



21 July 2016
EMA/CHMP/BWP/532517/2008
Committee for medicinal products for human use (CHMP)

Guideline on development, production, characterisation and specification for monoclonal antibodies and related products

3R's technical update	January 2016
Agreed by Biologics Working Party	January 2016
Adopted by CHMP	July 2016
Date for coming into effect	September 2016

* This is a technical update to reflect current best practice with regard to implementation of 3Rs approaches and it is not intended as a full revision of this guideline (only sections 1, 5.3 and 6.3 are affected). In addition, minor changes have been introduced to reflect the new Agency templates, for example addition of an Executive Summary. All these changes are considered to be minor and uncontroversial and consequently a consultation phase was considered to be unnecessary.

This guideline replaces the guideline on "Production and quality control of monoclonal antibodies" (3AB4A).

This guideline replaces the quality requirements for monoclonal antibodies set forth in the guideline on "Radiopharmaceuticals based on monoclonal antibodies" (3AQ21A).

This guideline replaces "Guideline on development, production, characterisation and specification for monoclonal antibodies and related products" (EMA/CHMP/BWP/157653/2007).

Keywords	<i>Monoclonal antibody, recombinant proteins, quality, characterisation, specification, hybridoma</i>
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Executive summary

This guideline addresses quality aspects of monoclonal antibodies for human use in the context of a marketing authorisation application. Guidance is provided in relation to development, production, characterisation and control for this class of products.

The concept of platform manufacturing is presented in the guideline to support the use, where appropriate and justified, of data derived from relevant experience.

The guideline explains the importance of characterisation and control of relevant glycosylation structures and biological activity.

This guideline does not include requirements regarding the use of specific analytical methods in order to allow flexibility in the selection of methods and take into account future technology evolutions. However, references to relevant European Pharmacopoeia monographs are present.

1. Introduction (background)

This guideline lays down quality requirements for monoclonal antibodies.

Monoclonal antibodies are immunoglobulins (Ig) with a defined specificity derived from a monoclonal cell line. Their biological activities are characterised by a specific binding characteristic to a ligand (commonly known as antigen), and may be dependent on immune effector function such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).

Monoclonal antibodies may be generated by recombinant DNA (rDNA) technology, hybridoma technology, B lymphocyte immortalisation or other technologies (e.g. display technology, genetically engineered animals).

This guideline covers principles and general requirements for development, production, characterisation and specifications for monoclonal antibodies to be used as, or in the production of, human medicinal products.

In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and Directive 2010/63/EU on protection of animals used for scientific purposes, the 3R principles (replacement, reduction and refinement) should be applied to production and control testing of medicinal products.

2. Scope

This guideline addresses quality issues for the marketing authorisation of monoclonal antibodies derived from a monoclonal cell line, and intended for therapeutic and prophylactic use (including *ex vivo* application), and *in vivo* diagnostic use.

The principles described in this document apply to monoclonal antibodies used as reagents, as well as monoclonal antibody-related products, such as fragments, conjugates, and fusion proteins. However, their applicability will be determined on a case-by-case basis, based on their specific properties, and may be addressed in specific annexes.

Polyclonal antibodies (fractionated or recombinant) are outside the scope of this guideline, although its principles should be applied where appropriate.

The scope of this guideline does not include:

- Monoclonal antibodies to be used for diagnostic purposes *in vitro*;
- Monoclonal antibodies used in clinical trials. However, the principles described in this document should be taken into account in the production and control of monoclonal antibodies in clinical trials, and their applicability will be determined on a case-by-case basis.

3. Legal basis

This guideline should be read in conjunction with the introduction and general principles and Part 2 of Annex I of Directive 2001/83/EC, as amended.

This guideline replaces the guideline on "Production and quality control of monoclonal antibodies" (3AB4a).

This guideline replaces the quality requirements for monoclonal antibodies set forth in the guideline on "Radiopharmaceuticals based on monoclonal antibodies" (3AQ21A).

This guideline replaces "Guideline on development, production, characterisation and specification for monoclonal antibodies and related products" (EMA/CHMP/BWP/157653/2007).

This guideline should be read in conjunction with all other relevant guidelines, especially those pertinent to the production and quality control of rDNA products. Furthermore, reference is made to the Ph. Eur. monograph on "Monoclonal antibodies for human use" (2031).

4. Development of the monoclonal antibody

The structure of the monoclonal antibody should be justified with respect to its mechanism of action, biological activity and stability. This justification should at least include discussion on the suitability of the product's immunochemical properties (e.g. affinity, cross-reactivity, isotype, allotype) and the importance and integrity of effector function. Furthermore, the risk of inducing antibody responses in patients should be carefully considered, especially when the product does not have a high homology with human immunoglobulin, or when potentially immunogenic epitopes are identified in the structure, as it may result in clinical adverse reactions and/or modify the therapeutic potential.

The cell substrate to be used for the production of the monoclonal antibodies should be a stable and continuous monoclonal cell line that has been developed by means of recombinant DNA and/or other suitable technologies. The rationale for selecting the cell substrate should be discussed with regards to its capability to produce the desired product quality, compared to other relevant approaches.

When the cell substrate is obtained by recombinant DNA technology, a description of the expression system used for the production of antibodies should be in accordance with relevant guidelines, especially "Production and Quality Control of medicinal products derived by recombinant DNA technology" (3AB1A), and the relevant ICH guidelines Q5A (viral safety), Q5B (expression constructs) and Q5D (cell substrates).

When one or more specific procedures are performed during development, prior to the isolation of the monoclonal cell line, such as cell fusion, viral transformation, gene library of phage display screening, application of *in silico*, *in vitro* or *in vivo* technologies, these procedures do not need to be described in great detail. However, sufficient information on these procedures should be provided to allow assessment of the identity and purity of the monoclonal cell line, when relevant to the safety and

efficacy of the product (e.g. amino acid or post-translational modifications to modulate immunogenicity or effector functions, and information regarding adventitious agents and potential contaminants).

The immortalisation of a human or non-human B-lymphocyte through cell fusion or transformation may be necessary to obtain a stable and continuous monoclonal cell line to be used for antibody production. The choice of using this approach should be cautiously considered and appropriately justified with regards to safety and efficacy.

The use of human B-lymphocytes as parental cell lines raises specific concerns with respect to the transmission of infectious agents, including the agent causing variant Creutzfeldt-Jakob disease (vCJD), as well as other human pathogens. The use of human lymphocytes transformed with Epstein-Barr virus (EBV) raises further concerns due to the presence of infectious EBV.

Hybridoma cell lines obtained through the fusion of human or non-human B-lymphocytes with myeloma cells may be used as cell substrates. The origin and characteristics of the parental cell should be documented, including information on the health history of the donors, the fusion partner used and the materials of human or animal origin to which it has been exposed (e.g. feeder cells and myeloma cells).

5. Production of monoclonal antibodies

5.1. 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.

5.2. 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 authorisation application. Validation studies should include data derived from the final manufacturing process and site(s) used to produce the product to be commercialised. 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.

5.3. *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" (EMA/410/01) should be consulted.

6. Characterisation 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.

6.1. Physicochemical characterisation

A physicochemical characterisation 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, mass spectrometry analysis). The variability of N- and C- terminal amino-acid sequences should be analysed (e.g. C-terminal lysine(s)).

Free sulphhydryl 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 characterised, 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 characterised by appropriate physicochemical methodologies.

6.2. Immunological properties

The immunological properties of the antibody should be fully characterised. 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. Crossreactivity with a range of human tissues should be determined using immunohistochemical procedures (see Annex I). 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 characterisation 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.

6.3. 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.

6.4. 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 analyse heterogeneity related to charged variants.

Multimers and aggregates should also be appropriately characterised 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.

6.5. 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.

7. Specifications

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.

7.1. Identity

The identity test(s) should be highly specific and should be based on unique aspects of the product's molecular structure and/or other specific properties (e.g. peptide map, anti-idiotypic immunoassay, or other appropriate method). 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.

7.2. 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.

7.3. 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 substance and/or drug product, and should ideally reflect the biological activity in the clinical situation.

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.

7.4. Quantity

The quantity of the drug substance, usually based on protein content (mass), should be determined using an appropriate assay.

7.5. General tests

Appearance, solubility, pH, osmolality, extractable volume, sterility, bacterial endotoxins, stabiliser and water, should be assessed where appropriate.

Visible and sub-visible particulate matter in drug product should comply with the requirements set forth in the European Pharmacopoeia.

8. Monoclonal antibody-related products

In addition to intact, non-modified monoclonal antibodies, the scientific principles described in this document can be applied to other monoclonal antibody related products, such as antibody fragments (including single-chain variable fragment (scFv)), fusion proteins, conjugated monoclonal antibodies, bispecific antibodies and radiolabelled antibodies. However, their applicability will be determined on a case-by-case basis, based on the specific properties of the product.

Additional monoclonal antibody-related product specific annexes will progressively be developed and will be made available on the EMA website, as appropriate.

References

- ICH Q5A (R1) "Viral safety Evaluation of Biotechnology Products derived from Cell Lines of Human or Animal Origin" (CPMP/ICH/295/95)
- ICH Q5B "Analysis of the Expression Construct in Cell Lines used for Production of r-DNA derived Protein Products" (CPMP/ICH/139/95)
- ICH Q5D "Derivation and Characterisation of Cell Substrates used for Production of Biotechnological/Biological Products" (CPMP/ICH/294/95)
- ICH Q5E "Comparability of Biotechnological/Biological Products subject to Changes in their Manufacturing Process" (CPMP/ICH/5721/03)
- ICH Q6B "Test Procedures and Acceptance Criteria for Biotechnological/Biological Products" (CPMP/ICH/365/96)
- Note for Guidance on "Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products" (EMA/410/01)
- Guideline on "Immunogenicity Assessment of Biotechnology-derived Therapeutic Proteins" (CHMP/BWP/14327/06)
- Guideline on "Strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal products" (EMA/CHMP/SWP/28367/2007)
- Ph. Eur. Monograph on "Monoclonal antibodies for human use" (2031)
- Ph. Eur. Monograph on "Human normal Immunoglobulin" (0338)
- Ph. Eur. Monograph on "Parenteral preparations" (0520): 2.9.19. Particulate contamination: sub-visible particles (20919)
- Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the "Community code relating to medicinal products for human use", as amended

Annex - suggested list of human tissues to be used for immunohistochemical or cytochemical investigations of cross reactivity of monoclonal antibodies.

This list should reflect the specificity of the antibody and its particular use.

- Tonsil, thymus, lymph node;
- Bone marrow, blood cells;
- Lung, liver, kidney, bladder, spleen, stomach including underlying smooth muscle, intestine,
- Pancreas, parotid, thyroid, parathyroid, adrenal, pituitary;
- Brain, peripheral nerve;
- Heart, striated muscle;
- Ovary, testis;
- Skin;
- Blood vessels.