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**DRAFT**

# GUIDELINE ON PRODUCTION AND QUALITY CONTROL OF MONOCLONAL ANTIBODIES AND RELATED SUBSTANCES

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

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

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# GUIDELINE ON PRODUCTION AND QUALITY CONTROL OF MONOCLONAL ANTIBODIES AND RELATED SUBSTANCES

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## EXECUTIVE SUMMARY

This guideline lays down the quality requirements for monoclonal antibodies and related substances in medicinal products. Furthermore, requirements for monoclonal antibodies and related substances used as reagents in the production of other medicinal products are discussed.

## 1. INTRODUCTION (background)

Monoclonal antibodies are immunoglobulins (Ig) with a defined specificity derived from a monoclonal cell line.

The classical method for obtaining monoclonal antibodies is the hybridoma technology, through fusing B cells from immunised mice with myeloma cells, followed by clonal expansion. It is currently state of the art to humanise monoclonal antibodies by recombinant DNA (rDNA) technology and express the monoclonal antibody in a recombinant cell culture system. In addition, a number of innovative methods to obtain monoclonal antibodies have been developed, for example those based either on display technology (phage, ribosome, yeast) or genetically engineered mice that produce human antibodies.

The field of monoclonal antibodies has matured. In the last years, several medicinal products with a monoclonal antibody as the active substance have successfully obtained a marketing authorisation. Moreover, a high percentage of proteins currently in phase II and III clinical studies are monoclonal antibodies. Finally, monoclonal antibody-related substances are being developed, such as fusion proteins, proteolytic fragments, single-chain variable fragments (scFv) and other fragments, and bispecific monoclonal antibodies (BsAb).

## 2. SCOPE

In this guideline, specific requirements for monoclonal antibodies for therapeutic and prophylactic use (including *ex vivo* application) and *in vivo* diagnostic use are described. In addition, requirements for monoclonal antibodies used as reagents, especially in the purification of other pharmaceutical products are described.

Besides monoclonal antibodies, the scope includes all monoclonal antibody-related substances, such as fragments, conjugates, and fusion proteins. It is noted that new classes of substances related to monoclonal antibodies are regularly being developed. The scientific principles of this guideline are relevant to these products. However, their applicability will be determined on a case-by-case basis, based on the specific properties of such a substance.

Monoclonal antibodies to be used for diagnostic purposes *in vitro* are outside the scope of this guideline.

Polyclonal antibodies are outside the scope of this guideline, although some principles may apply.

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*" (07/2005:2031).

## 3. LEGAL BASIS

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

## 4. MAIN GUIDELINE TEXT

### 4.1. INTRODUCTORY REMARKS

Monoclonal antibodies are characterised by a specific structure, which is based on the immunoglobulin structure, and a clearly defined functional activity, which is primarily based on a specific binding characteristic to a ligand (commonly known as the antigen).

Based on their structure, monoclonal antibodies can be non-human, chimeric/humanised or human antibodies.

Non-human monoclonal antibodies are mainly of murine origin, derived from hybridoma cell lines. A general problem with the therapeutic use of murine monoclonal antibodies in human is the induction of antibodies in the recipient against murine immunoglobulin (human anti-murine antibody or HAMA response). This may result in clinical adverse reactions. As a consequence, the use of antibodies with a full non-human sequence should be avoided, unless otherwise justified.

Human monoclonal antibodies are antibodies of entirely human sequence.

Chimeric monoclonal antibodies are monoclonal antibodies in which the variable heavy and light chain domains of a human antibody are replaced by those of a non-human species, which possess the desired antigen specificity. Humanised monoclonal antibodies are antibodies in which the three short hypervariable sequences (the complementary determining regions or CDRs) of non-human variable domains for each chain are engineered into the variable domain of a human antibody.

In addition to the full-length, non-modified monoclonal antibodies, there are other monoclonal antibody related substances that fall within the scope of this Guideline, such as antibody fragments (including scFv), fusion proteins, bispecific antibodies, conjugated monoclonal antibodies or radiolabelled antibodies.

This guideline covers the development, production, characterisation, formulation, comparability and specifications for monoclonal antibodies to be used as, or in the production of, human medicinal products. The principles and requirements for intact monoclonal antibodies will be addressed first, followed by recommendations for monoclonal antibody-related substances. The extent of these recommendations will be based on their specific properties: for example, requirements relating to the Fc part of a monoclonal antibody (including effector functions) are not relevant for Fab fragments and are therefore not applicable.

The final section deals with monoclonal antibodies used as reagents in the manufacture of other human medicinal products.

For preclinical investigations in animals, homologous monoclonal antibodies (monoclonal antibodies which recognise the same epitope but have a different structure, for example murine or simian instead of human IgG) are sometimes developed. Although using homologous monoclonal antibodies may be the only feasible way to perform pharmacological studies in animals, and the results may have scientific value, it should be noted that the use of these data is limited by the fact that quality characteristics of the clinical and homologous monoclonal antibody are different, and that it is therefore difficult to extrapolate the data obtained.

### 4.2. GENERATION OF THE MONOCLONAL CELL LINE

It is currently state of the art to humanise monoclonal antibodies by rDNA technology and express the monoclonal antibody in a recombinant cell culture system. For monoclonal antibodies produced by recombinant DNA technology, the guidelines on proteins from rDNA technology apply, 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).

### 4.2.1. Hybridoma

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.

Transformation of human lymphocytes with Epstein-Barr virus (EBV) has been used for many years to produce continuous, rapidly growing human B cells for antibody production. The presence of infectious EBV virus poses specific safety concerns. In addition, the use of continuous human B-lymphocyte as parental cell lines raises specific concerns with respect to the transmission of infectious agents including the agents causing variant Creutzfeldt-Jakob disease (vCJD), as well as other human pathogens. As a consequence, the choice of human cell lines (EBV-transformed or not) should be cautiously considered and appropriately justified with respect to these aspects.

Non-human B-lymphocytes may also be used to obtain the cell substrate. Hybridomas are normally cell lines created through the fusion of murine B-lymphocytes with myeloma cells to achieve immortalisation. Source cells include lymphocytes, myeloma cells, feeder cells and host cells for the expression of the monoclonal antibody. The origin and characteristics of the parental cell should be documented, including information on the health of the donors, and on the fusion partner used. Hybridoma cell lines are acceptable expression systems for monoclonal antibodies; however the choice of such system should carefully take into consideration the antigenicity issues related to non-human antibodies, as well as the viral safety issues related to the cell lines.

Ascites was used in the past as a production system for hybridoma-derived monoclonal antibodies. Its use is discouraged in view of the additional viral safety issues and in light of the principles of Directive 86/609/EC, which seeks to reduce, refine, and replace the use of animals

### 4.2.2. Recombinant DNA technology in antibody production

A description of the expression system used for the production of antibodies including the construction and characterisation of the rDNA expression vector and the parental cell line should be provided.

When one or more specific procedures, such as cell fusion, viral transformation, gene library or phage display screening, are performed prior to the isolation of the gene of interest to be used to obtained the production clone, these preceding procedures do not need to be described in great detail. However appropriate information on the source and cloning of the genes should be provided.

### 4.2.3. Other technologies

Other technologies, such as the development of transgenic mice to generate human B-lymphocytes or phage display, may be used to generate the monoclonal cell line. When such a technology is directly used to obtain the monoclonal cell line, appropriate details should be provided to allow the assessment of the identity and purity of the monoclonal cell line, especially with regard to adventitious agents and other potential contaminants (for example contaminant cell line).

## 4.3. PRODUCTION OF MONOCLONAL ANTIBODIES

### 4.3.1. General considerations

All parts of the drug substance manufacturing process (cell culture, purification, etc) should be fully validated. The following aspects deserve specific attention during validation:

- consistency of production with respect to heterogeneity;

- adequate removal of product- and process-related impurities (for example host cell protein (HCP)

and DNA, protein A, antibiotics, cell culture components);

- compliance with the requirements for the bacterial endotoxins test. In addition, where the manufacturing process itself or the data from the analysis of product- and process-related impurities indicates the likelihood of the presence of non-endotoxin contaminants, such as peptidoglycan, a monocyte activation test for pro-inflammatory and pyrogenic contaminants might provide valuable information. Such a test would also be expected to identify unwanted intrinsic activities secondary to the release of pro-inflammatory cytokines.

During development and validation, particular attention should be paid to genetic stability, optimal and validated time for fermentation and harvest (yield, product quality), etc.

With regard to the purification, protein A chromatography in particular is an almost universal part of a purification process, although other approaches are possible. Protein A is obtained from *S. aureus* or from recombinant sources. It may be purified with the use of human IgG purified from blood, in which case the quality of human IgG needs to be appropriately documented for the intended use (preferably by confirming compliance to the Ph. Eur. monograph on "*Human Normal Immunoglobulin*" (01/2007:0338).

### 4.3.2. Platform manufacturing

The structural and physiochemical characteristics of monoclonal antibodies are now well understood and together with many years of antibody process development and production in industry, a concept whereby different monoclonal antibodies are manufactured using an identical, pre-defined purification process has evolved. This concept is termed "platform manufacturing." However, this term does not represent identical purification processes throughout industry; it is rather used to describe each manufacturer's own unique "platform manufacturing" processes.

From the viewpoint of process development, a "platform manufacturing" approach can be accepted, because it is not necessary to develop each new manufacturing process from scratch, instead it is based on previously developed in-house knowledge and may also be appropriate to make efficient use of available or shared equipment.

However, each producer cell line (even if it is derived from the same parent cell line and a similar genetic construct is used) is likely to have different properties. Therefore, any product-specific process should be duly optimised and validated in its own right. This also applies to process changes.

The "platform manufacturing" process will never be identical for each monoclonal antibody and interference by the product cannot be excluded beforehand. Therefore each process should be separately validated for its ability to remove viruses. However, for a new product, the manufacturer may partly rely on viral validation data obtained with other products manufactured with the same "platform manufacturing" process. Such data may be considered supportive but the manufacturer will need to justify the relevance of the data and demonstrate that virus validation data obtained from the new product is comparable to data obtained for other products. With respect to data relating to virus carry-over and cleaning procedures, this may be extrapolated across from the same "platform manufacturing" processes provided this can be fully justified.

If a change is made to the whole "platform manufacturing" process, then the effects for each concerned product should be evaluated separately. In principle, revalidation of the process for each separate product should be performed, unless it can be justified that results can be extrapolated across the platform. Such an extrapolation might be successful if partial validation data for each separate product are obtained and demonstrate an equivalent effect of the change.

Simultaneous submission of related Variations to the Marketing Authorisation for several products is recommended to highlight changes to the "platform manufacturing" process. Each variation should contain a comprehensive data package, including relevant validation data obtained with other monoclonal antibodies if the Marketing Authorisation Holder wants to extrapolate these data to the monoclonal antibody for which the variation is submitted.

”Platform assays” are equally acceptable; however, for each product at least a partial revalidation to demonstrate suitability for the intended purpose should be performed (for example a platform assay for HCP might be feasible, especially if the parent cell line is identical for several products, but it should have the same sensitivity and specificity for HCPs from different producer cell lines.

#### 4.3.3. 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 (including when grown *in vivo* as ascites) or from cells genetically engineered to express a monoclonal antibody. Monoclonal antibodies derived from genetically engineered mice should also comply with ICH Q5A. The importance of good viral validation studies is emphasised. Source cells should undergo suitable screening for extraneous agents and endogenous agents. The choice of viruses for the tests is dependent on the species and tissue of origin.

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.

### 4.4. CHARACTERISATION OF MONOCLONAL ANTIBODIES

The contents of this section apply to monoclonal antibodies and antibody related substances as described under 4.7 produced both by rDNA and hybridoma technologies for *in vivo* use. However, characterisation and control requirements for monoclonal antibodies not used *in vivo* but in purification are described in chapter 4.8.

The monoclonal antibody should be characterised thoroughly. This characterisation must include both biochemical/physicochemical and biological/immunological properties of the antibody. In addition, the specificity and crossreactivity of the monoclonal antibody should be assessed.

A state-of-the-art characterisation should be performed in line with the ICH Q6B Guideline, which addresses the primary and higher-order structure and the physicochemical properties of the product. The primary sequence should be deduced by DNA sequencing and confirmed experimentally by peptide mapping and amino acid sequencing.

In addition to what is covered under sections 4.5 and 4.6, the following (additional) parameters may be specifically relevant for monoclonal antibodies:

#### 4.4.1. Physicochemical characterisation

##### 4.4.1.1. Structure

Characterisation should start with the determination of class, subclass, light chain composition and determination of the amount of half antibody molecules in case of a monoclonal antibody belonging to the IgG4 subclass.

##### 4.4.1.2. Heterogeneity

Monoclonal antibodies are subject to post-translational modifications or degradation at several independent sites. Such modifications may result in the presence of many different species in the final product. Monoclonal antibodies therefore display considerable heterogeneity that can be characterised by several orthogonal methods for example isoelectric focussing (IEF), ion exchange chromatography (IEC), or capillary electrophoresis (CE). The monoclonal antibody should be characterised as much as possible using relevant methods and batch-to-batch consistency with respect to heterogeneity should be shown. Because the immunoglobulin molecule is relatively robust, many of these species will have full bioactivity *in vitro*. When using certain methods (for example state-of-the art liquid

chromatography (LC) or CE methods), these species can be seen as many poorly resolved peaks. The Applicant should characterise the possible discrete modifications and the major peaks seen in chromatograms; however, a full identification of all the different minor species will often not be feasible.

A form of heterogeneity very specific for monoclonal antibodies is C-terminal charge heterogeneity. Lysine residues from the C-termini are often partially or completely removed by a carboxypeptidase B-like activity. The extent of Lys-removal should be addressed.

#### 4.4.1.3. Glycosylation

Typically, monoclonal antibodies have only one N-glycosylation site, on each heavy chain located in the Fc region, and the light chain is not glycosylated. The number of glycan structures found in most IgGs is limited and are primarily biantennary with so-called G0, G1, and G2 glycan structures which have a variable presence of terminal galactose. All glycan structures present should be fully characterised, paying attention to the degree of sialylation.

### 4.4.2. Biological/immunological characterisation

The immunological properties of the antibody should be described in detail and should include:
- antigenic specificity including the characterisation of the epitope that the antibody recognises;
- affinity and Kd;
- ability for complement binding and activation and other effector functions;
- cytotoxic properties, antibody-dependent cytotoxicity;
- the paratope (the part of the monoclonal antibody that recognises and binds to the epitope) should be identified;
- the immuno-reactivity of the antibody.

The specific activity of the purified monoclonal antibody should be determined (units of activity/mass of product).

### 4.4.3. Specificity and crossreactivity

The epitope (amino acid sequence or equivalent structural moiety) recognised by the monoclonal antibody should be determined. The analysis should further include the determination of unintentional reactivity with or cytotoxicity for human tissues distinct from the intended target, and crossreactivity with a range of human tissues by immunohistochemical procedures.

The following list of human tissues to be used for immunohistochemical or cytochemical investigations of crossreactivity of monoclonal antibodies is suggested: tonsil, thymus, lymph node, bone marrow, blood cells, lung, liver, kidney, bladder, spleen, stomach including underlying smooth muscle, intestine, pancreas, paratid, thyroid, parathyroid, adrenal, pituitary, brain, peripheral nerve, heart, striated muscle, ovary, testis, skin, and blood vessels.

### 4.4.4. Formulation

High amounts of monoclonal antibody are often necessary to obtain a therapeutic effect, and therefore the amounts of monoclonal antibody protein in the drug product are higher than for other biotechnological products (often > 100 mg/vial). Because of the high amounts and a natural tendency for immunoglobulins to aggregate, the formation of sub-visible and visible particulates (including aggregates) in the drug product is a significant issue. These particulates may be present at release or develop during storage. The presence of such visible particulates is unwanted. In addition, the drug product should always comply with the requirements set forth in the Ph. Eur. Monograph on "*Parenteral preparations*" (07/2005:0520): *2.9.19. Particulate contamination: sub-visible particles* (01/2005:20919) and other pharmacopoeial requirements on visible particles. Appropriate formulation studies should be performed to find an optimal formulation that is stable with respect to formation of particulates at release and during storage.

### 4.4.5. Filling based on mass (protein concentration)

Filling and dosing based on mass (in mg instead of Units) is a common approach. Labelling in Units is not always meaningful, unless these Units refer to a clinically relevant and accepted parameter or if labelling is stated in International Units, referring to an International Standard (for example Ph. Eur.). Furthermore, filling, dosing and labelling based on mass has the advantage of being more precise.

## 4. 5. SPECIFICATIONS

Appropriate release and shelf life specifications for both the drug substance and drug product should be defined in line with the principles described in ICH Q6B. Some issues specific to monoclonal antibodies are discussed below.

### 4.5.1. Identity

The determination of identity presents specific difficulties due to the great similarity of the constant domains of different antibodies. Identity can be determined either by one very specific test (e.g. peptide mapping) or by a combination of tests with sufficient specificity (e.g. a specific ELISA which also determines potency).

However, the Applicant should justify that identity is indeed demonstrated by the tests included in the specification, and that mix-ups with other monoclonal antibodies can be ruled out. The latter aspect in particular is important for manufacturers that produce several monoclonal antibodies at the same facility.

### 4.5.2. Potency

The potency/biological activity of the monoclonal antibody should be established by a biological assay. It is preferable to establish a link to a clinical relevant parameter.
With regard to potency testing, it is relevant to consider if the mode of action of the antibody only involves binding/neutralisation or if the effector functions are part of the therapeutic effect. For binding/neutralising antibodies, a potency assay that measures binding (an immunoassay for example in an ELISA format) might be deemed appropriate.
If effector functions are relevant, then a cell-based bioassay or another assay that takes effector functions duly into account is to be preferred. A combination of two separate methods, one measuring the specificity and one giving an indication of an effector function (for example complement activation), would be acceptable if a single cell-based assay is not feasible or if the combination of two methods gives more precise results.
Although the two types of potency assays (ELISA or cell-based) often yield comparable results, the two assays cannot be deemed interchangeable, because there are parameters that do not affect binding but which may affect effector functions (for example glycosylation, fragmentation).
Specific activity (biological activity per unit of mass) is of considerable value to demonstrate consistency of production.

### 4.5.3. Glycosylation

Glycosylation may influence:

- The binding capacity of the Fc region to specific receptors (for example Fc gIIIa and others) and a consistent glycosylation pattern are important for antibodies since triggering or modulating specific responses of various cells of the immune system may be an intrinsic part of their pharmacological effect.

- Immunogenic properties (especially if immunogenic structures like 1,3-α-gal sugars are detected).

- Pharmacokinetic and pharmacodymanic properties (including plasma half-life) *in vivo.*

Therefore, a specification for glycosylation (at least aimed at controlling the amount of G0, G1 and/or G2 relative to the total amount) should always be set. In addition, such a specification will function as an independent check on manufacturing consistency.

### 4.5.4. Consistency of heterogeneity

As noted in the characterisation section, monoclonal antibodies may display significant heterogeneity. The specification should include suitable tests to check consistency of manufacture with respect to heterogeneity, for example based on charge heterogeneity, such as capillary zone electrophoresis/IEF, IEC and/or by checking for the extent of a certain discrete modification. It is neither possible nor meaningful to have a limit for each of the possible species that can be present. However, the specification should assure that the mixture of the species has a consistent pattern.

### 4.5.5. Process-related impurities

In general, specifications for process-related impurities should be considered in relation to all reagents. For monoclonal antibodies, a specification for residual protein A should be considered, based on the principle that it is both a relevant process-related impurity and an independent consistency check of the performance of the purification process.

The amount of residual DNA should be tested, when (potentially tumorigenic) hybridomas are used for the production of the monoclonal antibody. A specification should be set based on batch analysis data and the WHO recommendation of no more than 10 ng/dose. For CHO and similar cell lines, a validation approach would in general be acceptable.

### 4.5.6. Other release tests for the drug substance and drug product

Besides pharmacopoeial and other appropriate tests as outlined above and in ICH Q6B, tests for the following parameters should be included:

-Molecular-size distribution (for example by size-exclusion high-performance liquid chromatography (SEC-HPLC));

- The structural integrity of modified (conjugated) monoclonal antibodies requires special consideration;

-For IgG4 isotype monoclonal antibodies, a test for the amount of half antibody should normally be included.

Appropriate tests can be identified from the results of the characterisation of the drug substance, as described in section 4.4 of this Guideline.

The analytical determination of particulate matter in the drug product raises difficult issues in practice. SEC-HPLC methods may not be suitable, visible particulates can be trapped in the void volume; or they may disintegrate due to shearing when the sample is injected. Analytical ultracentrifugation may be more suitable to isolate and characterise particulates. As noted in section 4.4.4., the presence of particulate matter should be prevented by acceptable formulation development.

## 4.6. COMPARABILITY

The issue of comparability after process changes is discussed in the ICH Q5E Guideline.
The characteristics which are specific for monoclonal antibodies, as described above, also deserve special attention when assessing comparability of monoclonal antibodies after a process change. Heterogeneity and glycosylation in particular require attention. In general, monoclonal antibodies are fairly robust and changes in the structure may not affect pharmacological properties *in vitro* (for example binding to epitope, effector functions like activation of Fc receptors), although they may influence pharmacokinetic properties and safety/immunogenicity *in vivo*. However, if there are theoretical or experimental indications that changes do affect pharmacological parameters, those should be fully assessed. If effector functions of the monoclonal antibody are part of the mechanism of action, then these should be fully re-assessed as part of a comparability exercise.

## 4.7. MONOCLONAL ANTIBODY-RELATED SUBSTANCES

In addition to the intact, non-modified monoclonal antibodies, there are other monoclonal antibody related substances that fall within the scope of this guideline. These include:

- antibody fragments (including scFv);
- fusion proteins;
- conjugated monoclonal antibodies;
- bispecific antibodies;
- radiolabelled antibodies.

Other modifications or combination of modifications are possible. The scientific principles described in this guideline are directly relevant for such monoclonal antibody-related substances. However, their applicability will be determined on a case-by-case basis, based on the specific properties of the substance.

Modified monoclonal antibodies may be obtained either as a different gene product (for example scFv) or through (bio)chemical modification after fermentation and purification, or both (for example bispecific antibodies).

If the antibody fragment is produced as an insoluble and inactive protein (inclusion bodies), a refolding step is required to recover the functional structure of the molecule. In that case, special attention should be given to process validation in order to ensure correct refolding and disulphide bond formation as well as the absence of misfolded and/or aggregated species from the final product. A relevant specification to control misfolded species or aggregates should be considered.

If a peptide tag (for example $His_n$-tag) fused to the antibody fragment for purification purposes is not cleaved off from the final product, attention should be given to its effect on both immunogenicity and folding of the antibody molecule. If such a peptide tag is cleaved off by proteolysis, the considerations described below for proteolytic fragments are relevant and should be followed.

If monoclonal antibodies are modified by chemical or biochemical means after a primary fermentation and purification stage, then the purified but unmodified monoclonal antibody is a critical intermediate, which should comply with an appropriate specification. In that case, the quality dossier should include a full description of the production and control of the unmodified monoclonal antibody.

### 4.7.1. Antibody fragments

Antigen-binding antibody fragments such as Fab fragments, scFv antibodies and other Fv fragments and their multivalent forms are minimised antibodies that retain the antigen-binding site and specificity but lack the Fc part of the antibody molecule. Antibody fragments can either be produced by recombinant methods or by proteolytic digestion of whole monoclonal antibodies.

#### 4.7.1.1. Recombinant fragments

Recombinant antibody fragments can be created directly by cloning the corresponding antibody genes from a monoclonal antibody derived from a hybridoma or they can be isolated from antibody gene libraries using different display platforms (phage, ribosome, yeast).

#### 4.7.1.2. Proteolytic fragments

Fragmentation can be performed by enzymatic digestion (papain or pepsin for Fab or $F(ab')_2$ fragments, respectively). For proteolytic fragments, a detailed description and validation of the digestion and subsequent purification should be given. The removal of the enzymes used for fragmentation should be validated.

### 4.7.2. Fusion proteins

For the purpose of this Guideline, a fusion protein is expressed as a single protein whose gene contains part or all of the IgG coding sequence along with part or all of a sequence encoding a distinct protein. A fusion protein should be differentiated from a modified monoclonal antibody, in which the

monoclonal antibody and another protein are coupled after they have been manufactured by separate processes (see section 4.7.3.).

### 4.7.3. Conjugated monoclonal antibodies

The therapeutic and diagnostic uses of monoclonal antibodies and antibody fragments can be enhanced by chemical conjugation to another compound or matrix with the desired properties, for example:

- Conjugation with a toxin or another protein;

- Conjugation with a chemical moiety for radiolabelling, targeting or prolonged half life such as pegylation;

- Chemical linking of two antibody molecules or their derivatives to generate a bispecific antibody.

For conjugated antibodies, a detailed description of their preparation and purification should be provided. The consistency of the modification should be validated. The removal of process-related impurities requires special attention because the modification is often performed at the final stage of the manufacturing process.

The site(s) of conjugation should be determined, if feasible. The stability of the conjugate should also be studied. As part of the characterisation studies, the effect of conjugation on the biological activity should be determined.

If conjugation at more than one site of the monoclonal antibody is possible, the effect of various extents of conjugation on the biological activity should be investigated. The specification should normally include a test for determining the extent of conjugation and the amount of free monoclonal antibody and conjugate remaining in the preparation as product-related impurities.

### 4.7.4. Bispecific antibodies

BsAb are antibody-based molecules that can simultaneously bind two different antigens or two different epitopes of the same antigen. BsAb may be produced from a cell line (by fusion of two hybridomas or by DNA co-transfections), or obtained by chemical conjugation of two antibodies, fragments or scFv.

### 4.7.5. Radiolabelled antibodies

Reference is made to the guideline on "*Radiopharmaceuticals*" (3AQ20A), which is currently under revision.

Monoclonal antibodies may be radiolabelled directly, but normally monoclonal antibodies are conjugated with a chelating group (see section 4.7.3.) that will bind the radiolabel with high affinity. The properties (both physicochemical and immunological) of the radiolabelled complex should be sufficiently characterised; this may be done with a 'cold' (non-radioactive) label. If the radiolabelling is performed by the manufacturer, this process should be validated and subject to batch release. If the radiolabelling is to be done by the end user, the manufacturer should validate that labelling, purification (if necessary) and control can be performed according to clear, precise instructions in the product information. Preferably, the manufacturer should present the product as a kit for radiolabelling containing all the components (monoclonal antibody, buffer, etc), except for the 'hot' radiolabel.

## 4.8. MONOCLONAL ANTIBODIES USED AS REAGENTS

A monoclonal antibody can be used as a reagent in the production of other medicinal products (including cell-based products), especially for purification because they bind to a protein with high selectivity and specificity.

For monoclonal antibodies used in purification, the following points should be addressed:

- The production should be described and validated. The most important aspects are consistency with regard to functional aspects and absence of adventitious agents.

- The monoclonal antibody should be sufficiently characterised, especially with regard to its functional properties, i.e. its binding to the product to be purified. This binding should be quantitatively characterised.

- The immobilisation reaction and its effect on binding of the ligand to the antibody should be studied. If feasible, the amino acid residues of the monoclonal antibody coupling to the resin should be determined.

- Control/release testing: both the free monoclonal antibody and the immobilised monoclonal antibody should be tested for functional aspects.

- Stability of the monoclonal antibody should be demonstrated especially after it has been immobilised. If monoclonal antibody columns/resins are reused, reuse studies should be considered and the effect of use, sanitisation, and regeneration should be studied. Leaching of the monoclonal antibody from the columns should be evaluated, and a specification of leached monoclonal antibody in the product which is purified should be considered.

- The production process should sufficiently ensure that no virus is introduced into a product by using a monoclonal antibody as reagent. However, it is acknowledged that the coupling reaction, and the subsequent purifications and regenerations of the column will add a further theoretical margin to the viral safety of a product purified with a monoclonal antibody column.

The use of ascites as a production system is discouraged in light of the principles of Directive 86/609/EC which seeks to reduce, refine, and replace the use of animals.

**DEFINITIONS**

- Monoclonal antibody: an immunoglobulin with a defined specificity derived from a monoclonal cell line. For the purpose of this Guideline, closely related substances (for example fragments, fusion proteins) shall be considered monoclonal antibodies.

- $F(ab)_2$: an antigen-binding bivalent (or bispecific) antibody fragment that consists of two Fab fragments linked covalently together. $F(ab)_2$ fragments can be produced from the intact monoclonal antibody by enzymatic (pepsin) digestion or by chemical conjugation of two Fab fragments.

- Fab: antigen-binding monovalent antibody fragment that consists of the light chain and the two first domains of the heavy chain linked together by disulphide bond. A Fab fragment can be produced either from the intact monoclonal antibody by enzymatic (papain) digestion or by rDNA technology. A Fab fragment can be created by cloning the corresponding antibody genes from a monoclonal antibody or it can be isolated from an antibody display library.

- Fv: an antigen-binding antibody fragment that consists of the variable domains of the heavy and the light chains non-covalently linked together. Fv fragments are produced by rDNA technology.

- ScFv: antigen-binding single-chain antibody fragment that consists of the variable domains of the heavy and the light chains linked together by a flexible polypeptide. A scFv is produced using rDNA technology by cloning the corresponding antibody genes from a monoclonal antibody or it can be isolated from an antibody display library.

- Fusion protein: for the purpose of this Guideline, a fusion protein is a protein which contains parts of the IgG molecule, merged with another molecule by rDNA technology, and expressed as one protein by a cell line.

## REFERENCES (SCIENTIFIC AND / OR LEGAL)


- 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*" (EMEA/410/01)

The above-mentioned guidelines and other relevant guidelines are available at the following address on the EMEA website: http:/www.emea.europa.eu/htms/human/human guidelines/biologicals.htm

- Draft Guideline on "*Requirements for first-in-man clinical trials for potential high-risk medicinal products*" (EMEA/CHMP/SWP/28367/2007)

- Concept Paper on the "*Revision of the Note for Guidance on Radiopharmaceuticals*" (CHMP/92877/2005)

Ph. Eur. monograph on "*Monoclonal antibodies for human use*" (07/2005:2031)

Ph .Eur. monograph on "*Human Normal Immunoglobulin*" (01/2007:0338)

Ph. Eur. Monograph on "*Parenteral preparations*" (07/2005:0520): *2.9.19. Particulate contamination: sub-visible particles* (01/2005:20919)

- 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

- Council Directive 86/609/EC of 24 November 1986 on the "*Approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other specific purposes*"