are limited in nature so the rare adverse events are unlikely to be encountered. Hence, a comprehensive pharmacovigilance plan should be prepared by manufacturer to further evaluate the clinical safety in all the approved indications in the post marketing phase. The pharmacovigilance plan should include the submission of periodic safety update reports (PSURs). The PSURs shall be submitted every six months for the first two years after approval of the Similar Biologic is granted to the applicant. For subsequent two years the PSURs need to be submitted annually to DCGI office as per NDCT Rules 2019. Post-marketing safety reports should include all information on product safety received by the marketing authorization holder. The safety information must be evaluated in a scientific manner and this should include evaluation of the frequency and cause of adverse events.

14.2 Adverse Drug Reaction (ADR) Reporting

All cases involving serious unexpected adverse reactions must be reported to the licensing authority as per NDCT Rules 2019.

14.3 Post Marketing Studies (Phase IV Study)

Finally, in order to further reduce the residual risk of the Similar Biologics, additional safety data may need to be collected after market approval through a pre-defined single arm study and compared to historical data of the Reference Biologic. The study should be completed preferably within 2 years of the marketing permission /manufacturing license unless otherwise justified.

The primary aim of the post marketing phase IV study is safety and hence following parameters should be considered for the post marketing phase IV study protocol:

- Primary endpoint: Safety
- Secondary endpoint: Efficacy and Immunogenicity
- The phase IV protocol should be submitted along with marketing authorization application for approval.
- The clinical studies done on similar biologics prior to market authorization are limited in nature so post marketing studies should be conducted and the reports be submitted to DCGI. The plan of post market studies should be captured in Pharmacovigilance plan and update on the studies should be submitted to the CDSCO.
- Regarding post-marketing safety and immunogenicity study at least one non- comparative post-marketing clinical study with focus on safety and immunogenicity (on case-by-case basis) should be performed. This study must be designed to confirm that the Similar Biologic does not have any concerns with regard to the therapeutic consequences of unwanted immunogenicity.

- It is not mandatory to carry out additional non-comparative immunogenicity studies in post marketing studies, if immunogenicity is evaluated in clinical studies. The immunogenicity of the Similar Biologics should be evaluated using appropriately designed studies with state-of-the-art methods, taking into consideration the potential impact on both safety and efficacy.
- Rationale on the strategy for testing immunogenicity should be provided.
- Assay methods should be validated and should be able to characterize antibody content (concentration or titer) as well as the type of antibodies formed.
- Of most concern are those antibodies that have potentially serious impact on safety and efficacy, such as neutralizing antibodies and antibodies with cross reactivity. When neutralizing antibodies are detected in patients in clinical studies (either in pre-approval clinical studies or post-approval clinical studies), the impact of the antibodies on the PK/PD parameters of the Similar Biologics should be analyzed, where the data is available.
- Furthermore, an assessment of the impact of the neutralizing antibodies and cross-reacting antibodies (if applicable) on the overall safety and efficacy of the Similar Biologics should be conducted.

15. Labelling and Prescribing Information

The labelling of the similar biologic should be in accordance to Rule 96 and Rule 97 of the Drugs and Cosmetics Act 1940 and rules made thereunder and prescribing information must align the format as prescribed in Table 8 of NDCT Rules 2019.

The prescribing information for a similar biologic should be as similar as possible to that of the RBP except for product-specific aspects such as use of different excipient(s) and/or presentations. This similarity is particularly important for posology and for safety-related information, including contraindications, warnings and known adverse events. However, if there are fewer indications for the similar biologic than for the RBP, the related text in various sections may be omitted unless it is considered important in informing doctors and patients of certain risks – for example, as a result of potential off-label use. In such cases it should be clearly stated in the prescribing information that the similar biologic is not intended for use in the specific indication(s) and the reasons why.

16. Application Forms

Various application forms for submitting request to regulatory agencies are as

Stage	Agency Involved	Application	Approval
Manufacturing permission for test, analysis and examination	CDSCO - HQ	Form CT-10/12/13	Form CT-11/14/15

Manufacturing License for test, analysis and examination (After CDSCO permission)	State FDA	Form 30	Form 29
Import license for test, analysis and examination	CDSCO-HQ	CT-16	CT-17
Cell bank import / export /transfer/received	RCGM	Form B1/B3/B5/B7	IBSC / RCGM permission
Clinical Trial Permission	CDSCO	CT-04	CT-06
Import and marketing permission	CDSCO	CT-18 (separate for DS and DP)	CT-19- DS CT-20- DP
Registration certificate for import	CDSCO	Form 40 (with schedule DI and DII)	Form 41
Import License for imported product	CDSCO	Form 8 & 9	Form 10
Manufacturing and marketing permission	CDSCO	CT-21 (separate for DS and DP)	CT-22- DS CT-23- DP
Manufacturing License	State FDA/ CDSCO- (countersignature)	Form 27 D	Form 28 D

The applicant should comply with the established pharmacopoeia requirements while testing the excipients and as well as Biological Product for which monograph is available in Indian Pharmacopoeia. Refer Drugs and Cosmetic Act,1940 and Rules 1945 for the application format.

17. Archiving of Data/Retention of Samples:

The manufacturer should establish the SOP for data archival as well as sample retention. The applicant should archive all the data (quality, preclinical and clinical documentation) for a period of at least five years after marketing approval by competent authority in India. Important samples such as test substance, vehicle, plasma / serum, tissues, paraffin blocks, microscope slides, electronic material, etc., should be retained till the period of expiry. The designated authority, which will be responsible for archiving and can be approached for inspection or retrieval if required, should be indicated in the data archival and sample retention SOP.

18. Glossary

The definitions given below apply to the terms used in this guideline. They may have different meanings in other contexts

a. **Comparability/similarity exercise**: direct head-to-head comparison of a biological product with a licensed reference product with the goal of establishing similarity in quality, safety and efficacy.

b. **Comparability margin**: the largest difference that can be judged as being clinically acceptable.

c. **Drug**: Drug includes (as defined in Drugs and Cosmetics Act, 1940).

   i. all medicines for internal or external use of human beings or animals and all substances intended to be used for or in the diagnosis, treatment, mitigation or prevention of any disease or disorder in human beings or animals, including preparations applied on human body for the purpose of repelling insects like mosquitoes;

   ii. such substances (other than food) intended to affect the structure or any function of human body or intended to be used for the destruction of (vermin) or insects which cause disease in human beings or animals, as may be specified from time to time by the Central Government by notification in the Official Gazette

   iii. All substances intended for use as components of a drug including empty gelatine capsules; and

   iv. Such devices intended for internal or external use in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals, as may be specified from time to time by the Central Government by notification in the Official Gazette, after consultation with the Board.

d. **Drug substance**: Any substance or mixture of substances intended to be used in the manufacture of a drug (medicinal) product and that, when used in the production of a drug, becomes an active ingredient of the drug product. Such substances are intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease or to affect the structure and function of the body.

e. **Drug product:** The dosage form in the final immediate packaging intended for marketing. A pharmaceutical product type that contains a drug substance, generally in association with excipients.

f. **Efficacy study:** a clinical trial to compare the efficacy of the biosimilar to the reference product.

g. **Excipient:** a constituent of a medicine other than the drug substance, added in the formulation for a specific purpose. While most excipients are considered inactive, some can have a known action or effect in certain circumstances (for example, hyaluronidase).

The excipients may differ for a biosimilar and its reference product and need to be declared in the labelling and package leaflet of the medicine to ensure its safe use.

h. **Equivalent:** equal or highly similar in the parameter of interest. Equivalent quality, safety and efficacy of two medicinal products denotes that they can be expected to have similar (no better and no worse) quality, safety and efficacy, and that any observed differences are of no clinical relevance.

i. **Generic medicine**: a medicine that is structurally identical to an originator product (comparator) for which the patent and/or data protection period has expired.

j. **Genetic engineering:** The technique by which heritable material, which does not usually occur or will not occur naturally in the organism or cell concerned, generated outside the organism or the cell is inserted into said cell or organism. It shall also mean the formation of new combinations of genetic material by incorporation of a cell into a host cell, where they occur naturally (self-cloning) as well as modification of an organism or in a cell by deletion and removal of parts of the heritable material (Rules, 1989).

k. **Head-to-head comparison:** direct comparison of the properties of a biosimilar with its corresponding reference product. Comparison based on historical data is not acceptable.

l. **Highly Similar:** Highly similar means that the characteristics of quality, biological activity, safety and efficacy of the similar biologic and its RBP have been shown to be comparable to the degree such that SBP can be called a version of the RBP.

m. **Immunogenicity:** The ability of a substance to trigger an immune response or reaction (e.g., development of specific antibodies, T cell response, allergic or anaphylactic reaction).

n. **Impurity:** Any component present in the drug substance or drug product that is not the desired product, a product-related substance, or excipient including buffer components. It may be either process- or product-related.

o. **Manufacture:** “Manufacture” in relation to any drug includes any process or part of a process for producing, altering, ornamenting, finishing, packing, labelling, breaking up or otherwise treating or adopting any drug with a view to its sale or distribution but does not include the compounding or dispensing in the ordinary course of retail business; and “to manufacture” shall be construed accordingly.

p. **New Drug:** “New Drug” means,

(i) a drug, including active pharmaceutical ingredient or phytopharmaceutical drug, which has not been used in the country to any significant extent, except in accordance with the provisions of the Act and the rules made thereunder, as per conditions specified in the labelling thereof and has not been approved as safe and efficacious by the Central Licencing Authority with respect to its claims; or

(ii) a drug approved by the Central Licencing Authority for certain claims and proposed to be marketed with modified or new claims including indication, route of administration, dosage and dosage form; or

(iii) a fixed dose combination of two or more drugs, approved separately for certain claims and proposed to be combined for the first time in a fixed ratio, or where the ratio of ingredients in an approved combination is proposed to be changed with certain claims including indication, route of administration, dosage and dosage form; or

(iv) a modified or sustained release form of a drug or novel drug delivery system of any drug approved by the Central Licencing Authority; or

(v) a vaccine, recombinant Deoxyribonucleic Acid (r-DNA) derived product, living modified organism, monoclonal anti-body, stem cell derived product, gene therapeutic product or xenografts, intended to be used as drug;

Explanation. The drugs, other than drugs referred to in sub-clauses (iv) and (v), shall continue to be new drugs for a period of four years from the date of their permission granted by the Central Licencing Authority and the drugs referred to in sub-clauses (iv) and (v) shall always be deemed to be new drugs

q. **Non-inferior**: not clinically inferior to a comparator in the parameter studied. A non-inferiority clinical trial is one that has the primary objective of showing that the response to the investigational product is not clinically inferior to that of a comparator within a pre-specified margin.

r. **Originator product:** a medicine that has been licensed by an NRA on the basis of a full registration dossier – that is, the approved indication(s) for use were granted on the basis of full quality, efficacy and safety data.

s. **Pharmacodynamic study**: a clinical study that measures a pharmacodynamic (PD) response that effectively demonstrates the characteristics of the products target effects. PD biomarkers for biosimilars do not need to be surrogate end-points for clinical efficacy outcomes.

t. **Pharmacovigilance:** The science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other drug related problems.

u. **Posology**: dosage for each indication and each method/route of administration. Information includes dose recommendation (for example, in mg, mg/kg or mg/m2), frequency of dosing (for example, once or twice daily, or every 6 hours) and treatment duration.

v. **Reference Biological Product:** A Reference Biological product is used as the comparator for comparability studies with the Similar Biologic in order to show Similarity in terms of safety, efficacy and quality. The Reference Biologic should be licensed / approved in India or ICH countries and should be the innovator's product. The Reference Biologic should be licensed based on a full safety, efficacy and quality data. Therefore, another Similar Biologic cannot be considered as a choice for Reference Biologic.

w. **Reference standard**: a measurement standard such as an international, pharmacopoeial or national standard – it should be noted that reference standards are distinct from reference products and serve a different function.

x. **Similar Biologic**: Similar biologic means a biological product which is similar in terms of quality, safety and efficacy to reference biological product licenced or approved in India, or any innovator product approved in International Council of Harmonisation (ICH) member countries.

y. **Similarity:** absence of any relevant difference in the parameter(s) of interest.

19. References

I. World Health Organization (WHO) TRS No. 1043 Annex 3, Guidelines on evaluation of biosimilars, 2022 (Replacement of Annex 2 of WHO Technical Report Series, No. 977)

II. Medicines & Healthcare products Regulatory Agency, Guidance on the licensing of biosimilar products, November 2022

III. Health Canada, Guidance Document Information and Submission Requirements for Biosimilar Biologic Drugs, 2022

IV. EMA Guideline on Similar Biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues, 2014 (EMEA/CHMP/BMWP/42832/2005 Rev1)

V. EMA guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins, 2007 (CHMP/BMWP/14327)

VI. ICH guideline on preclinical safety evaluation of biotechnology-derived pharmaceuticals (S6), 1997 and addendum, 2011

VII. Guideline for Safety Study of Biological Products, (KFDA, 2010)

VIII. World Health Organization (WHO) Guidelines on Evaluation of Similar Biotherapeutic Products (SBP), 2009

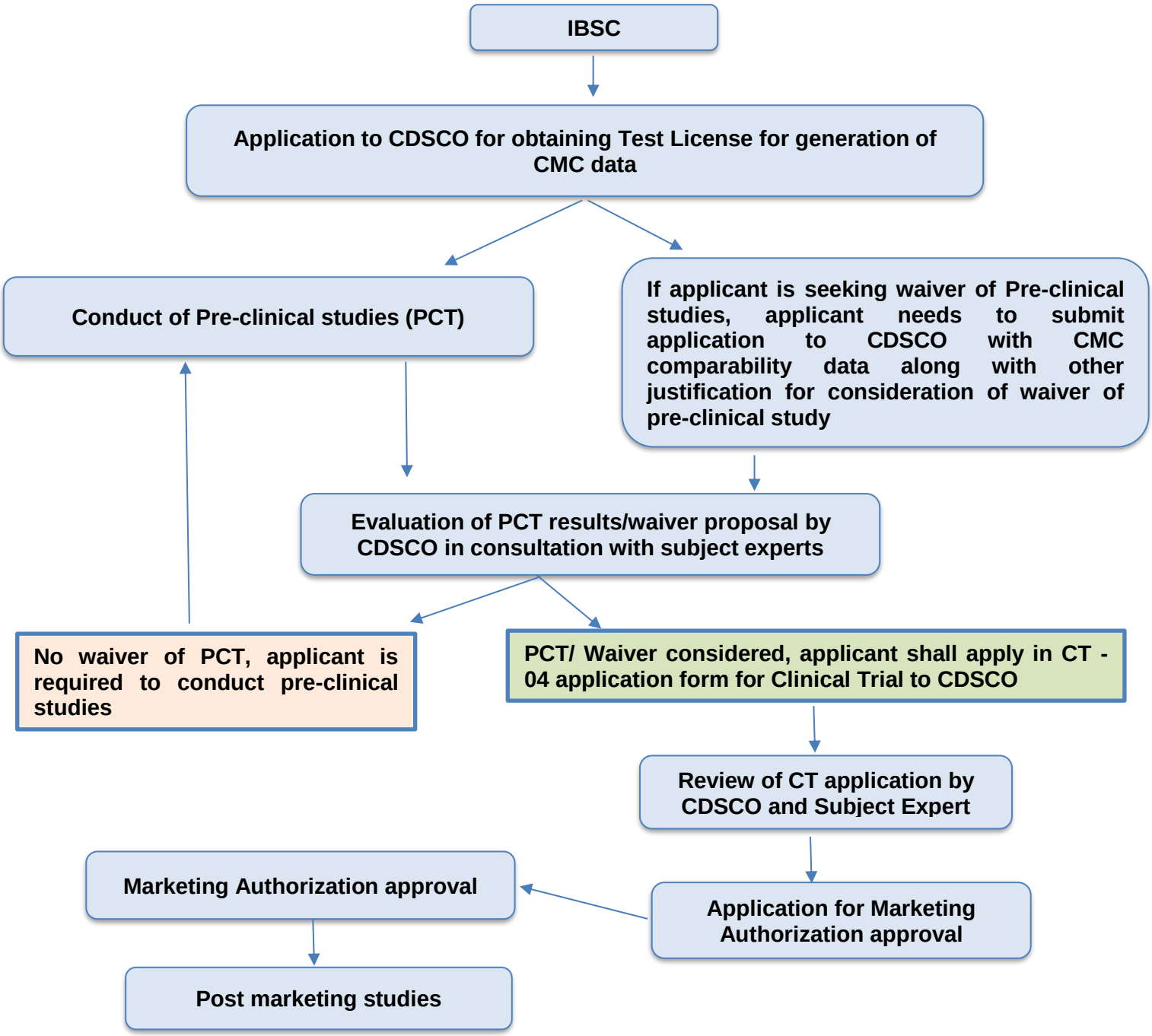
IX. World Health Organization (WHO), Guidelines on the quality, safety and efficacy of bio-therapeutic protein products prepared by recombinant DNA technology, 2013

X. EMA- DNA and Host cell protein impurities routine testing versus validation studies, 1997

XI. ICH Q1 A(R2)- Stability Testing of New Drug Substances and Products, 2003

XII. The Regulations & Guidelines for Recombinant DNA Research and Biocontainment, 2017

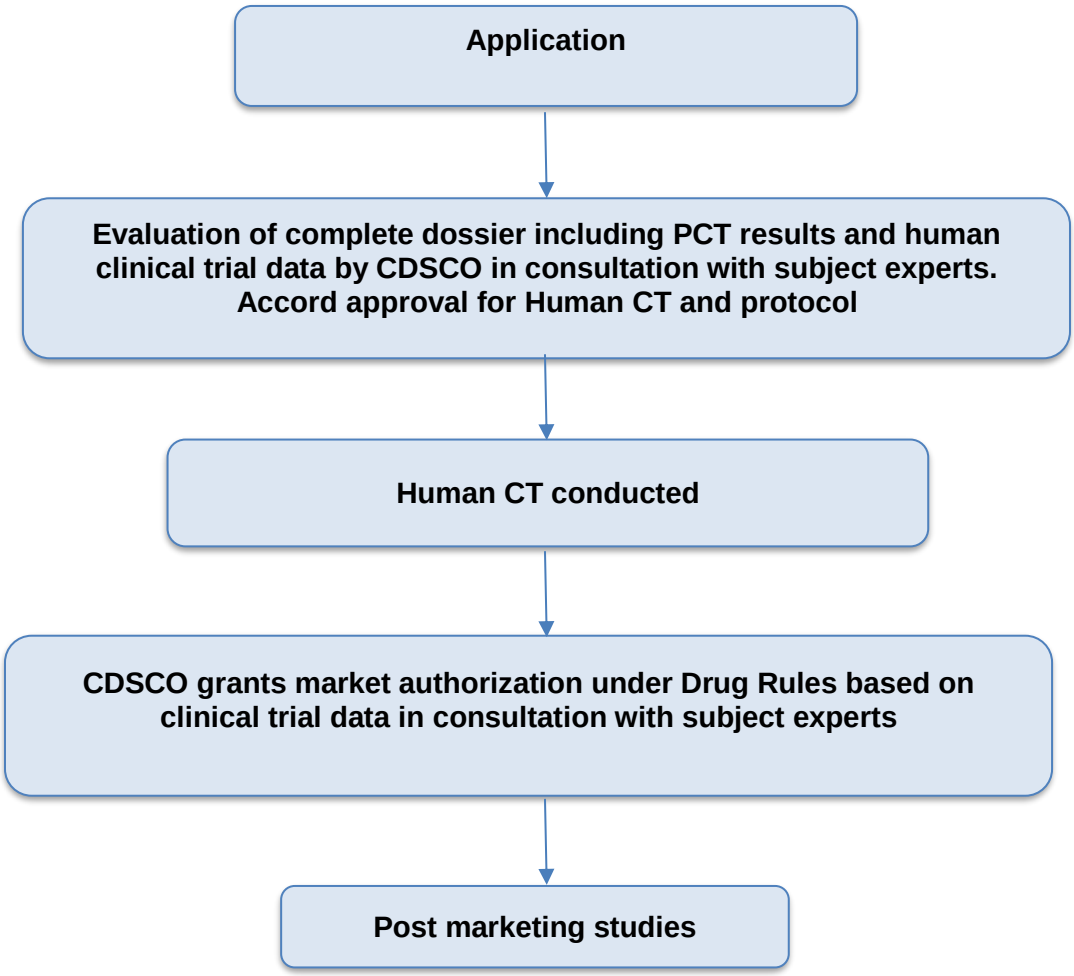
Annexure I: Pathway for approval to manufacture and market indigenously developed Similar Biologics



Note:

1. Application for seeking waiver of Pre clinical studies/ for conduct of clinical studies is required to be submitted to CDSCO and decision of waiver/MA permission will be granted by Licensing Authority.
2. Firm should obtain a valid license/permission from Licensing Authority under D&C Act and Rules thereunder for generation of data for regulatory submission.
3. The approval of RCGM is required for experiments involving Risk Group 3 and 4 organisms. (Reference: The Regulations & Guidelines for Recombinant DNA Research and Biocontainment, 2017)

Annexure IA: Pathway for approval to import and market Similar Biologics



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Annexure II: Critical Quality Attributes (CQA)

Physicochemical and biological characterization of nucleic acid based recombinant products (Vector for expression of recombinant protein, siRNA/ snRNA etc.), recombinant therapeutic Proteins, recombinant mAbs, recombinant therapeutic Enzymes

Quality Attributes	**Analytical Methodology**
Protein content	Absorbance
Primary structure/Identity	Peptide mapping by LC-MS/MS (CID/ETD/HCD)
	Amino acid sequence by LC-MS/MS or Edman degradation
	Intact mass (Native/deglycosylated) by LC-MS
	Subunit mass (Native/deglycosylated) by LC-MS
	N-terminal and C-terminal sequence by LC-MS/MS
Higher order structure (Secondary structure)	Far UV Circular Dichroism (CD)
	Fourier transform infrared spectroscopy (FTIR)
Higher order structure (Tertiary structure)	Near UV Circular Dichroism (CD)
	Fluorescence spectroscopy
	1D/2D Nuclear Magnetic Resonance (NMR)*
	Hydrogen/Deuterium eXchange Mass Spectrometry (HDX-MS)*
Higher order structure (Disulfide bridging)	Free thiol group analysis by Ellman/LC-MS
	Non-reduced LC-MS/MS
	Melting temperature by DSC/DSF
Higher order structure (Conformational stability)	Differential scanning calorimetry (DSC)/NanoDSC or Time-Correlated Single-Photon Counting (TCSPC)*
	Nano Differential Scanning Fluorimetry (nanoDSF)*
	Ion Mobility Mass Spectrometry (IM-MS)
Product related substances and impurities	Charge variants by CEX /cIEF/CZE-UV/LC-MS/CE-MS
	Size variants by SEC, DLS/MALLS (aggregates) Sub visible particles by MFI, AUC or equivalent
	Size-variants by reduced and non-reduced CE-SDS /

	SDS-PAGE
	PTMs by LC-MS
	N-Glycan relative quantitation by HILIC (labelling methods)
	Glycan characterization at intact or subunit level using LC-MS/CZE-LIF/CE-MS
Fab-mediated biological assays	Cell based assay
	Major target (receptor/ligand) binding assay by BLI/SPR
Fc-mediated biological assays	FcRI, FcRIIa(R and H)/b, FcRIIIa(V and F)/ b, FcRn binding kinetics if applicable
Fc effector functions	ADCC, if applicable
	CDC, if applicable
	Apoptosis, if applicable
DP Physical attributes	pH
	Appearance Concentration (Drug and excipient)
Process related impurities	HCP by ELISA/2D-PAGE/CZE-MS/LC-MS
	HCD by qPCR/Picogreen
	Residual Protein A
	BET Endotoxins (if applicable) Bioburden

* These next generation analytical methodologies are not mandatory and can be used if feasible.

**To ensure the statistical analysis, each quantitative experiment should be done atleast three times and data should be represented in terms of mean and standard deviation. Appropriate statistical significance should be represented throughout the characterization data.

Annexure III: Statistical tools for Biosimilarity assessment

NOTE: The following text elaborates the utilities of below statistical approaches. These are meant to be illustrative and nor prescriptive.

There are 3 tests recommended by regulatory agencies (World Health Organisation) for biosimilarity assessment, 1. x-sigma test, 2. min-max interval test, 3. tolerance Intervals test.

1. X-sigma interval: This tool calculates the similarity ranges based on the mean and standard deviation of the reference product batch data as shown in below equations.

    1.1. $\text{Mean } (\overline{X}) = \frac{\sum x_i}{n}$

    where, xi = lots of RBP; BS

    n = number of lots of RBP; BS

    1.2. $\text{Standard deviation } (\sigma) = \sqrt{\frac{1}{n-1}\sum(x_i - \overline{X})^2}$

    1.3. $\text{Interval} = (\overline{X} \pm 3 . \sigma)$

2. Min-Max Range: It establishes similarity ranges using the observed minimum and maximum values of the RBP quality attribute data.

    2.1. Min- Max Range: (x_{min}, x_{max})

    2.2. $\%\text{Within Range} = \frac{\text{Count of BS within range}}{\text{Total BS samples}} \times 100$

    where, xmin = Minimum value of RBP; xmax = Maximum value of RBP;

    BS represents biosimilars

3. Tolerance Intervals: It defines a range within which a specified percentage of future observations are expected to fall, given a certain confidence level.

    3.1. $\text{Tolerance Interval} = (\mu \pm k. \sigma)$

    where:

    $k = \sqrt{\sum \frac{n(1-\propto)}{\propto}} . t_{\frac{\propto}{2}, n-1}$

    $\propto$ = Significance level ($\propto$ = 1 – Confidence level)

    $t_{\frac{\propto}{2}, n-1}$ = Critical value of the student's t-distribution with

    (n-1) = degrees of freedom at $\propto/2$

Case 1: Glycosylation

Table 1: Glycan attributes with their criticality, tier ranking, and data from reference product and biosimilar lots

Glycan Attribute	Tier	RBP Lot 1	RBP Lot 2	RBP Lot 3	BS Lot 1	BS Lot 2	BS Lot 3
High mannose	Highly critical	5.91	5.06	4.61	4.55	4.26	5.17
Total Afucosylated	Highly critical	10.03	9.73	8.36	7.75	8.79	7.72
Galactosylation	Moderate	41.46	39.17	41.07	44.02	40.49	41.83
GlcNAc	Low	52.63	55.76	54.32	53.43	55.25	53.0
Sialylation	Low	1.2	0.6	0.8	0.9	0.8	1.2

Results:

Table: Summary of Mean, Standard Deviation, and Calculated Ranges for x-Sigma, Min-Max, and Tolerance Interval Tests.

Glycan Attribute	RBP Mean	Standard Deviation (SD)	Test -1	Test -2	Test -3
			X sigma (Mean ± (3. SD)	(Min – Max)	Tolerance Interval
High mannose	5.19	0.66	(3.21, 7.17)	(4.61, 5.91)	(4.70, 5.69)
Total Afucosylated	9.37	0.89	(6.7, 12.04)	(8.36-10.03)	(8.71, 10.04)
Galactosylation	40.57	1.23	(36.88, 44.26)	(39.17-41.46)	(39.65, 41.48)
GlcNAc	54.27	1.57	(49.56, 58.98)	(52.63-55.76)	(53.06, 55.41)
Sialylation	0.87	0.31	(-0.06, 1.8)	(0.6 -1.2)	(0.64, 1.10)

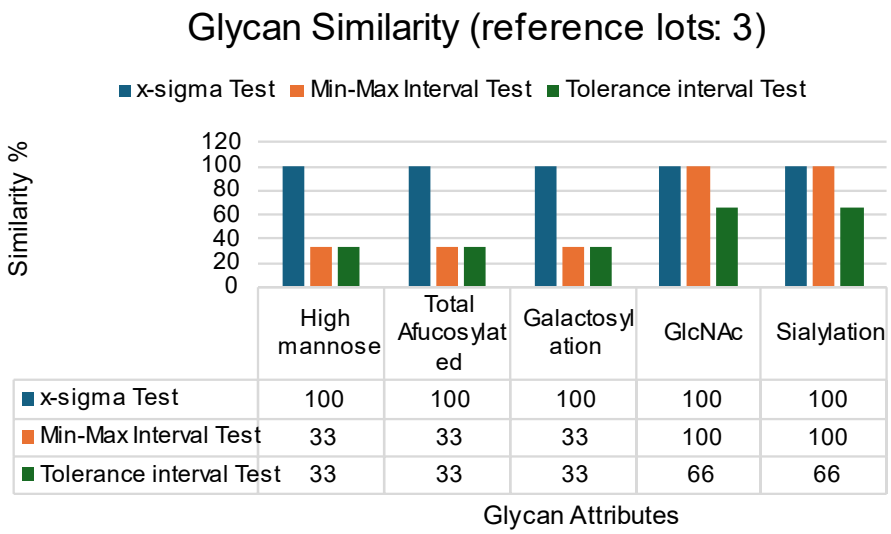

Fig 1: Illustration of biosimilarity scores for each quality attribute (glycan) assessed using three statistical methods: (1) x-sigma test, (2) min-max interval test, and (3) tolerance interval test for 3 lots for reference lots. The comparison highlights the percentage of biosimilar batches falling within the similarity ranges established by each method.

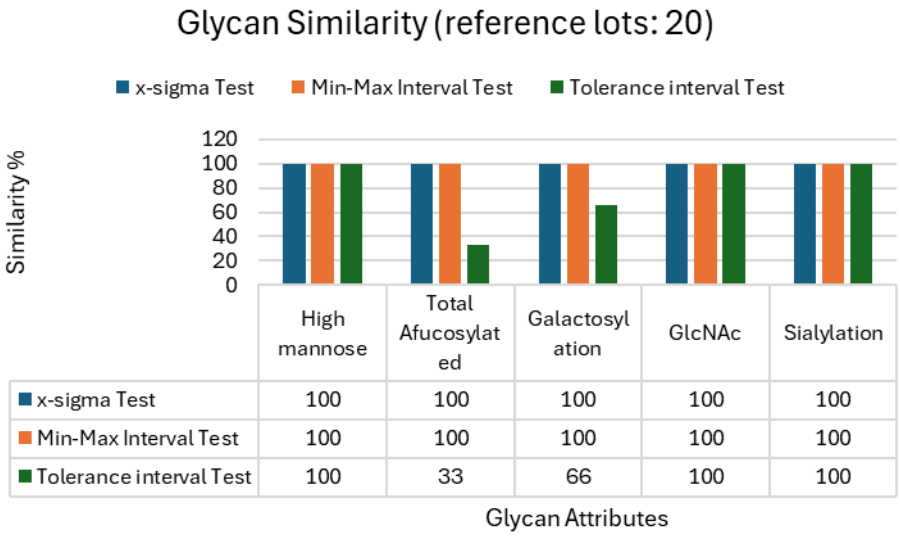

Fig 2: Illustration of biosimilarity scores for each quality attribute (glycan) assessed using three statistical methods: (1) x-sigma test, (2) min-max interval test, and (3) tolerance interval test for 20 lots of reference lots. The comparison highlights the percentage of biosimilar batches falling within the similarity ranges established by each method.

Key Observations

- For n=3 (less lots of reference)

- In this case study, X-sigma is widely accepted approach with 100% similarity for all the glycan attributes (Fig 1). For min-max approach and tolerance interval approach (highly critical and moderate attributes) showed only 33% of the similarity and batches fall within the calculated tolerance intervals, indicating tighter thresholds.
- For low criticality attributes in both the tests (min-max), 100% of the BS batches fall within the tolerance intervals, reflecting good fit to the range.
- For low criticality attributes in both the tests (tolerance interval), 66% of the BS batches fall within the tolerance intervals, reflecting less stringent requirements for these attributes.
- For n=20 (more lots of reference): As the number of lots, there is an improvement in the similarity of both min-max approach and tolerance approach as can be seen from fig 1 and 2. The increased the tolerance interval method provides a statistically robust framework for evaluating similarity but may lead to stricter conclusions when sample sizes are small.

Case 2: Size Heterogeneity

	Criticality	RBP Lot 1	RBP Lot 2	RBP Lot 3	BS Lot 1	BS Lot 2	BS Lot 3
HMW	moderate	1.12	2.42	0.95	1.62	1.81	2.05
Monomer	moderate	97.25	95.55	96.98	96.26	96.4	95.79
LMW	moderate	1.63	2.03	2.07	2.12	1.79	2.16

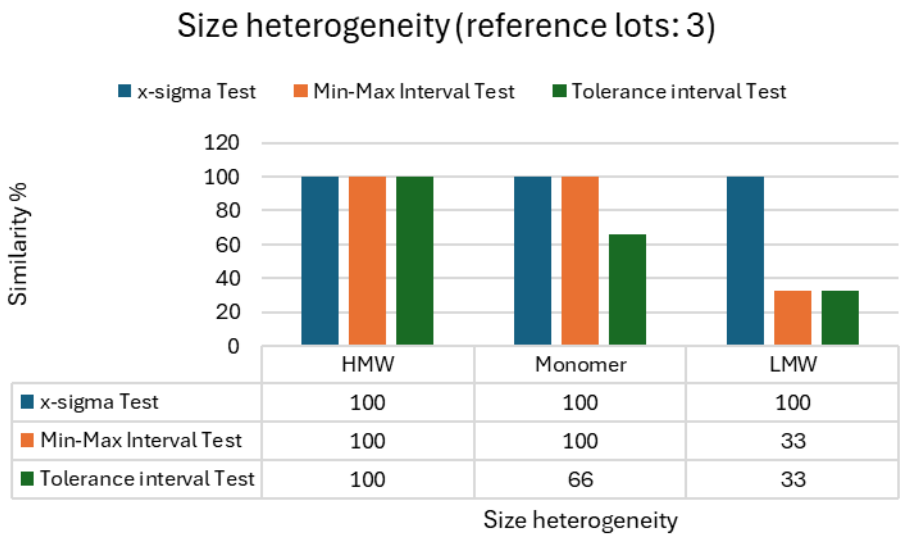

Fig 3: Illustration of biosimilarity scores for each quality attribute (size heterogeneity) assessed using three statistical methods: (1) x-sigma test, (2) min-max interval test, and (3) tolerance

interval test for 3 lots of reference lots. The comparison highlights the percentage of biosimilar batches falling within the similarity ranges established by each method

Key Observations

For n=3 (less lots of reference), The criticality of size attributes (monomer, high and low molecular weight species) are placed in the moderate range of criticality. X-sigma showed a good acceptance to the biosimilarity for all size attributes with 100% similarity (Fig 3). For min-max approach and to tolerance interval test showed similar similarity.

Case 3: Charge Variant

	criticality	RBP Lot 1	RBP Lot 2	RBP Lot 3	BS Lot 1	BS Lot 2	BS Lot 3
Acidic	moderate	6.92	6.18	8.16	7.62	7.48	6.56
Main	moderate	67.46	68.9	63.93	65.83	65.9	67.95
Basic	moderate	25.62	24.92	27.91	26.55	26.48	25.49

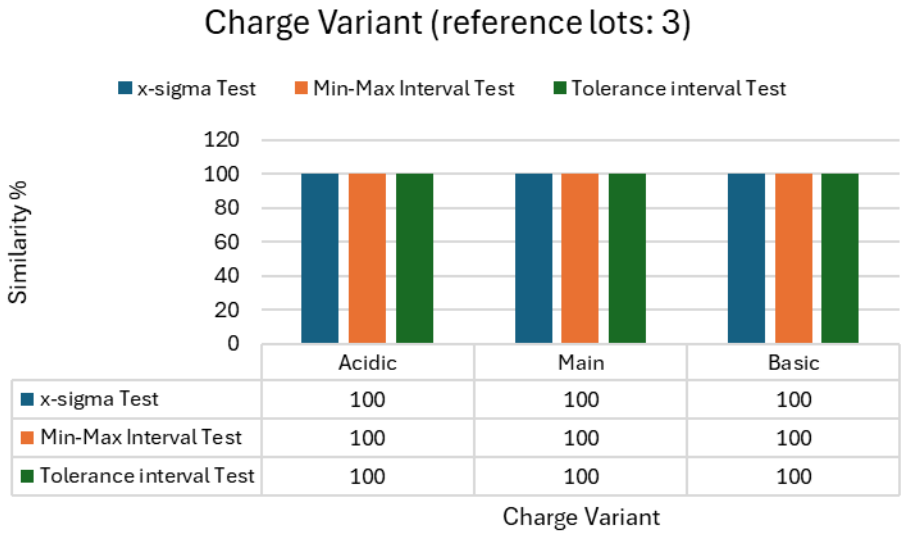

Fig 4: Illustration of biosimilarity scores for each quality attribute (Charge variant) assessed using three statistical methods: (1) x-sigma test, (2) min-max interval test, and (3) tolerance interval test for 3 lots of reference lots. The comparison highlights the percentage of biosimilar batches falling within the similarity ranges established by each method

Key Observations

For n=3, the criticality of size attributes (acidic, main and basic variant) are placed in the moderate range of criticality. All the 3 tests (X-sigma, min-max and tolerance interval) showed a good acceptance to the biosimilarity for all attributes with 100% similarity (Fig 4).

Overall Recommendation:

For Small Reference Datasets

The x-sigma method is the most effective, showing high acceptance for biosimilarity with 100% similarity across all attributes.

Limitations of Other Methods: The min-max approach and tolerance interval tests may yield lower similarity percentages due to stricter thresholds or overly conservative ranges, especially for highly critical and moderate attributes.

For Larger Reference Datasets

The tolerance interval method becomes more statistically robust and reliable as more RBP batches reduce variability-related artifacts.

The min-max approach also improves in similarity acceptance, but care must be taken to prevent overly conservative conclusions.

Other recommendations

- Apply stricter thresholds using scientifically justified multipliers in the x-sigma method or tighter tolerance intervals.
- Avoid reliance on min-max ranges, as they may be overly restrictive and prone to false-negative conclusions.

Annexure IV: Requirements of Toxicological Studies

In case of in vivo toxicity studies, at least one repeat dose toxicity study in a pharmacologically relevant species is required to be conducted with an intended route of administration.

Regarding the animal models to be used, the applicant should provide the scientific justification for the choice of animal model(s) based on the data available in scientific literature. However, if the pharmacologically relevant animal species is not available and has been appropriately justified, toxicity studies need to be undertaken either in rodent or nonrodent species as per requirements of NDCT Rules 2019.

Regarding route of administration either in pharmacologically relevant or pharmacologically non-relevant animal model the route of administration would include only the intended route as per NDCT Rules 2019.

The duration of the study would be generally not less than 28 days with 14 days recovery period. However, the duration may vary depending on the dosage and other parameters on case-by-case basis.

The dose should be calculated based on the therapeutic dose of the Reference Biologic. If required a pilot dose response study should be conducted prior to initiating the toxicity studies. Generally, there would be three levels of doses (viz. low, medium and high) used in the animal toxicology studies corresponding to 1X, 2X and 5X of human equivalent dose or higher test dose for repeated-dose toxicity studies. In the toxicity study the Similar Biologic should be compared with Reference Biologic at least at 1X of human equivalent dose (HED). Any difference in the levels of doses should be justified and approved prior to the studies. Regarding the schedule of administration, the therapeutic schedules may be used as the basis.

Depending on the route of administration, local tolerance should be evaluated. This evaluation, if feasible may be performed as a part of above mentioned repeated-dose toxicity study.

Accordingly, the study groups of animals in repeated-dose toxicity testing will consist of:

i. Historical Control (Optional)
ii. Vehicle Control
iii. Vehicle Control for recovery group
iv. Formulation without protein (for vaccines) if multiple adjuvants - each to be checked independently
v. 1X Similar Biologic for study duration (lowest dose)
vi. 1X Reference Biologic for study duration
vii. 2X Medium dose Similar Biologic
viii. 5X High dose Similar Biologic
ix. Similar Biologic with a recovery group going beyond the end of study period for 7 to 14 days

The protocols and the study reports should provide complete details of various steps in the toxicity testing as indicated below:

- Procedures prior to euthanasia e.g. blood drawing, body weight, etc.

- Events immediately after euthanasia, necropsy, gross – description, organ weights and organs sampled for histopathology.
- Biochemical parameters – Equipment and methods used - units of measurement and expression.
- Haematology procedures and parameters – method to be used (automated or manual).
- Statistical methods used.
- Bone marrow either examined as an aspirate /smear or on histopathology section.

In case of histopathological observations, the applicants should consider the following points:

- Every observation considered as deviation from described normal histology needs to be documented and the incidence of each of these in the different groups should be denoted.
- Whether such a feature is significant or not can be decided on review of statistical significance or dose response or if it is within or outside the normal range of values in case of biochemical and haematological observations.
- If all organs from all animals were not examined e.g. in 5 animals only 4 livers were examined, the reason for the 1 liver not being examined should be documented.
- In case of premature death or morbidity the proposed course of action is to be included in the protocol.

The final report of the study should reflect all the aspects approved in the protocol and the following additional sections/documents:

- IBSC approval of report
- IAEC approval for animal use and for the procedures • QA statement
- Signatures of study director and all investigators who were involved in the study
- All quality analytical reports on the test material and vehicle
- Animal feed and animal health certifications.

Protocol deviations if any

- Discussion on the results.
- Individual animal data, summary data and any other data like computer analysis outputs etc.
- Conclusion.

Annexure V: Statistical consideration in sample size determination for Clinical Study

Determining the number of subjects (sample size) in a clinical trial is a critical step in the design of the study. The sample size must be large enough to reliably detect the effect of the intervention. The statistical criteria for deciding the number of subjects typically include the following key elements like Primary Objective and Endpoint, Effect Size, Statistical Power, Significance level, Variability, Equivalence / non inferiority margins, incidence rate, Dropout & Compliance Rates, Study design, Multiplicity adjustments etc.

Commonly following choices are made:

- Power (1 - β): 80% or 90%.
- Type II Error (β): Typically 20% or 10%.
- Type I Error (α): Set at 5% (0.05).
- Variability estimated from previous studies or pilot data.
- Dropout and Compliance Rates to increase the sample size to ensure sufficient power after adjustment
- Stratification and Subgroup Analysis requires adequate numbers in each subgroup.

Various statistical software packages (e.g., SAS, R, Stata, PASS, nQuery) can be used to perform sample size calculations by Biostatistician. These tools often allow for more complex designs and adjustments.

Determining the number of subjects in a clinical trial involves a careful balance of statistical criteria, clinical relevance, and practical considerations. Proper sample size calculation ensures that the trial is adequately powered to detect meaningful effects while minimizing risks and resource use. The comparability Phase III clinical trials intended for seeking marketing approval of Similar Biologics falling under the category of new drugs as per Drugs and Cosmetics Rules, 1945 shall be conducted in accordance with the Indian Good Clinical Practice (GCP) guidelines and should be adequately powered to evaluate the safety, efficacy and comparability. Based on the statistical calculation of sample size, the number of subjects in test arm should not be less than 100 evaluable patients. Based on the results of such Clinical trials, the marketing approval may be considered if safety, efficacy and comparability are established. Further, Phase IV clinical trials may be required to be conducted, generally in more than two hundred patients in continuation of comparability clinical trials. In general, if the firm conducts pre approval comparative studies that included more than 100 patients on the proposed Similar Biologics drug and statistically proportionate number of patients in reference biologic arm, the number of patients in the Phase IV study can be modified accordingly so that the safety data (from both Phase III and IV) is derived from not less than 300 patients.

Exceptions:

In the case of Similar Biologics that can be evaluated for rare diseases, the clinical trial population size can be reduced as per the rarity and severity of the disease as well as the limitation of access to therapeutic options.

Acknowledgement