ETHIOPIAN FOOD AND DRUG AUTHORITY

GUIDELINE FOR REGISTRATION OF MONOCLONAL ANTIBODIES AND RELATED PRODUCTS

Document No.	**EFDA/GDL/079**	**Version No.**	**001**
Date of Approval	**01/11/2025**	**Date of first Issue**	**07/11/2025**

Document History

Version No.	Reason for amendment	Effective Date
001	New document developed	07/11/2025

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Table of Contents

Abbreviations

ADCC	Antibody-Dependent cell-mediated Cytotoxicity
ADCP	Antibody-Dependent Cellular Phagocytosis
C1q	Complement Component 1q CDC complement-dependent cytotoxicity
CDR	Complementarity Determining Region
CHO	Chinese Hamster Ovary
CPP	Certificate of Pharmaceutical Product
ELISA	Enzyme-Linked Immuno-Sorbent Assay
Fab	Fragment antigen-binding (region)
Fc	Fragment crystallizable (region)
Fv	variable fragment(s)
GMP	Good Manufacturing Practices
hcDNA	host cell DNA
HCP	Host Cell Protein
HPLC	High-Performance Liquid Chromatography
HVAC	Heating, Ventilation and Air Conditioning
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
LAL	Limulus Amoebocyte Lysate (test)
mAb	monoclonal antibody
MCB	master cell bank
mRNA	messenger RNA
MSB	master seed bank
NCL	national control laboratory
PCR	Polymerase Chain Reaction
PEG	Polyethylene Glycol
PPQ	Process Performance Qualification
QbD	Quality by Design
rDNA	recombinant DNA
scFv	single-chain variable fragment(s) SEC size-exclusion chromatography

Definition

The definitions given below apply to the terms as used in this Guideline. These terms may have different meanings in other contexts.

Adventitious agents: Contaminating microorganisms that can include bacteria, fungi, mycoplasmas, and endogenous and exogenous viruses, and that have been unintentionally introduced into the manufacturing process.

Antibody fragments: Proteins that consist of regions, or sections, of antibody molecules. These are usually single-chain variable fragments (scFv), fragment antigen binding (Fab) regions or single domain antibodies.

Applicant: is the person or entity who submits a registration application of product to the Authority and responsible for the product information.

Biological activity: The ability or capacity of a mAb substance or product to elicit a defined biological effect in vitro (for example, in cultured cells or viruses) or in vivo (in animal models and/or humans).

Bispecific or multi-specific antibodies: a single mAb in which each binding domain recognizes different epitopes of the same antigen or different antigens.

Co-formulated mAbs: A final product formulated to contain two or more mAbs, mAb conjugates and/or mAb fragments each of which recognizes a different epitope or antigen. These may also be referred to as “antibody cocktails”, “antibody mixtures”, “pooled antibody products” or “oligoclonal products

Drug product: A final product in a defined container closure system that contains one or more drug substances and which may be formulated with excipients.

Drug substance: The active pharmaceutical ingredient and associated molecules that may subsequently be formulated with excipients to produce the drug product.

Final bulk: A formulated preparation from which the final containers are filled. The final bulk is prepared from one or more purified mAb substances, formulated to contain all excipients and homogenous with respect to its composition.

Impurities: materials present in the mAb substance or product which are either:

(a) product-related (for example, mAb molecular variants, aggregates or fragments) and which do not have properties comparable to the desired product with respect to its safety, activity and efficacy; or

(**b) process-related** (for example, reagents, media components, host cell proteins (HCPs) or leachates) and not considered to be the active ingredient.

Intermediate product: a material produced during the production of an active pharmaceutical ingredient or drug substance that undergoes further molecular change or purification before it becomes the active pharmaceutical ingredient or drug substance.

Recombinant DNA technology: technology that joins (that is, recombines) DNA segments from two or more different DNA molecules that are then inserted into a host organism to produce new genetic combinations. It is also referred to as gene manipulation, gene editing or genetic engineering because the original gene is artificially altered. These new genes, when inserted into the expression system, form the basis for the production of rDNA derived protein(s).

1. Introduction

The Ethiopia Food and Drug Authority (EFDA) is responsible for ensuring the safety, efficacy, and quality of medicines in Ethiopia, as mandated by the Food and Medicine Administration Proclamation No. 1112/2019. Article 19(1) of the proclamation states that the rigor of regulatory assessment shall commensurate with the type, nature, and potential risk of the product to human health.

A biological product is a medicine whose active substance is made by or derived from a living organism such as a plant, human, animal, or microorganism and may be produced using biotechnology or other advanced technologies. These products are typically large, complex, and difficult to characterize. They include vaccines, monoclonal antibodies, blood products, recombinant proteins, and cell and gene therapy products.

Monoclonal antibodies (mAbs), a major class of recombinant deoxyribonucleic acid (rDNA)-derived biotherapeutics, are produced through methods such as hybridoma, phage display, humanized transgenic mouse technologies, single B-cell cloning, or recombinant DNA technologies. mAbs have achieved remarkable success in treating many life-threatening and chronic diseases.

Monoclonal antibodies (mAbs) are a category of medicines that require rigorous regulatory assessment. Due to their complex and unique nature, a specific guideline outlining the safety, efficacy, and quality requirements for mAbs is essential. Therefore, this guideline has been developed to address these needs.

This guideline provides technical guidance for both assessors and applicants on the documentation required for the registration of monoclonal antibodies and related products. It outlines the requirements and recommendations concerning the quality, safety, and efficacy information necessary for these products.

It should be noted that mAbs represent a broad spectrum of products and manufacturing processes and it is therefore not possible to establish a fixed set of attributes or testing methods that would necessarily apply to all of them. Therefore, the authority will assess on a case-by-case basis for each product and manufacturing process with flexibility to allow for the introduction of innovative approaches based on sound scientific principles and practices.

It also needs to be noted that mAb manufacturing processes and control strategies should be described in detail in the dossier of the registration application. Any subsequent manufacturing and/or control strategy changes must be assessed for their potential impact on product quality, safety and efficacy using a risk-based approach and in accordance with the EFDA guideline on procedures and data requirements for changes to approved biotherapeutic products. Such changes may require assessment and approval by the authority.

To facilitate the assessment process of monoclonal antibodies, applicants should organize product dossiers following the structure of the Common Technical Document (CTD). The structure of Module 3 in the CTD is provided in Annex 2 of this guideline. Applicants are also encouraged to consult the following key references:

- **WHO**: *Guidelines for the Production and Quality Control of Monoclonal Antibodies and Related Products Intended for Medicinal Use* (Annex 4, WHO TRS 1043, 2022)
- **EMA**: *Guideline on Development, Production, Characterisation, and Specification for Monoclonal Antibodies and Related Products*
- **ICH**: *Quality (M4Q), Safety (M4S), and Efficacy (M4E) Guidelines*
- Other relevant **WHO non-clinical and clinical guidelines**.

2. Scope

The scope of this guideline is applicable to monoclonal antibodies including but not limited to:

- mAbs of all isotypes, whether they are humanized, human, or chimeric, and regardless of the intended therapeutic mechanism of action;
- Antibody fragments, such as single-chain variable fragments (scFv), and fragment antigen-binding (Fab) and fragment crystallizable (Fc) regions;
- Single domain antibodies;
- Bispecific or multi specific antibodies;
- Fc-fusion proteins;
- mAbs or related antibody proteins that have been chemically modified, such as through conjugation to polyethylene glycol (PEG) or an active drug substance; and
- Multiple mAb substances co-formulated within a final product (“antibody cocktail”)

- mAbs used for in vivo diagnostics use
- Small recombinant mAb mimetic proteins and pathogen specific plasma-derived immunoglobulins

The guideline is not applicable to:

- ❖ Monoclonal antibodies for in vitro diagnostic purpose
- ❖ Monoclonal antibodies for clinical trials
- ❖ Immunomodulatory antibodies, which have little or no immunoglobulin structure (for example, DARPins, affimers and anticalins)
- ❖ Polyclonal antibodies (fractionated or recombinant)

3. Administrative and Product Information

3.1. Covering Letter

Dated and signed letter for submission of the dossier by mentioning the product included in the dossier from the applicant responsible for registration. The letter should declare that the information provided in the dossier is true and correct.

3.2. Table Contents of the Dossier

Table of contents should be provided.

3.3. Application Form

Completed and signed application form as provided in Annex I of this Guideline should be submitted.

3.4. Agency Agreement

The agency agreement should be submitted as per the current version of Guideline for Registration of Medicines.

3.5. Good Manufacturing Practice and Certificate of Pharmaceutical Product

A valid Good Manufacturing Practice (GMP) Certificate and market authorization certificate should be provided from the country of origin. Certificate of pharmaceutical product as a requirement for registration could be optional provided that valid cGMP Certificate and Market Authorization Certificate is submitted. The format of the CPP is provided in Annex II of Guideline for Registration of Medicines.

GMP compliance of the manufacturing site should be confirmed by the authority before the MA certificate being issued by the authority.

3.6. Regulatory Situation in Other Countries

The countries should be listed in which the product under application has been granted a marketing authorization, withdrawn from the market and/or rejected or deferred. Evidence of registration (with copy of certificates) in other countries should be provided.

3.7. Product Information

Product information should be provided as per the current Guideline for Medicine Product information of the Authority.

3.8. Evidence for an Application Fee

Each application should be accompanied by a relevant service fee for registration. The application fee shall be made per application and the payment receipt shall mention the application number issued by the eRIS.

Applicants are advised to consult the current Rate of Service Fees Regulation of the Authority for the amount to be paid for application and contact the Authority for details of mode of payment.

4. PRODUCTION OF MONOCLONAL ANTIBODIES

4.1. General considerations

The manufacturing process should be appropriately described and validated. Validation studies should at least include

i. the demonstration that the process is capable of producing product of consistent quality, in line with an appropriately defined control strategy,
ii. an evaluation of the process capability (e.g. elimination of process-related impurities, viruses), and
iii. the demonstration that each operational unit performs appropriately (e.g. validation of purification column, aseptic filling).

Attention should be focused on the setting of in-process controls (including product quality attributes and process parameters), as well as the drug substance and drug product specifications. These controls should be capable of monitoring relevant quality attributes, such as product-related substances and impurities (e.g. disulfide bond integrity or mismatch, deamidation, oxidation, truncation, aggregates) or process-related impurities (e.g. host cell protein, DNA, protein A, bovine serum and culture media residues), as well as relevant process parameters (e.g. column loads, pH, temperature).

When protein A is used in the purification process, the source of the protein A (e.g. S. aureus, recombinant) and its preparation method (e.g. purified using human IgG) should be appropriately documented. Where human IgG has been used in the preparation, it should be demonstrated that the quality of human IgG is suitable for its intended use, especially with regards to viral safety.

4.2. Platform manufacturing

The development of processes used for the production of monoclonal antibodies very much depends on the manufacturer's knowledge of the product and manufacturing process.

Some manufacturers have gained considerable experience in the production of monoclonal antibodies, and have developed a production strategy based on similar manufacturing processes (i.e. using a pre-defined host cell, cell culture and purification process). This approach is often referred to as “platform manufacturing”.

As for any medicinal product, the manufacturing process of a product that has been developed using a platform manufacturing approach should be appropriately validated at the time of marketing authorization application. Validation studies should include data derived from the final manufacturing process and site(s) used to produce the product to be commercialized. Nevertheless, when appropriately justified and documented, data derived from other relevant experience may be used to support or reduce the data derived from the final commercial process to be submitted.

Considering that quality attributes are specific for a given product and its manufacturing process, the suitability of analytical methods, and more generally the control strategy, should be specifically demonstrated for the product and process being registered. As a consequence, the suitability of the control strategy, demonstrated to be suitable for the analysis of other product(s) derived from the same platform manufacturing approach, should be carefully re-considered, as it may not be adapted to the product and process being submitted. For instance, process-related impurities, such as host cell proteins (HCP), are highly dependent on the process, and the controls applied for a given product and process may not be suitable for other products using the same platform manufacturing (e.g. different cell substrates derived from a common parenteral cell line, similar culture and purification conditions).

When a change is made to an already authorised process following a platform manufacturing approach, the impact of this change should be specifically evaluated for the concerned product and process. Nevertheless, when appropriately justified and documented, data derived from relevant experience may be used to support or reduce the data derived for the post-changed product and process to be submitted. Furthermore, when several products are derived from a common platform manufacturing process, and modifications (e.g. process optimisation, improvement) are introduced in only one or some of them, the rationale for the harmonisation strategy adopted or for the lack of harmonisation should be discussed.

4.3. Viral safety and Transmissible Spongiform Encephalopathy (TSE)

Viral safety aspects of monoclonal antibodies covered by this guideline should comply with ICH Q5A. The scope of this guideline includes monoclonal antibodies derived from hybridoma cell lines or from cells genetically engineered to express a monoclonal antibody. Whenever production of monoclonal antibodies is performed using animals (e.g. engineered animals), ICH Q5A should be followed with particular reference to Appendix 1. Source cells (e.g. host cells) should undergo suitable screening for adventitious agents (i.e. extraneous agents and endogenous agents). The choice of viruses for the tests is dependent on the species and tissue of origin of the production cell and the nature of any other biological raw material used in production.

The importance of good studies on the validation of viral reduction is emphasised. The virus-reducing capacity of manufacturing steps should be validated for the submitted product and its manufacturing process according to ICH Q5A. These validation studies are usually performed using intermediates from the specific production process in order to cover potential or unexpected product-specific factors affecting virus reduction. Nevertheless, when appropriately justified and documented, relevant studies (e.g. derived from a platform manufacturing approach) can also be helpful to establish and evaluate virus-reducing process steps, and thus may help to reduce the number of validation studies to be submitted. Such data may be considered supportive, e.g. for investigation of the potential influence of varying process parameters on virus reduction, performance of columns after multiple production cycles, virus carry-over studies or studies on column sanitisation. In all cases, the manufacturer should justify the relevance of these data for the specific product.

A rationale should be provided why prior in-house data can be applied to the new product, e.g. referring to viral reduction data of a particular process step would be possible when the product intermediate at the stage before such a step has comparable biochemical properties and is purified by identical methods. The manufacturer should provide a critical analysis of the manufacturing step for which these supportive in-house data will be applied and on the composition of the respective product intermediate. The analysis should provide confidence in the conclusion that in both cases the established manufacturing step is similar in its capacity to inactivate/remove potential virus contaminants. If the comparison of the step is not entirely convincing, or if the database cannot rule out a product-specific effect on virus reduction capacity, confirmatory runs using product-specific process intermediates are expected.

Where materials of bovine or other TSE-relevant animal species have been used in development or manufacture, the Note for Guidance on "Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products" (EMEA/410/01) should be consulted.

5. Characterization of monoclonal antibodies

The monoclonal antibody should be characterised thoroughly. This characterisation should include the determination of physicochemical and immunochemical properties, biological activity, purity, impurities and quantity of the monoclonal antibody, in line with ICH Q6B guideline. At the time of submission, the manufacturer should have established appropriately characterised in-house reference materials which will serve for biological and physicochemical testing of production lots.

5.1. Description

A clear description of the drug substance should be provided. This description may include, but not be limited to, any of the following: chemical structure, primary and subunit structure, molecular weight, molecular formula, established name, antibody class/subclass (if appropriate), etc.

5.2. Physicochemical Characterization

A physicochemical characterization program will generally include a determination of the class, subclass, light chain composition (kappa and/or lambda chain) and primary structure of the monoclonal antibody.

The amino acid sequence should be deduced from DNA sequencing and confirmed experimentally by appropriate methods (e.g. peptide mapping, amino acid sequencing, and mass spectrometry analysis).

The variability of N- and C- terminal amino-acid sequences should be analysed (e.g. C-terminal lysine(s)).

Free sulphydryl groups and disulfide bridges should be determined. Disulfide bridge integrity and mismatch should be analysed.

The carbohydrate content (neutral sugars, amino sugars and sialic acids) should be determined. In addition, the structure of the carbohydrate chains, the oligosaccharide pattern (antennary profile), the glycosylation site(s) and occupancy should be analysed.

Typically, monoclonal antibodies have one N-glycosylation site on each heavy chain located in the Fc region. The light chain is usually not glycosylated. However, additional glycosylation site(s) in the heavy chains may occur, and thus their presence or absence should be confirmed.

Glycan structures should be characterized, and particular attention should be paid to their degree of mannosylation, galactosylation, fucosylation and sialylation. The distribution of the main glycan structures present (often G0, G1 and G2) should be determined.

Higher-order structure of the monoclonal antibody should be characterized by appropriate physicochemical methodologies.

Furthermore, applicants can refer Summary of potential sources of heterogeneity in recombinant mAbs and examples of possible characterization methods (Refer: Appendix 2: WHO TRS 1043, 2022).

5.3. Immunological property

The immunological properties of the antibody should be fully characterized. Binding assays of the antibody to purified antigens and defined regions of antigens should be performed, where feasible, to determine affinity, avidity and immunoreactivity (including cross reactivity with other structurally homologous proteins).

Unintentional reactivity/cytotoxicity for human tissues distinct from the intended target should be documented. Cross-reactivity with a range of human tissues should be determined using immunehistochemical procedures. Where appropriate, cross reference to non-clinical and/or clinical section(s) may be made.

The complementary determining regions (CDR) should be identified, unless otherwise justified.

The epitope and molecule bearing the relevant epitope should be defined. This should include a biochemical identification of these structures (e.g. protein, oligosaccharide, glycoprotein, glycolipid), and relevant characterization studies (amino acid sequence, carbohydrate structure) to the extent possible. The ability for complement binding and activation, and/or other effector functions should be evaluated, even if the intended biological activity does not require such functions.

5.4. Biological Activity

The biological activity (i.e. the specific ability or capacity of a product to achieve a defined biological effect) should be assessed by appropriate in vitro assay(s). Where in vivo assays are necessary, the use of such assays should be thoroughly justified. The mechanism of action and the importance (or consequences) of the product effector functions with regards to the safety and efficacy of the product should be discussed.

For antibodies where effector function may play a role in the mechanism of action, and/or have an impact on the product safety and efficacy, a detailed analysis of ADCC, cytotoxic properties (e.g. apoptosis), ability for complement binding and activation and other effector functions, including Fc gamma receptor binding activity, and neonatal Fc receptor (FcRn) binding activity should be provided, as appropriate.

5.5. Purity, Impurity and Contaminants

Monoclonal antibodies commonly display several sources of heterogeneity (e.g. C-terminal lysine processing, N-terminal pyroglutamate, deamidation, oxidation, isomerisation, fragmentation, disulfide bond mismatch, N-linked oligosaccharide, glycation), which lead to a complex purity/impurity profile comprising several molecular entities or variants. This purity/impurity profile should be assessed by a combination of orthogonal methods, and individual and/or collective acceptance criteria should be considered for relevant product-related variants. These methods generally include the determination of physicochemical properties such as molecular weight or size, isoform pattern, extinction coefficient, electrophoretic profiles, chromatographic data and spectroscopic profiles. In addition, suitable methods should be proposed to qualitatively and quantitatively analyze heterogeneity related to charged variants.

Multimers and aggregates should also be appropriately characterized using a combination of methods. The formation of aggregates, sub-visible and visible particulates in the drug product is important and should be investigated and closely monitored on batch release and during stability studies. In addition to the pharmacopoeial test for particulate matter, other orthogonal analytical methods may be necessary to determine levels and the nature of particulates. Potential process-related impurities (e.g. HCP, host cell DNA, cell culture residues, downstream processing residues) should be identified, and evaluated qualitatively and/or quantitatively, as appropriate. Contaminants, which include all adventitiously introduced materials not intended to be part of the manufacturing process (e.g. microbial species, endotoxins) should be strictly avoided and/or suitably controlled. Where non-endotoxin pro-inflammatory contaminants, such as peptidoglycan, are suspected, the use of additional testing, such as the monocyte activation test, should be considered.

5.6. Quantity

Quantity should be determined using an appropriate physicochemical and/or immunochemical assay.

It should be demonstrated that the quantity values obtained are directly related to those derived using the biological assay. When this correlation exists, it may be appropriate to use measurement

of quantity rather than the measurement of biological activity in the product labelling and manufacturing processes, such as filling.

6. Control of mAb active substance

The specification for the mAb active substance should be provided. Copy of the active substance specifications dated and signed by authorized personnel (e.g., the person in charge of the quality control or quality assurance department) should be provided in the product dossier. The specification should at least the parameters with their acceptance criteria should be listed where appropriate:

- Appearance and description
- Identity
- Purity and impurities
- Potency
- Quantity

Justification for the mAb active substance specification should be provided. A discussion should be provided on the inclusion of certain tests, evolution of tests, analytical procedures and acceptance criteria, differences from the officially recognized compendial standard(s), etc. Applicant may refer applicable WHO and ICH guidelines, pharmacopoeia and other relevant documents for justification of the specification

The analytical procedures used for testing mAb active substance should be provided. If the manufacturer adopts in-house analytical method, analytical validation information, including experimental data for the analytical procedures used for testing the mAb active substance, should be provided.

7. Composition of finished products

A tabulated list of all components with their unit dose and batch quantities for the drug product or diluent should be submitted. The composition of all ancillary products that might be included in the final product should be included.

8. Specifications and test methods of the final product

Specifications are one part of a total control strategy designed to ensure product quality and consistency, and when tested, the product should be in compliance with its specification. Specifications should be set and take into account relevant quality attributes identified in characterisation studies. Selection of tests to be included in the specifications is product specific. The rationale used to establish the acceptable range of acceptance criteria should be described. Acceptance criteria should be established and justified taking into account data obtained from lots used in preclinical and/or clinical studies, data from lots used for demonstration of manufacturing consistency, data from stability studies and relevant development data, in accordance with ICH Q6B.

8.1. Appearance

The appearance of the final container and its contents should be verified using a suitable method, and should meet the established criteria with respect to physical state (for example, solid or liquid) and colour, taking into consideration the nature of the container (for example, a dark amber container). The appearance of lyophilized or freeze-dried products should be verified both before and after reconstitution with the intended diluent, and should meet the established criteria.

MAbs are prone to the formation of visible particles, especially at high protein concentrations. Although appropriate formulation development should prevent this from occurring in the final product, the presence of visible particles may not always be avoidable.

8.2. Identity

Identity tests on the mAb, mAb conjugate or co-formulated mAb product should be performed on each final lot. The identity tests selected should be specific and may be based on the antigen target specificity, molecular structure, isotype, lightchain composition and/or other specific properties of the mAb product. Considering the great similarity of the constant domains of different antibodies, more than one test (physicochemical, biological and/or immunochemical) may be necessary to establish identity, and such test(s) should be able to discriminate other antibodies that may be manufactured in the same facility.

For mAb conjugate products, the presence of its conjugated payload must be verified. For co-formulated mAb products, release testing methods should include an identity method that

demonstrates the presence of each individual antibody and a quantitative method to confirm their ratio.

8.3. Purity and impurities

As noted in the characterisation section, monoclonal antibodies may display a complex purity/impurity profile that should be assessed by a combination of orthogonal methods, and for which individual and/or collective acceptance criteria should be established for relevant product-related variants. For example, separation methods based on charge heterogeneity should be considered to quantitatively and qualitatively monitor charge variants.

Chromatographic and/or electrophoretic methods capable of detecting product truncation, dissociation and polymerisation should be included, and quantitative limits should be proposed for these, as appropriate.

Particular attention should be paid to the demonstration of the suitability of the analytical methods used to control multimers and aggregates.

Considering that glycosylation may have an impact on the pharmacokinetics of the product, and may modulate its immunogenic properties, appropriate acceptance criteria should be considered for this attribute. In addition, such control will further confirm the consistency of the product.

As a consequence, tests and acceptance limits for relevant glycosylation structures should be carefully considered (e.g. relative amounts of G0, G1 and/or G2 of Fc fragments, levels of galactosylation, fucosylation and sialylation) taking into account the intended and potential impact of this attribute on the biological activity in the context of the clinical situation (e.g. the presence of functional effector functions not being required for the intended mechanism of action, Fab glycosylation).

The control of relevant process-related impurities should be included in the control strategy. In some situations, and where appropriately demonstrated, their control may be performed on an intermediate product, at an appropriate process step. Routine testing may not be necessary for some impurities for which the process has been demonstrated to achieve high reduction levels. Control of residual protein A, HCP, residual DNA and other potential culture or purification residues are typically part of the drug substance specification, as appropriate. In addition, such control provides valuable information on process consistency and performance. Applicant may

also refer WHO TRS 1043 Annex 4 "Guidelines for the production and quality control of monoclonal antibodies and related products intended for medicinal use" for the methods employee to determine product-related impurities and process-related impurities.

8.4. Potency

Potency is the quantitative measure of biological activity based on an attribute of the product which is linked to the relevant biological properties. A relevant potency assay should be part of the specifications for drug product, and should ideally reflect the biological activity in the clinical situation.

Potency testing should be conducted for each final product lot. The test method(s) used should reflect the activity/activities of the mAb or mAb conjugate. Potency should be expressed as a value relative to a reference material, and the assay should be sufficiently sensitive to detect functional differences in the product. Any potential effect(s) on the potency assay(s) caused by excipients contained in the product formulation should be considered.

For co-formulated mAbs, the potency methods used should account for all mAb substances present in the final product.

For antibodies for which the clinical activity is only dependent on binding/neutralising properties, a potency assay that measures binding to the target (i.e. binding assay) may be deemed acceptable, if appropriately justified. Where effector functions are relevant for clinical activity, a cell-based bioassay or another assay that takes effector functions into account should be performed. A combination of two separate methods, one measuring the specificity and one giving an indication of an effector function (e.g. complement activation, C1q binding, Fc gamma receptor binding) may be acceptable if a cell-based assay is not feasible or if the combination of two methods gives more precise results.

Although the two types of potency assays (binding or cell-based) often yield comparable results, these assays cannot be deemed interchangeable, because there are product attributes that may not affect binding to target (e.g. glycosylation, fragmentation) but may affect further signalling or receptor expression.

Specific activity (biological activity per mass) is of considerable value to demonstrate consistency of production.

8.5. Protein content

Protein concentration should be measured using a validated method of suitable sensitivity and specificity – such as determination of the absorbance at 280nm, using the protein-specific absorbance. The protein concentration of the final product must be within ±10% of the labelled claim. For co-formulated mAb products, the protein content of each of the individual mAbs should be measured.

8.6. pH and Osmolality

If the mAb product is a liquid preparation, the pH of each lot should be controlled and the results should be within the range considered to be safe for parenteral administration. For a lyophilized preparation, the pH should be measured after reconstitution using the same diluent recommended for clinical use.

The osmolality of the final lots should be determined and shown to be within the range considered to be safe for parenteral administration to humans.

8.7. Moisture content (if applicable)

If the final product is a lyophilized preparation, the level of residualmoisture should be determined and the results should be within the limit.

8.8. Test for ratio of combined mAbs (if applicable)

If two or more mAbs and/or mAb conjugates are co-formulated in the final product, a test must be in place to ensure the proper ratio of the combined mAbs. This test may not be required on the final product if the ratio of the combined mAbs was verified in the final bulk.

8.9. Heterogeneity profile

The heterogeneity profile of the final product should be confirmed as being similar to that of the purified mAb substance. Some differences in the heterogeneity profile might occur during substance storage and final product manufacturing (for example, formation of aggregates) and should be justified in such cases. Attributes which should be considered during final product consistency assessment include the size distribution, charge heterogeneity and other post-translational modifications. Conjugated mAbs should also be verified in terms of the heterogeneity of the payload-to-mAb ratio. The number of methods used to assess heterogeneity

may be reduced if the impact of the formulation and filling processes are clearly characterized and demonstrated to have little effect – however, this should be appropriately justified.

The measurement of some product-related post-translational modifications in the drug substance may be sufficient and not require further retesting if the drug product manufacturing process is demonstrated to not have an impact on the post-translational modifications.

8.10. Excipients

The presence and concentration of excipients critical to product stability and sterility (such as surfactants or preservatives) should be controlled. With the exception of compendial grade excipients, testing requirements for all excipients should be based on risk assessment.

8.11. Sterility

The contents of the final containers should be tested for bacterial and fungal sterility. If the final product contains a preservative, then appropriate measures should be taken to prevent it from interfering with the tests.

8.12. Endotoxin or pyrogen content

The endotoxin content of each lot of the final product should be consistent with levels found to be acceptable in product lots used during clinical trials. Suitable in vitro methods include the test for bacterial endotoxins using recombinant factor C or the LAL test. The test selected for assessing endotoxin content must be validated for its intended purpose.

The authority expects a parenterally administered drug product to have an endotoxin content of ≤ 5 EU/kg/h in its final presentation, or ≤ 0.2 EU/kg/h for intrathecally administered products. Therefore, the potential contribution of endotoxin from a reconstitution buffer, diluent or other co-administered product should also be considered.

The need for pyrogenicity testing should be determined during the manufacturing development process based on an appropriate risk assessment. This may need to be re-evaluated following any changes in the production process or relevant reported production inconsistencies that could influence the quality of the product with regard to its pyrogenicity. A monocyte activation test may be used for monitoring the potential pyrogenic activity in the final product after a product-specific validation. A rabbit pyrogenicity test is discouraged due to the inherent variability, high re-testing rates and interspecies differences in pyrogenic responses compared to humans.

8.13. Reconstitution time (if applicable)

The reconstitution time should conform to specification if the final product is presented as a freeze-dried or lyophilized formulation.

8.14. Extractable volume

It should be demonstrated that the nominal volume indicated on the label can consistently be extracted from the containers, whether single-dose or multi-dose.

9. Stability testing, storage and expiry date

9.1. Stability testing

Stability programmes for drug products should be initiated early in the pharmaceutical development process. When relevant, in-use stability studies should be conducted to establish the time period during which a drug product may be used after the container is opened while still retaining acceptable quality specifications. Stability study protocols and results supporting the stability claims over the shelf-life must be provided.

Recommended storage conditions for drug products should be based on the stability data. Stability programmes for lyophilized products should be conducted following reconstitution with the intended diluent. Appropriate studies should be considered for multi-dose containers to demonstrate maintenance of product quality and microbial control during the in-use period. Appropriate stability-indicating parameters should be defined or selected according to the stage of production.

When changes are made in the production procedure that may affect the stability of the product, further stability studies may need to be conducted to determine the validity period of the new product. For radio-labelled mAbs, stability studies may be conducted using non-radioactive labels and limited to the expected duration over which the radioisotope is considered to be active.

A minimum of 12 month long-term and 6 months accelerated stability studies for final products are required. At the time of dossier submission a minimum of 6 months accelerated stability study data should submit. Stability studies under accelerated and stress conditions are strongly advised in WHO and ICH guidelines. Such studies provide additional information on the overall characteristics of the mAb substance(s) and product, and help identify stability-indicating

methods suitable for ongoing stability studies. This information may also be useful in assessing comparability should the manufacturer plan to make future changes to the manufacturing process.

For mAb product licensure, the stability and expiry date of the product in its final container, when maintained at the recommended storage temperature should be demonstrated using final containers from at least three final lots made from different mAb bulks. For products filled in more complex containers (for example, in a device) stability testing might be considered after the final container closure is secured but prior to the addition of non-container closure parts.

Following licensure, ongoing monitoring of mAb product stability will be required to support shelf-life specifications and to refine the stability profile.

The final stability-testing programme should include an agreed set of stability-indicating parameters, as well as procedures for the ongoing collection and sharing of stability data. In-use stability and, where applicable, compatibility (for example with infusion sets) should also be specified and justified with adequate data generated under real-time conditions.

9.2. Storage conditions and shelf life

Storage conditions and shelf life should be defined as per the stability data generated. The mAb product should have been shown under these conditions to maintain its potency for a period equal to that from the date of release to the expiry date.

10. Non-clinical studies

10.1. General considerations in nonclinical evaluation

The primary objectives of both in vitro and animal nonclinical studies are to define the pharmacological and toxicological effects of investigational products prior to the initiation of human studies. This will involve:

- Functional characterization of the product, such as its ability to prevent disease, reduce pathogen load, impair toxin activity, promote pathogen clearance from the blood and tissues, improve clinical signs, prevent or reduce weight loss, or reduce severity of infection.
- Identification of possible toxicities, their potential for reversibility and likelihood of potential adverse or undesirable effects.
- Identification of a safe starting dose for first-in-human (FIH) studies and of safe dose escalation when possible.

There are several important factors to consider when designing nonclinical studies for mAbs intended to prevent or treat a human infectious disease. Knowledge of the mAb target antigen of the infecting pathogen and its biology is expected, as is characterization of the binding site/epitope and evaluation of the specificity and selectivity of the mAb to the pathogen. Unwanted and unexpected cross-reactivity with animal or human cells and/or tissues need to be explored.

In addition, naturally occurring changes to the antigen (that is, through antigenic drift or shift) may occur through the course of some epidemics and result in reduced affinity of the mAb to the target antigen. The potential for such reduced affinity through epitope mutation should therefore be considered and prospectively evaluated, if relevant, before a mAb is committed to clinical study, and should be monitored by the sponsor (for example, through in vitro tests using antigens derived from circulating and emerging strains).

Nonclinical study design should be guided by, and tailored to, the type of data needed, and by whether it is a PK, PD or safety study. Data derived from PD, PK and short-term toxicity studies help to approximate the FIH dose and dosing margins.

PD studies in animals help to define the lower range of the efficacious therapeutic dose (for example, minimum effective dose) whereas short-term toxicity studies provide an indication of the upper range for a safe FIH dose. PK studies provide information on the blood concentration–time profile of the mAb following administration that can help refine the therapeutic dose range. In some cases, PK data may also provide an estimate of the lower dose range for use in FIH studies where PD data are not available.

In vitro and modelling studies for mAbs for which there are sufficient data and experience may be acceptable alternatives for estimating FIH doses, but this should be discussed with the NRA in advance. In vitro and modelling studies for estimating FIH doses may not be sufficient for novel mAb products for which there is limited experience. The selection of a suitable animal species for use in evaluating mAbs against an infectious disease could prove challenging, and may not necessarily be the same species across the different study types. Scientific justification should be provided for the animal species selected for use in each study and should take into account the likely suitability of the resulting data in guiding human clinical studies.

This is particularly important where established animal models of infection do not exist, are not relevant to human physiology or do not reflect the pathology of the infection in humans. The nature of the mAb product itself should also inform species selection since this may also influence the study results. Although the target antigen for anti-infective mAbs is unique to the infecting pathogen, regardless of the host, the subsequent response by the host to the mAb-bound pathogen can vary significantly in nonclinical studies depending on the host species and on the species from which the mAb has been derived. For example, the use of a humanized mAb in a mouse model would not necessarily predict the activity or safety of the same humanized mAb in humans.

For this reason, understanding the impact of host species and mAb differences will be crucial in the preclinical development programme and in the translation of nonclinical data to the clinical situation. The induction of anti-drug antibodies (ADAs) is species specific, and their occurrence in animal studies is generally not relevant in terms of predicting the potential immunogenicity of mAb products in humans. Nevertheless, the detection of ADAs in animals may provide some insight as to potential complications, particularly for mAb-related products, and may also assist in the interpretation of data derived from animal toxicity studies. For example, ADA formation can increase the clearance of the mAb and impact its PK and/or toxicokinetic (TK), which in turn

can reduce its pharmacological and/or toxicological effects. The induction of ADAs could also result in other pharmacological and/or toxicological changes including the emergence of new toxic effects.

Therefore, all such PK and TK effects of ADA formation should be considered. In addition, consideration should be given to situations where the mechanism of action of the mAb involves a secondary response such as ADCC, ADCP or CDC, which may vary greatly depending on antibody Fc and animal model Fc receptors. Such pharmacological properties, and whether or not they are species specific, should be considered when interpreting exposure–response relationships, PK parameters and tissue toxicity in animal studies. The degree of similarity of the animal infection model to human infection must also be taken into consideration. In all animal studies it is important to sequence, characterize and standardize the pathogen challenge strain and its dose on administration. Where the passage of pathogenic strains may lead to the development of variants it is vital to use challenge material at defined and standardized passage levels . It may also be informative to genotype pathogens isolated from animals that succumb to infection despite mAb exposure in order to assess whether the susceptibility to such infection correlated with antigenic drift or shift in the pathogen.

The applicant should submit the non-clinical studies reports as per the structure indicated below. The data should contain the table of content. As appropriate the non-applicability of some studies should clearly indicated as such. Applicants are strongly advised refer WHO Guideline on the non-clinical and clinical evaluation of monoclonal antibodies and related products intended for prevention of or treatment of infectious disease, Annex 2, WHO TRS 1048, 2023 and ICH Safety and efficacy guidelines.

10.2. Table of Contents of Module 4

A Table of Contents should be provided that lists all of the nonclinical study reports and

gives the location of each study report in the PD.

10.3. Study Reports

The study reports should be presented in the following order:

10.3.1. Pharmacology

10.3.1.1 Primary Pharmacodynamics

10.3.1.2 Secondary Pharmacodynamics

10.3.1.3 Safety Pharmacology

10.3.1.4 Pharmacodynamic Drug Interactions

10.3.2. Pharmacokinetics

10.3.1.5 Analytical Methods and Validation Reports (if separate reports are available)

10.3.1.6 Absorption

10.3.1.7 Distribution

10.3.1.8 Metabolism

10.3.1.9 Excretion

10.3.1.10 Pharmacokinetic Drug Interactions (nonclinical)

10.3.1.11 Other Pharmacokinetic Studies

10.3.3. Toxicology

10.3.1.12 Single-Dose Toxicity (in order by species, by route)

10.3.1.13 Repeat-Dose Toxicity (in order by species, by route, by duration; including supportive toxicokinetic evaluations)

10.3.1.14 Genotoxicity

10.3.1.14.1 In vitro

10.3.1.14.2 In vivo (including supportive toxicokinetic evaluations)

10.3.1.15 Carcinogenicity (including supportive toxicokinetic evaluations)

10.3.1.15.1 Long-term studies (in order by species, including range-finding studies that cannot appropriately be included under repeat-dose toxicity or pharmacokinetics)

10.3.1.15.2 Short- or medium-term studies (including range-finding studies that cannot appropriately be included under repeat-dose toxicity or pharmacokinetics)

10.3.1.15.3 Other studies

10.3.1.16 Reproductive and Developmental Toxicity (including range-finding studies and supportive toxicokinetic evaluations) [If modified study designs are used, the following sub-headings should be modified accordingly.]

10.3.1.16.1 Fertility and early embryonic development

10.3.1.16.2Embryo-fetal development

10.3.1.16.3Prenatal and postnatal development, including maternal function

10.3.1.16.4Studies in which the offspring (juvenile animals) are dosed and/or further evaluated

10.3.1.17 Local Tolerance

10.3.1.18 Other Toxicity Studies (if available)

10.3.1.18.1Antigenicity

10.3.1.18.2Immunotoxicity

10.3.1.18.3Mechanistic studies (if not included elsewhere)

10.3.1.18.4Dependence

10.3.1.18.5Metabolites

10.3.1.18.6Impurities

10.3.1.18.7Other

10.4. Literature References

11. Clinical studies

The applicant should submit the clinical studies reports as per the structure indicated below. As appropriate the non-applicability of some studies should clearly indicate as such and as applicable, the authority may request additional study reports based the intended use of monoclonal antibody product under application. Applicants are strongly advised refer WHO Guideline on the non-clinical and clinical evaluation of monoclonal antibodies and related products intended for prevention of or treatment of infectious disease, Annex 2, WHO TRS 1048, 2023 and ICH Safety and efficacy guidelines.

11.1. Table of content

11.1.1. Phase I studies

11.1.2. Pharmacokinetics

11.1.3. Pharmacodynamics

11.1.4. Efficacy–Phase II and III studies

11.1.5. Clinical end-points

11.1.6. Phase II studies

11.1.7. Phase III studies

11.2. Safety

11.3. Pharmacovigilance

List of References

1. *Guidelines for the production and quality control of monoclonal antibodies and related products intended for medicinal use, Annex 4, WHO TRS 1043, 2022.*
2. *Guideline on development, production, characterization and specification for monoclonal antibodies and related products, 21 July 2016 EMA/CHMP/BWP/532517/2008 Committee for medicinal products for human use (CHMP).*
3. *Guidelines for the production and quality control of monoclonal antibodies and related products intended for medicinal use Replacement of Annex 3 of WHO Technical Report Series, No. 822.*
4. *Guidance for industry for the submission of chemistry, manufacturing, and controls information for a therapeutic recombinant DNA-derived product or a monoclonal antibody product for in vivo use center for biologics evaluation and research (cber) center for drug evaluation and research (cder) august 1996.*
5. *Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products*
6. *Guidelines on the nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of infectious diseases, Annex 2, WHO Technical Report Series, 2023.*
7. *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin Q5A(R1), International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use.*
8. *Biological Products Registration Guideline, Version 1.0, Drug Safety Center, Kingdome of Saudi Arabia*

LIST OF ANNEXES

ANNEX I: APPLICATION FORM FOR REGISTRATION

Food and Drug Authority of Ethiopia P.O. Box 5681, Addis Ababa, Ethiopia

A. Type of application (check the box applicable)

New Application	
Renewal	
Variation to existing marketing authorization (If selected, complete the information below.)	
• Previous registration number	
• Previous registration condition	
• Brief description of change intended	
• Reasons for variations	

B. Details on the product

Proprietary name (trade name)	
Approved generic name (s) (use INN if any)	
Standard claimed (BP, Ph.In, Ph. Eur., USP, IH, etc.)	
Strength(s) per dosage unit	
Dosage form	
Route of administration	
Shelf life (months)	
Visual description	
Description of container closure	
Packaging and pack size	
Therapeutic category	
Use category	

Complete qualitative and quantitative composition (indicate per unit dosage form, e.g., per 5ml, etc.)** ** Add/delete as many rows and columns as needed.	Composition	Strength	Function
Complete qualitative and quantitative Composition (indicate per batch in Kg, L, etc.)	Composition	Strength	Function
Statement of similarity and difference of clinical, bio-batch, stability, validation, and commercial batch sizes			
Regulatory situation in other country (Provide a list of countries in which this product has been granted a marketing authorization and the			

restrictions on sale or distribution, e.g., Withdrawn from the market, etc.)	

c.Details on the applicant

Name	
Business address	
Street number and postal address	
Telephone number	
Fax number	
E-mail and website address	
Contact person in a company	Name:
	Position:
	Postal address:
	Telephone number:
	Fax number:
	E-mail:
Details of Manufacturer, if different from above	Insert the required information as indicated Above---

A. Details on active pharmaceutical(s) ingredient(s)

Name of manufacturer	
Street and postal address	
Telephone	
Fax number	
E-mail	
Name of the active ingredient	
Retest period/Shelf life	

E. Details on local agent (representative) in Ethiopia

Name of local agent	
Sub-city and postal address	
Telephone	
Fax number	
E-mail	
Contact person in company	

F. Details on dossiers submitted with the application

Section of dossier	Annex, page number
Module 1	
Module 2	
Module 3	
Module 4	
Module 5	

CERTIFICATION BY A RESPONSIBLE PERSON IN THE APPLICANTCOMPANY

I, the undersigned, certify that all the information in the accompanying documentation concerning an application for a marketing authorization for:

Proprietary name (trade name)	
Approved generic name(s) (INN)	
Strength(s) per dosage unit	
Dosage form	
Applicant	
Manufacturer	

… is correct and true, and reflects the total information available. I further certify that I have examined the following statements and I attest to their accuracy.

1. The current edition of the WHO Guideline, “Good manufacturing practices for biological

products," is applied in full in all premises involved in the manufacture of this product.

2. The formulation per dosage form correlates with the master formula and with the batch manufacturing record forms.

3. The manufacturing procedure is exactly as specified in the master formula and batch manufacturing record forms.

4. Each batch of all starting materials is either tested or certified against the full specifications in the accompanying documentation and comply fully with those specifications before it is released for manufacturing purposes.

5. All batches of active pharmaceutical ingredient(s) are obtained from the source(s) specified in the accompanying documentation.

6. No batch of active pharmaceutical ingredient will be used unless a copy of the batch certificate established by the active ingredient manufacturer is available. Each batch of the container/closure system is tested or certified against the full specifications in the accompanying documentation and complies fully with those specifications before it is released for manufacturing purposes.

8. Each batch of the finished product is either tested or certified against the full specifications in the accompanying documentation and complies fully with the release specifications before it is released for sale.

9. The person releasing the product for sale is an authorized person as defined by the WHO guideline "Good manufacturing practices: Authorized person - the role, functions and training."

10. The procedures for control of the finished product have been validated for this formulation.

11. The market authorization holder has a standard operating procedure for handling adverse reaction reports on its products.

12. The market authorization holder has a standard operating procedure for handling batch recalls of its products.

13. All the documentation referred to in this Certificate is available for review during a GMP inspection.

14. Any clinical trials were conducted according to WHO's "Guidelines for good clinical practice (GCP) for trials on pharmaceutical products."

Signature ----------

Name ----------

Position in company (print or type) ----------

Date: ----------

ANNEX 2: ORGANIZATION OF MODULE 3 SECTION OF DOESSIERS

3.2.S. Drug Substance:

3.2.S.1 General Information	
3.2.S.1.1 Nomenclature	Information on Recommended International Nonproprietary Name (INN); Compendial name if relevant; Chemical name(s), Company or laboratory code
3.2.S.1.2 Structure	Brief description of active substance structure or content including the followings:
	• Molecular weight/ mass • Glycosylated /non glycosylated • Primary structure (In case of monoclonal antibody; Amino acid sequence of light chain and heavy chain should be mentioned)/ • Product variant /Heterogeneity • High order structure
3.2.S.1.3 General Properties	The details of the Characterization which includes the determination of physicochemical properties, biological activity and immunochemical properties of the drug substance by appropriate techniques should be stated. 1. **Physicochemical:** A physicochemical characterization program will generally include a determination of the composition, physical properties, and primary structure of the desired product. 2. **Biological:** it is the biological activity that describes the specific ability or capacity of a product to achieve a defined biological effect. Examples of procedures used to measure biological activity include: Animal-based biological assays, Cell culture-based biological assays, Biochemical assays. 3. **Immunological:** When an antibody is the desired

3.2.S.2.1 Manufacturer(s) • Name & address of API manufacturer	The name, address, and responsibility of each manufacturer, including contractors, and each proposed production site or facility involved in manufacturing and testing should be provided.
3.2.S.2.2 Description of Process and Process Controls	• A flow diagram of all manufacturing process including (upstream and downstream process) and their description. • Quantities of raw materials, solvents, catalysts and reagents reflecting the representative batch scale for commercial manufacture, identification of critical steps, process controls, equipment and operating conditions (e.g., temperature, pressure, pH, time). • Alternate processes should be explained and described with the same level of detail as the primary process. Reprocessing steps should be identified and justified. • A description of the manufacturing process including information on cell bank and cell culture, harvest(s), purification and modification reaction including filling storage and shipping conditions should be provided. The in-process controls for each step or stage of the process should be indicated. ✓ **Cell culture** The following information should be provided: • Flow diagram from working cell bank (WCB) through harvest. • Information for each stage should be provided (population doublings, cell concentrations, volumes, pH, cultivation time, temperature) and transfers between steps. • Description of each step including any media, materials or additives used for both cell growth and for induction. • Information with respect to operating parameters for each stage with links to section 3.2.S.2.4 (in-process controls) or specifications. ✓ **Purification**

	The following information should be provided: • Flow diagram from crude harvest, extraction and purification to final step of obtaining final active substance. • Information for each stage should be provided (pH, conductivity, processing times, hold times, elution profiles, fraction (selection) including viral inactivation step(s). • In-process controls, including acceptance criteria, should be described in detail and should be validated. Special attention should be given to the removal of viruses, nucleic acid, host cell proteins and impurities considered to pose a risk of immunogenicity. • Particular attention should be given to demonstrating the removal and/or inactivation of possible contaminating viruses and residual DNA from products manufactured using continuous cell lines. • Description of each step including scale (columns, membranes), lifetime usage for resins/membranes, regeneration, buffers used, and transfer between steps.
3.2.S.2.3 Control of Materials	• Materials used in the manufacture of the drug substance (e.g., raw materials, starting materials, solvents, reagents, catalysts) should be listed identifying where each material is used in the process. • Information on the quality and control of these materials should be provided. • Biological raw materials or reagents may require careful evaluation to establish the presence or absence of deleterious endogenous or adventitious agents.
3.2.S.2.4 Control of Critical Steps and Intermediates	• **Critical Steps**: Tests and acceptance criteria (with justification including experimental data) performed at critical steps identified in 3.2.S.2.2 of the manufacturing process to ensure that the process is controlled should be provided.

	• **<u>Intermediates</u>**: Information on the quality and control of intermediates isolated during the process should be provided.
Under section 3.2.S.2.2 or 3.2.S.2.3 or 3.2.S.2.4 additional information should be provided	Host cell line: • Origin of cell line • Source • Cell strain • Vector (plasmid): An explanation of the source and function of the component parts of the vector, such as the origins of replication, promoters, or antibiotic markers, should be provided in addition to a restriction-enzyme map indicating at least those sites used in construction. • Clone selection • Cell culture media
Under section 3.2.S.2.2 or 3.2.S.2.3 or 3.2.S.2.4 additional information should be provided	• Cell bank system (MCB) Information on the cell banking system; quality control activities and cell line stability during production and storage (including procedures used to generate the Master and Working Cell Bank(s) should be provided in detail. In addition, information about the cell bank origin and storage condition, details of life expectancy and any new working cell bank should be fully characterized.
	• Characterization and control of the host cell & cell bank system (MCB) including: ✓ Genetic and phenotypic stability ✓ Cell viability ✓ Absence of adventitious agent ✓ Absence of endogenous and exogenous viruses.
3.2.S.2.5 Process Validation and/or Evaluation	• Process validation and/or evaluation studies for aseptic processing and sterilization should be included.
3.2.S.2.6 Manufacturing Process Development	• A description and discussion should be provided of the significant changes made to the manufacturing process and/or

	manufacturing site of the drug substance used in producing nonclinical, clinical, scale-up, pilot, and, if available, production scale batches
3.2.S.3.1 Elucidation of Structure and Other Characteristics	Confirmation of Physicochemical Characteristics: Elucidation of product structure, including primary structure, post-translational modifications (PTMs), and higher-order structure. Confirmation of Biological Characteristics: Studies assessing binding and biological activity Preparation of Stress Materials for Characterization: Materials and methods used to prepare stress samples
3.2.S.3.2 Impurities • List of Potential Impurities.	The details of purity profile of the drug substance that is assessed by a combination of analytical procedures should be provided including: • Product-related impurities which are molecular variants arising during manufacture and/or storage, which do not have properties comparable to those of the desired product with respect to activity, efficacy, and safety. The accurate detection of degradation changes that may result from deamidation, oxidation, sulfoxidation, aggregation or fragmentation during storage should be included. • Process-related impurities encompass those that are derived from the manufacturing process, i.e., cell substrates (e.g., host cell proteins, host cell DNA), cell culture (e.g., inducers, antibiotics, or media components), or downstream processing. • Contaminants include all adventitiously introduced materials not intended to be part of the manufacturing process, such as chemical and biochemical materials (e.g., microbial proteases), and/or microbial species. (e.g., endotoxins, bioburden, mycoplasma, and adventitious viruses). • Elemental impurities include elements which are added during cell culture processing and purification steps.
3.2.S.4 Control of Drug Substance	

3.2.S.4.1 Specifications	The following tests and their acceptance criteria should be listed where appropriate: • Appearance and description • Identity • Purity and impurities • Potency • Quantity
3.2.S.4.5 Justification of Specification	Justification for the active substance specification should be provided.
3.2.S.4.2 Analytical Procedures	The analytical procedure used for testing the active substance should be provided in sufficient detail to enable reproducible testing by another laboratory.
3.2.S.4.3 Validation of Analytical Procedures	Analytical validation information, including experimental data for the analytical procedure used for testing the drug substance should be provided. Typical validation characteristics to be considered are selectivity, precision (repeatability, intermediate precision and reproducibility), accuracy, linearity, range, limit of quantitation, limit of detection, robustness, and system suitability.
3.2.S.4.4 Batch Analyses	Description of batches and results of three batch analyses should be provided. Results should be presented for three commercial batches against acceptance criteria
3.2.S.5 Reference Standards or Materials	• Information on the reference standards or reference materials used for testing of the drug substance should be provided. • At the time of submission, the manufacturer should have established an appropriately characterized in-house primary reference material, prepared from lot(s) representative of production and clinical materials. • Where an international or national standard is available and appropriate, reference materials should be calibrated against it.

	• Documentation of the characterization, storage conditions and formulation supportive of reference material(s) stability should also be provided.
3.2.S.6 Container/Closure Systems	• A description of the container closure system(s) should be provided, including the identity of materials of construction of each primary packaging component, and their specifications. The specifications should include description and identification (and critical dimensions with drawings, where appropriate). Non-compendial methods (with validation) should be included, where appropriate. • For non-functional secondary packaging components (e.g., those that do not provide additional protection), only a brief description should be provided. For functional secondary packaging components, additional information should be provided. • The suitability should be discussed with respect to, from moisture and light, compatibility of the materials of construction with the drug substance, including sorption to container and leaching materials of construction.
3.2.S.7 Stability	
3.2.S.7.1 Stability Summary and Conclusions	A minimum of six months' stability data at the time of submission should be submitted in cases where storage periods greater than six months are requested. For drug substances with storage periods of less than six months, the minimum amount of stability data in the initial submission should be determined on a case-by-case basis.
3.2.S.7.2 Post-approval Stability Protocol and Commitment	Should be submitted for active substance
3.2.S.7.3 Stability Data	Stability studies should include: Storage conditions i.e temperature and relative humidity for accelerated and stress conditions.

3.2.P. Drug Product

3.2.P.1 Description and Composition of the Drug Product	The information provided should include, for example: • Description of the dosage form. • Composition, i.e., list of all components of the dosage form, and their amount on a per unit basis (including overages, if any) the function of the components, and a reference to their quality standards (e.g., compendial monographs or manufacturer's specifications) • Description of accompanying reconstitution diluent(s) • Type of container and closure used for the dosage form and accompanying reconstitution diluent, if applicable.
3.2.P. Pharmaceutical Development	
3.2.P.2.1 Components of the Drug Product	
3.2.P.2.1.1Drug substance	The compatibility of the drug substance with excipients listed in 3.2.P.1 should be discussed. Additionally, key physicochemical characteristics (e.g., water content, solubility, and particle size distribution, polymorphic or solid-state form) of the drug substance that can influence the performance of the drug product should be discussed. For combination products, the compatibility of drug substances with each other should be discussed.
3.2.P.2.1Excipients	The choice of excipients listed in 3.2.P.1, their concentration, and their characteristics that can influence the drug product performance should be discussed relative to their respective functions.
3.2.P.2.2Drug Product	
3.2.P.2.2.1 Formulation Development(O)	A brief summary describing the development of the drug product should be provided, taking into consideration the proposed route of administration and usage. The differences between clinical formulations and the formulation (i.e. composition) described in 3.2.P.1 should be discussed.
3.2.P.2.2.2 Overages	Any overages in the formulation(s) described in 3.2.P.1 should be justified.

3.2.P.2.2.3 Physiochemical and Biological Properties	Parameters relevant to the performance of the drug product, such as pH, ionic strength, dissolution, re-dispersion, reconstitution, particle size distribution, aggregation, polymorphism, rheological properties, biological activity or potency, and/or immunological activity, should be addressed.
3.2.P.2.3 Manufacturing Process Development	The selection and optimization of the manufacturing process described in 3.2.P.3.3, in particular its critical aspects, should be explained. Where relevant, the method of sterilization should be explained and justified. Differences between the manufacturing processes (es) used to produce pivotal clinical batches and the process described in 3.2.P.3.3 that can influence the performance of the product should be discussed.
3.2.P.2.4 Container Closure System	The suitability of the container closure system (described in 3.2.P.7) used for the storage, transportation (shipping) and use of the drug product should be discussed. This discussion should consider, e.g., choice of materials, protection from moisture and light, compatibility of the materials of construction with the dosage form (including sorption to container and leaching) safety of materials of construction, and performance (such as reproducibility of the dose delivery from the device when presented as part of the drug product).
3.2.P.2.5 Microbiological Attributes	Where appropriate, the microbiological attributes of the dosage form should be discussed, including, for example, the rationale for not performing microbial limits testing for non-sterile products and the selection and effectiveness of preservative systems in products containing antimicrobial preservatives. For sterile products, the integrity of the container closure system to prevent microbial contamination should be addressed
3.2.P.2.6 Compatibility(O)	The compatibility of the drug product with reconstitution diluent(s) or dosage devices (e.g., precipitation of drug substance in solution, sorption on injection vessels, stability) should be addressed to provide appropriate and supportive information for the labeling.
3.2.P.3 Manufacture	

3.2.P.3.1 Manufacturer(s)	The name, address, and responsibility of each manufacturer, including contractors, and each proposed production site or facility involved in manufacturing and testing should be provided.
3.2.P.3.2 Batch Formula	A batch formula should be provided that includes a list of all components of the dosage form to be used in the manufacturing process, their amounts on a per batch basis, including overages, and a reference to their quality standards.
3.2.P.3.3 Description of Manufacturing Process and Process Controls	A flow diagram should be presented giving the steps of the process and showing where materials enter the process. The critical steps and points at which process controls, intermediate tests or final product control are conducted should be identified.
3.2.P.3.4 Controls of Critical Steps and Intermediates	Critical Steps: Tests and acceptance criteria should be provided (with justification, including experimental data) performed at the critical steps identified in 3.2.P.3.3 of the manufacturing process, to ensure that the process is controlled. Intermediates: Information on the quality and control of intermediates isolated during the process should be provided.
3.2.P.3.5 Process Validation and/or Evaluation	Description, documentation, and results of the validation and/or evaluation studies should be provided for critical steps or critical assays used in the manufacturing process (e.g., validation of the sterilization process or aseptic processing or filling). Viral safety evaluation should be provided in 3.2.A.2, if necessary
3.2.P.4 Control of Excipients	
3.2.P.4.1 Specifications	Information on the specifications for all the excipients employed in the formulation should be provided. List of raw materials meeting in-house specifications including the tests performed and specifications of biological starting materials (human or animal origin) with information on the requirements to avoid risk of transmissible spongiform encephalopathies (TSEs) and human diseases (HIV, hepatitis, etc) in the final product including Certificate of Suitability (CEP) should be included.
3.2.P.4.2 Analytical Procedures	
3.2.P.4.3 Validation of Analytical Procedures	
3.2.P.4.4 Justification of Specifications	

	Description of reference of the analytical methods used to control all the excipients employed in the formulation should be submitted. Justification for the proposed specifications of the excipients should be provided.
3.2.P.4.5 Excipients of Human or Animal Origin	For excipients of human or animal origin, information should be provided regarding adventitious agents (e.g., sources, specifications; description of the testing performed; viral safety data).
3.2.P.4.6 Novel Excipients	For excipient(s) used for the first time in a drug product or by a new route of administration, full details of manufacture, characterization, and controls, with cross references to supporting safety data (nonclinical and/or clinical) should be provided according to the drug substance format.
3.2.P.5 Control of Drug Product	
3.2.P.5.1 Specifications	The following parameters should be considered for all biological drug products: 1. Appearance and description 2. Identity 3. Purity and impurities 4. Potency 5. Protein Quantity 6. General physical test (pH, osmolality) 7. Additional test based on dosage form (liquid or solid)
3.2.P.5.6 Justification of Specifications	To justify each chosen criteria used in the specification above.
3.2.P.5.2 Analytical Procedures	Detailed information on the analytical procedures used for quality control of the drug product should be provided.
3.2.P.5.3 Validation of Analytical Procedures	Information on the validation of the analytical procedures for the drug product, including experimental data should be provided. This information should include complete description of the protocol used for each

	bioassay, the control standards, the validation of inherent variability of test and the establishment of acceptance limits for each assay.
3.2.P.5.4 Batch Analyses	Provide certificates of analysis and analytical results for at least three consecutive batches signed by authorized personnel
3.2.P.5.5 Characterization of Impurities	Details on the characterization and/or determination of impurities, as applicable, depending on the nature of active substance and method used to manufacture the Biotherapeutics product should be provided.
3.2.P.6 Reference Standards or Materials	Information on the reference standards or reference materials used for testing of the drug product should be provided, if not previously provided in "3.2.S.5 Reference Standards or Materials".
3.2.P.7 Container Closure System	• A description of the container closure systems should be provided, including the identity of materials of construction of each primary packaging component and its specification. The specifications should include description and identification (and critical dimensions, with drawings where appropriate). Non-compendial methods (with validation) should be included where appropriate. For non-functional secondary packaging components (e.g., those that neither provide additional protection nor serve to deliver the product), only a brief description should be provided. For functional secondary packaging components, additional information should be provided. Suitability information should be located in 3.2.P.2 • When a delivery device is presented as part of the drug product (e.g. prefilled syringe, single-use autoinjector), it is important to demonstrate the functionality of such a combination, such as the reproducibility and accuracy of the dispensed dose under testing conditions which should simulate the use of the drug product as closely as possible. • For multi-use containers such as vials or cartridges for a pen injector, proper in-use stability studies should be performed to

	evaluate the impact of the in-use period of the vial or the assembled device on the formulation and the functionality of the pen injector. • Dose accuracy should be demonstrated for the first and last dose delivered. • In addition, the effect of multiple injections/withdrawals on the closure system should be demonstrated.
3.2.P.8 Stability	
3.2.P.8.1 Stability Summary and Conclusions	• Stability study report including the study protocol, specifications, and analytical methods, detailed description of the container closure system for the product evaluated, storage conditions (temperature and relative humidity) and results for at least three lots of drug product prepared from different lots of drug substances should be provided and the reports should contain conclusions as well as proposed validity period. • A minimum of twelve months' data at the time of submission should be provided in cases where storage periods greater than six months are requested, unless otherwise justified. • For storage periods of less than six months, the stability data should cover the whole proposed shelf life. • Stability studies under accelerated and stress conditions, including the impact of the container closure system, should also be provided. • The stability program may be selected on the basis of a matrix system and/or by bracketing. The manufacturer should state the stability program design. • In liquid products (other than sealed ampoules), stability studies should include samples maintained in the inverted or horizontal position (i.e., in contact with the closure), as well as in the upright position, to determine the effects of the closure on product quality.

3.2.P.8.2 Post-Approval Stability Protocol and Stability Commitments	Include the stability program or stability commitment to be carried out once the drug product is on the market, including the number of batches to be included in the study each year and the tests to be performed. These results should be submitted periodically to update the information on the stability of the drug product.
3.2.P.8.3 Stability Data	Evidence should be provided to demonstrate that the product is stable for the proposed validity period under the indicated storage conditions. Stability data submitted should be for at least three batches and include the following: 1) Information on stability of drug product, quality control methods for determining stability. 2) Information on the dates of manufacture of the lots, the lot numbers, the vial and dose size, and the scale of production. 3) For lyophilized products the data supporting the shelf-life of the product following reconstitution should be included. If the drug product is frozen, data supporting the stability of the product through a stated number of freeze-thaw cycles should be provided.