PRODUCTION AND QUALITY CONTROL OF MONOCLONAL ANTIBODIES

Guideline Title	**Production And Quality Control Of Monoclonal Antibodies**
Legislative basis	**Directive 75/318/EEC as amended**
Date of first adoption	**See previous titles/other references** **This version adopted December 1994**
Date of entry into force	**July 1995**
Status	**Last revised December 1994**
Previous titles/other references	**Originally published as two guidelines: *Production and Quality Control of Human Monoclonal Antibodies* (July 1990) and *Production and Quality Control of Monoclonal Antibodies of Murine Origin* (June 1987). The previous reference of the combined version was III/5271/94**
Additional Notes	**This guideline outlines the requirements for murine, human and engineered monoclonal antibodies for therapeutic (including ex vivo application) and in vivo diagnostic use in humans. It concerns the application of Part 2, sections A, B, C, D and E of the Annex to Directive 75/318/EEC as amended with a view to the granting of a marketing authorisation for a new medicinal product.**

CONTENTS

PRODUCTION AND QUALITY CONTROL OF MONOCLONAL ANTIBODIES

1. INTRODUCTION

In this document the requirements for murine, human and engineered monoclonal antibodies for therapeutic (including ex vivo application) and in vivo diagnostic use in humans are outlined. Monoclonal antibodies intended for use in the purification of other products should be shown to be pure and free from adventitious agents by the methods described. Monoclonal antibodies to be used for diagnostic purposes in vitro are not the concern of this note for guidance.

Monoclonal antibodies are antibodies with a defined specificity derived from cloned cells or organisms. They can be obtained from immortalised B lymphocytes that are cloned and expanded as continuous cell lines (murine and human monoclonal antibodies) or from rDNA-engineered mammalian or bacterial cell lines (engineered monoclonal antibodies).

Important considerations for the clinical use of monoclonal antibodies include the possible unintentional immunological cross-reactivity of the antibody with human tissue antigens other than those desired, and the possible presence of viruses in the products.

1.1 Monoclonal antibodies of murine origin

Murine monoclonal antibodies are obtained from murine hybridomas produced by fusion of B-lymphocytes from immunised mice or rats with murine myeloma cells.
A general problem with the therapeutic use of murine monoclonal antibodies in man is the possible induction of antibodies in the recipient against murine immunoglobulin (**h**uman **a**nti **m**urine **a**ntibody or HAMA response). This may result in adverse reactions and limit the duration of effective antibody therapy. In addition the in vivo half life of murine monoclonal antibodies is relatively short. It may be prudent to minimise the murine protein load administered to the patient by the use of a parental myeloma cell lines which does not itself synthesise immunoglobulin chains.

1.2 Human monoclonal antibodies

The advantages of human monoclonal antibodies over murine monoclonal antibodies are that human recipients are less likely to develop antibodies against them (although anti-idiotypic and possibly anti-allotypic antibodies may still be produced) and that human antibodies are likely to have the full range of biological functions, such as those of the Fc region which may be species specific. There may be other advantages such as selection of a subclass of antibody with particular properties.

Murine monoclonal antibodies are almost always prepared using cell lines (hybridomas) made by fusion of lymphocytes from an immunised donor with myeloma cells. This is not the case for human monoclonal antibodies as, despite encouraging early reports, there is still no really satisfactory human myeloma fusion partner. As a result, a major difficulty with the production of human monoclonal antibodies has been the generation of hybridoma lines of acceptable stability. It is also difficult in many cases to obtain antigen-primed

lymphocytes suitable for fusion. In view of this, a number of alternative strategies have been devised for production of human monoclonal antibodies. These are:

a) Fusion of human lymphocytes (usually peripheral blood or lymph-node derived) with a murine myeloma or hybrid human-murine myeloma line. This procedure is essentially similar to the hybridoma technique used to produce murine monoclonal antibodies, but presents some technical problems in that a lower fusion efficiency is usually found and human chromosomes are lost preferentially. This procedure may be regarded as a compromise due to the absence of a suitable human myeloma fusion partner.

b) Transformation of human lymphocytes with Epstein-Barr virus (EBV). This procedure has been used for many years to produce continuous, rapidly growing human B cells.

c) Fusion of human B-lymphocytes with a human lympho-blastoid B-cell line.

d) Fusion of an EBV-transformed human B-lymphocyte line with a mouse myeloma cell line.

Other methods for generating stable lines secreting human antibodies may be developed or exploited in future.

1.3. Engineered monoclonal antibodies

An alternative approach to circumvent the HAMA response, the limited duration of effective murine antibody therapy and several manufacturing problems in the production of human monoclonal antibodies is the production of so called chimeric and humanised monoclonal antibodies using recombinant DNA (rDNA) technology and eukaryotic gene expression methods. Both types of rDNA-engineered monoclonal antibodies contain human sequences. In chimeric antibodies the variable heavy and light chain domains of a human antibody are replaced by those of a rodent (usually murine) antibody, which possesses the desired antigen specificity. In humanised antibodies only the three short hypervariable sequences (complementarity determining regions or CDR's) of the rodent variable domains for each chain are engineered into the variable domain framework of a human antibody producing mosaic variable regions. Humanised antibodies contain a minimum of rodent sequence.

Suitable cells for expression of the rDNA monoclonal antibody genes are mammalian cell lines such as immunoglobulin non-producing myeloma cell lines, that are capable of high-level expression of exogenous heavy and light chain genes and the glycosylation, assemblage and secretion of functional antibodies.

Engineered monoclonal antibodies may have the advantages of decreased immunogenicity, enhanced in vivo circulating half life in combination with optimised specificity and effector functions.

Certain aspects of the control requirements likely to apply to rDNA derived chimeric and humanised monoclonal antibody usage will be similar to those already described for products derived by rDNA technology (Note for guidance *Production and Quality Control of Medicinal Products derived by rDNA Technology*) with which the applicants should be familiar. These control requirements concern e.g. status of the rDNA within the host cell, expression regulation and stability of the expression system and the purification procedure.

2. POINTS TO CONSIDER IN MANUFACTURE

Several of the requirements relating to establishments in which biological products are manufactured (e.g. WHO technical Report series 822, 1992: Annex 1 Good Manufacturing Practices for Biological Products) apply to the manufacture of monoclonal antibodies. Additional information can be found in WHO technical Report Series 822, 1992: Annex 3 Guidelines for Assuring the Quality of Monoclonal Antibodies for use in Humans. Manufacturers should also refer to the EU Guide for Good Manufacturing Practice for Medicinal Products. Attention is drawn to the following points.

2.1. Production process

Many of the general requirements for the quality control of biological products, such as potency, abnormal toxicity testing, freedom from contaminants, stability and freedom from detectable levels of antibiotics will apply to monoclonal antibodies. It is undesirable to use agents which are known to provoke sensitivity reactions in certain individuals such as, for example, penicillin or other beta-lactam antibiotics.

2.2. Biological materials used in the production

Any reagents of biological origin (e.g. sheep erythrocytes, foetal calf serum, bovine serum albumin, human transferrin, insulin, trypsin) used in the generation of the monoclonal antibody producing cell line and/or during routine production, should be free of microbial contamination such as mycoplasma, fungi and bacteria. Special consideration should be given to possible viral contamination and tests for relevant viruses should be performed, e.g. trypsin should be tested for porcine parvovirus.

Bovine sera should be checked and found negative for potentially dangerous viruses (at least bovine diarrhoea virus, infectious bovine rhinotracheitis and parainfluenza 3). In addition, bovine sera and other bovine derived biologicals should comply with the requirements in the note for guidance *Minimising the Risk of Transmitting Agents causing Spongiform Encephalopathy via Medicinal Products*.

The following points, set out below, should de considered.

3. SOURCE CELLS

Whenever possible, murine tissue and animals used as source materials should be shown to be free of viruses as indicated in Annex I (a), table 2.

Monoclonal antibodies obtained from human cells present particular concerns regarding safety. Human monoclonal antibodies for use in humans are currently unique in that they are often derived from cells which are likely immortalised by the deliberate introduction of EBV, a potential human pathogen. They are likely to be obtained from a transformed human cell line which is potentially oncogenic. Evidence of contamination with viruses originating from the donor is cause for concern, as they will by definition be viruses capable of infecting humans. Cells from human origin should be shown free of viruses indicated in Annex I (a) table 3.

3.1 Characterisation of non-specific cells

3.1.1 Feeder cells

Whenever appropriate, the origin of feeder cells used should be defined. Feeder cells should be derived from SPF (**s**pecific **p**athogen **f**ree) animals or cell seed stocks shown to be free of microbial contamination such as mycoplasma, bacteria and fungi and special consideration should be given to possible exogenous viral contamination.

3.1.2 Fusion partner(s)

The fusion partner used (e.g. myeloma, human lymphoblastoid B-cell line) should be fully described and documented. The source, name and characteristics of the parental cell line should be given. It should be shown that the cell line is a pure culture and is not contaminated with cells of other types. If possible the cell line used as fusion partner should be selected as one which does not synthesise any immunoglobulin chains. Cryopreserved samples of the cell line used as fusion partner should be retained in case retrospective investigations become necessary.

3.1.3 Host cell for the expression of the recombinant monoclonal antibody

A description of the starting host strain or cell line should be provided including the history of the strain or cell line, its identification characteristics and potential viral contaminants. Special attention should be given to the possibility of unintended cross-contamination with other cell lines or viruses not endogenous to a particular cell line.

The cell line used should not synthesise any endogenous immunoglobulin chains before and after transfection.

Cryopreserved samples of the host cell line should be retained in case retrospective investigations become necessary.

3.2 Generation and characterisation of the specific parental cell (murine and human monoclonal antibodies)

The source of the immune parental cells should be documented. If an immunogen has been deliberately used, information on its source and preparation and on the immunisation procedure should be provided.

If the immune parental cells are derived from a human donor, information concerning the health of the donor should be provided. Any relevant clinical data on the donor must be reported, especially data on possible virological infections. Preferably, the description of the state of health of the donor should cover a period of some months before and after derivation to establish that blood borne viruses such as HIV, hepatitis B and hepatitis C were not in the process of incubation. If these conditions can not be completely fulfilled, this should be justified and it should be demonstrated that the cell bank system is devoid of any relevant viruses (e.g. HIV 1/2, HBV, HCV).

For the production of monoclonal antibodies of major therapeutic value it may be necessary to use cells potentially contaminated by a virus. In such a case, it will be necessary to look at the possible detection of the virus in the cell bank and to add one or more steps dedicated to inactivate this virus in the processing of monoclonal antibody.

4. CELL LINE PRODUCING THE MONOCLONAL ANTIBODY

4.1. Generation of the cell line

A complete description of the production of the cell line secreting the monoclonal antibody should be provided including details of cell fusion, EBV transformation and cloning procedures where appropriate. Sufficient data should be given to allow an assessment of the efficiency of the cloning procedure.

Agents used in the fusion and selection procedure should be described (e.g. PEG).

4.2. Characterisation of the cell line

The characteristics of the monoclonal antibody producing cell line should be detailed. These should include the specificity, class and, where appropriate, subclass of the immunoglobulin secreted, together with any distinguishing features, such as isoenzyme/immunochemical markers. The production of immunoglobulin chains originating from the fusion partner should be determined. The antibody secretion should be stable in respect to both the type of antibody (class switch) and level of expression up to and beyond the population doublings used for routine production. Appropriate precautions should be taken to avoid cross-contamination with other cells.

5. CELL LINE PRODUCING THE RECOMBINANT MONOCLONAL ANTIBODY

5.1 Cloning and characterisation of the DNA coding for the non-specific part of the recombinant mAb

For both chimeric and humanised monoclonal antibody a description of the origin, isolation and cloning strategy of the heavy and the light chain genes should be provided. In addition the following information is required:

i) the introduced framework residue substitutions in humanised monoclonal antibodies to improve the CDR conformation (where applicable).

ii) a justification of the choice of the immunoglobulin isotype.

iii) a characterisation of the human constant domain genes (e.g. by restriction endonuclease maps).

5.2 Selection, cloning and characterisation of the DNA coding for the specific part of the recombinant mAb

The origin of the hybridoma cell line and the characteristics of the rodent monoclonal antibody used should be described.

A description of the cloning of the rodent heavy and light chain variable region genes from the hybridoma cell line and the characterisation of the coding regions of the cloned genes should be provided. For humanised monoclonal antibodies a description of the identification, the method of isolation, either by cloning or synthesis, and the characterisation of the rodent CDR genes for both heavy and light chain should be provided.

5.3 Construction of the gene coding for the recombinant mAb

A description of the strategy followed either to join the rodent variable fragment to the human constant region, or, in the case of humanised antibody, to insert the rodent CDR genes into the human framework region sequences, is required. This documentation should include:

i) cell lines and vectors used in the generation of the monoclonal antibody, and a description of the expression vectors used for the transfection of rDNA antibody genes into the mammalian host cell line, including the origin, structure and selection markers.

ii) for both the heavy and light chain expression vectors the nucleotide sequences of the genes of interest and the flanking control regions. A detailed map indicating the regions which have been sequenced during construction and those deduced from the literature should be given. All the junction regions created by ligation during construction should be confirmed by sequencing.

iii) a clear identification of all known expressed sequences.

iv) an indication of any additional modifications.

5.4 Generation of the cell line expressing the recombinant monoclonal antibody

In addition to the documentation concerning the starting host strain or cell line, the following information is required:

i) the methods used for introducing the vector into the host cell, the selection and cloning of the transformants.

ii) the status of the vectors within the host.

iii) a detailed study using various restriction enzymes and Southern blot analysis providing convincing data on the integrity in the host cell. Useful information is provided on the expression system by Northern blot analysis.

iv) a detailed description of the strategy by which expression of the relevant gene is promoted and controlled (during production).

5.5 Genetic stability

The stability of the host/vector genetic and phenotypic characteristics should be investigated up to and beyond the population doubling level or generation number expected during full scale production. Such stability studies should provide detailed information on:

i) gene copy number in relation to productivity of the culture.

ii) characterisation of the monoclonal antibody. Analysis at the protein level and/or DNA level can be envisaged. Whichever method is used, it should be validated and the detection limit should be given.

iii) the level and consistency of expression of both the heavy and light chain.

6. CELL BANK SYSTEM

6.1 Establishment of the cell bank system (MCB and MWCB)

It is essential that production is based on a well defined cell bank system. This will normally involve a Master Cell Bank (MCB) and a Manufacturer's Working Cell Bank (MWCB). During the establishment of the cell bank no other cell lines should be handled simultaneously in the same laboratory suite or by the same persons. The origin, form, storage, use, and details of life expectancy at the anticipated rate of use must be described in full for all cell banks. New working cell banks should be fully characterised.

Samples of the working cell bank should be retained until at least after the expiry date of the resulting final lot.

6.2 Control of virological and microbial contamination

The various cell levels, including MCB, MWCB and PPCB (Post Production Cell Bank; see 6.5) should be examined for adventitious agents (viral, bacterial, fungal or mycoplasmal). Special attention should be given to viruses which can commonly contaminate the species from which the cell line has been derived. Appropriate tests to demonstrate the absence of virus contamination as described in Annex I should be performed.

Certain cell lines contain endogenous viruses, e.g. retroviruses, which may not readily be eliminated. Furthermore, potential viral contamination may take the form of complete viral genomes or subgenomic fragments resulting in the expression of infectious viral particles. Therefore the possibility of mutations of endogenous viruses during prolonged culture should be considered. The presence of sequences from viral genomes may not disqualify use of the cells, but any exogenous viral nucleic acid found should be identified. If the heterohybridoma approach is used for construction of the antibody secreting cell line the cell bank should be examined for the presence of murine and human viruses.

A cell line which produces any infectious virus capable of infecting human cells would be acceptable only in exceptional circumstances. All products derived from such lines would have to be considered on a case by case basis. If the cell line secretes infectious virus, appropriate precautions should be taken to protect personnel involved in production from infection.

There is special concern about the use of cell lines transformed by the deliberate introduction of EBV for the production of human monoclonal antibodies. Despite the fact that EBV transformed human B cells in general do not secrete viral particles these cells contain complex copies of the viral genome and EBV sequences should be sought by PCR or by co-cultivation with suitable indicator cell lines.

6.3 Characterisation

A critical part of quality control will involve the full characterisation of cells. The identity of the cells should be established by distinguishing markers of the cell, such as specific isoenzyme and immunological features, and phenotypic characterisation.

If the EBV transformation procedure is used alone for the generation of a cell line for the production of human monoclonal antibodies difficulties can arise in the cloning procedure. It is therefore essential that manufacturers show convincing evidence that the cell line is monoclonal.

6.4 Secretion of cytokines

Manufacturers should be aware that lymphocytes and/or feeder cells can secrete a number of biological mediators which have diverse functions and may cause adverse effects when administered to humans. Consideration should be given to the ability of the production process to remove immune mediators such as interferons and other cytokines.

6.5 Establishment of the post production cell bank

For validation purposes a post production cell bank should be established. For single harvest production 10 or more population doublings beyond the maximum population doubling level used for routine production should be used. For multiple harvest production at a time which exceeds the total length of the cultivation period by one third is suggested.

7. CHARACTERISATION OF THE MONOCLONAL ANTIBODY

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

7.1 Biochemical/physico-chemical characterisation

The biochemical/physico-chemical properties of the antibody should be described in detail. At least the following parameters should be determined: class, subclass (when appropriate) and light-chain composition, molecular weight and either N- and C-terminal amino acid sequence, secondary and tertiary structure.

7.2 Biological/immunological characterisation

The immunological properties of the antibody should be described in detail. Therefore the biological/immunological characterisation should include: antigenic specificity including the characterisation of the epitope the antibody recognises, binding capacity, ability for complement binding and activation, cytotoxic properties, antibody dependent cytotoxicity, ability to modify relevant antigens, capacity to stimulate immunocompetent cells and the ability to induce secretion of cytokines or other mediators.

7.3 Specificity and crossreactivity

The analysis should include the determination of unintentional reactivity with or cytotoxicity for human tissues distinct from the intended target, and cross-reactivity with a range of human tissues (listed in Annex II) by immunohistochemical procedures.

8. PRODUCTION

In vitro production is the preferred method of production. During the last few years the technology for in vitro production of monoclonal antibodies has been considerably improved. In vitro production of monoclonal antibodies offers a high degree of control and

standardisation and has important advantages over in vivo production with respect to viral safety, consistency of production and the absence of contaminant immunoglobulins in the crude harvest. In vitro production in serum free medium is also now feasible. Another advantage of this method of production is the considerable reduction of animal usage. Manufacturers should be aware of Directive 86/609/EEC concerning the protection of animals used for experimental and other scientific purposes.

If in vivo production is chosen it must be justified by the manufacturer.

A clear definition of a "batch" of product for further processing should be provided. A production batch should normally originate from a fresh ampoule of the MWCB. Details of the culture with the in-process controls should be provided. Criteria for rejection of the harvests and premature termination of the culture should be defined.

8.1 In vitro production

For each production run, the presence, extent and nature of any microbial contamination in the culture vessels immediately prior to all harvesting must be thoroughly examined. Detailed information to confirm the adequate sensitivity of the methods used to detect contamination should be provided and acceptable limits of contamination set. The bulk culture fluid should be shown to be free from mycoplasmal, mycotic and bacterial contamination and should be tested for the presence of viruses using a general test involving inoculation into suitable cell substrates (see Annex I, b).

The composition and source of the cell culture medium used for production should be recorded. If animal serum-derived additives are used, they should be shown to be free from adventitious agents (See 2.2.).

Ideally not more than one cell line should be cultivated simultaneously in the same production area. If other cell lines are cultivated in parallel, records must be kept of the cell lines handled and evidence presented for the absence of cross contamination between them.

8.1.1 Single harvest production

The maximum permitted generation number for production should be defined and should be based on information concerning the stability of the cell line or the up to and beyond the level of production. Data on consistency of growth of the culture and on the maintenance of yield within specified limits should be presented. Appropriate monitoring of the cell line characteristics at the end of the production cycles should also be undertaken. Evidence should be provided that the yield does not vary beyond defined limits and that the nature and quality of the product does not change with respect to specific parameters.

8.1.2 Multiple harvest production

The period of continuous cultivation should be specified and this should be based on information concerning the stability of the system and consistency of the product up to and beyond this limit. Monitoring of the production system is necessary throughout the life of the culture. The required frequency and type of monitoring will depend upon several factors including the nature of the expression system and monoclonal antibody, as well as the total length of the period of continuous cultivation undertaken. The acceptance of harvests for further processing should be clearly linked to the schedule of monitoring applied. Evidence

should be provided that the yield does not vary beyond defined limits and that the nature and quality of the monoclonal antibody does not change with respect to specific parameters.

8.2 In vivo production

In vivo production should comply to the additional requirements set below.

8.2.1 Characterisation of the animals used

The strain and origin of the animals used for production should be specified, together with their genotype and age. They should be from a closed, specific pathogen-free (SPF) colony which is routinely monitored for those viruses listed in Annex I Table 2. The long term records of the breeding colony in respect of freedom from viral contamination should be considered in relation to the reliability of maintenance of the colony. Evidence should also be presented that animals are maintained under SPF conditions at all times during transfer and use.

8.2.2 Harvest and handling of ascitic fluid

Each production batch should originate from a fresh ampoule of the MWCB. The maximum permissible number of serial passages in vivo during normal production should be defined and restricted: justification of this limit should include information concerning the yield of monoclonal antibody and the stability of the hybridoma characteristics on in vivo passage up to beyond that used in production. Indefinite passage in animals is not acceptable. A scheme of priming, inoculation and harvesting should be provided.

The number of animals and the procedure used to prepare the bulk ascitic harvest should be given in detail. Full details should be provided on any substances used to pre-treat mice or rats to facilitate growth of hybridomas. Description, volume and concentration of cell inoculum should be given. Data concerning the titre of the antibody in and storage conditions of the bulk ascitic fluid should be provided (e.g. temperature, duration, details of any proteolytic enzyme inhibitors added). Particular attention should be paid to the degree and nature of any microbial contamination (bacterial, mycotic and mycoplasmal) in the bulk ascitic fluid. Testing procedures capable of detecting all of the murine viruses listed in Annex I Table 2 should be performed, as indicated in Annex I(a) and (b), on at least the first five bulk harvests of the product. However, it may be expected that general testing methods for viruses may be sufficient as experience of production is gained. Consequently, after the first five production runs, general testing for viruses, limited to those described in Annex I (b), may be considered adequate. Any infectious agent should be identified and tests for viruses in Group I Table 2 should be negative. If the source of mice is changed to a different colony or supplier, tests described in Annex I(a) should be performed on at least the first five bulk harvests to re-establish consistency of freedom from contaminant agents.

8.3 Virological aspects: in-process controls

The bulk harvest should be tested for the presence of viruses using a general test involving inoculation into suitable cell substrates as described in procedures given in Annex I (b).

9. PURIFICATION OF THE ANTIBODY

9.1 Methods

Methods used to purify the product and their in-process controls including their specification limits should be described in detail, justified and validated. It is important to ensure that purification procedures do not impair relevant immunobiological features of the immunoglobulin. Procedures which make use of affinity chromatography, for example employing monoclonal antibodies, should be accompanied by appropriate measures to ensure that these substances, or any additional potential contaminants arising from their use, do not compromise the quality and safety of the final product. Cross reference is made to the note for guidance *Virus Validation Studies: The Design, Contribution and Interpretation of Studies Validating the Inactivation and Removal of Viruses.*

The criteria for reprocessing of any intermediate or final bulk should be carefully defined, validated and justified.

Consideration should be given to incorporating procedures which inactivate/eliminate potential viral contaminants where such methods will not compromise the biological activity of the product.

9.2 Validation of the purification

The capacity of the purification procedure to remove unwanted host cell derived proteins, nucleic acids, carbohydrates, viruses and other impurities including product-related proteins should be investigated thoroughly. Any inactivation process used should be shown to be effective and not compromise the biological activity of the product. The reproducibility of the purification process with respect to its ability to remove specific contaminants, should also be demonstrated. Studies using, for example, a carefully selected group of viruses which exhibit a range of physico-chemical features relevant to their behaviour on purification (see note for guidance on *Virus Validation Studies: The Design, Contribution and Interpretation of Studies Validating the Inactivation and Removal of Viruses*), host-cell proteins, other potential impurities derived from the production process (e.g. heavy or light chain immunoglobulin fragments) and DNA intentionally mixed with the crude preparation (spiking) should be undertaken. The choice of the nucleotide probe to detect DNA contamination should be relevant to the system used. A reduction factor for such contaminants at each stage of purification, and overall, should be established by using, if necessary, concentrations of viruses, host cell proteins, other potential impurities and DNA in excess of that expected during normal production.

Where a cell line contains viral subgenomic fragments (see section 6.2) consideration should be given to using appropriate viral nucleic acid in DNA spiking studies. Where a hybridoma line has been established by transformation with Epstein-Barr virus, specific EBV sequences should be sought by sensitive techniques such as the polymerase chain reaction.

Validation of the purification process should also include justification of the working conditions such as column loading capacity, column regeneration and sanitisation and length of use of the columns.

10. The bulk final processed product

10.1 The monoclonal antibody

Rigorous characterisation of the purified monoclonal antibody by chemical and biological methods will be essential. At least the following parameters should be determined: class, subclass and light-chain composition, glycosylation pattern, integrity of the molecule by analysis of the ratio heavy/light chain, microheterogeneity, molecular weight, N- and C-terminal amino acid sequence, and secondary and tertiary structure of the antibody. With increasing experience, the tests for subclass, light chain composition, N- and C- terminal amino acid sequence and secondary and tertiary structure could be omitted. The total protein content, the degree of aggregation and molecular fragmentation of the immunoglobulin should be determined. Appropriate specifications for these parameters, with acceptance limits, should be set. Especially for engineered and humanised antibodies sufficient sequence information to characterise the gene product adequately should be obtained by peptide mapping or amino acid sequencing.

Particular attention should be given to use a wide range of analytical techniques exploiting different physico-chemical properties of the molecule. Examples of suitable techniques are: SDS-polyacrylamide gel electrophoresis under reducing and non reducing conditions, isoelectric focusing, column chromatography (including HPLC), peptide mapping, amino acid analysis, circular dichroism and carbohydrate mapping. The manufacturer should provide clear photographs of the gels, etc..

The immuno-reactivity of the antibody should be assessed. The specific activity of the purified monoclonal antibody should be determined (units of activity/weight of product).

A clear difference should be made between the analytical tests performed during development, in order to fully characterise the monoclonal antibody and tests performed routinely on each batch of purified bulk product. Quality control tests should be carried out routinely on each batch of purified bulk product according to the Guide to GMP.

10.2 Purity

Data should be provided on contaminants whose presence is anticipated in the final processed product. The level of contamination considered as acceptable should be justified, and criteria of acceptance or rejection of a production batch should be given. It is important that the techniques used to demonstrate purity be assessed using as wide a range of methods as possible, including physico-chemical and immunological techniques. These should include tests for viral and cellular nucleic acid and protein contamination of parental, hybridoma, or host cell origin, as well as on materials derived from tissue culture medium or materials which have been added during the production or purification processes.

Measurements of total protein and cellular DNA concentrations, specific activity, microbiological and chemical purity should be reported for the final product. Assays of endotoxin level should also be carried out.

10.3 Adventitious agents

The final bulk product should be shown to be free from bacterial, fungal and mycoplasmal contamination. Evidence should be presented to show that any viral contaminant known to be possibly present in the bulk harvest has been eliminated or inactivated (see Annex I).

11. CONSISTENCY AND ROUTINE BATCH CONTROL OF BULK PROCESSED PRODUCT

A comprehensive analysis of the initial batches of a product should be undertaken to establish consistency with regard to identity, purity and potency. Thereafter, a more limited series of tests may be appropriate as outlined below.

11.1 Consistency

Evidence should be provided on the consistency of production, for example on at least five consecutive full scale production batches. This should include information on the bulk harvest and final dosage form as well as on in-process controls. In the case of a production where multiple harvests are applied, batches from different fermentation runs are needed. The studies should include biological, chemical and immunological methods to characterise and assay the monoclonal antibodies and methods to detect and identify impurities. Any differences which occur between batches should be noted.

11.2 Routine batch control analysis

11.2.1 Identity

A selection of the tests used to characterise the purified monoclonal antibody should be used to confirm the product identity for each batch. The methods employed should include tests for the biological activity as well as physico-chemical and immunological methods. Engineered antibody should be subjected to sequence verification of the peptide backbone by adequate methods such as peptide mapping.

11.2.2 Purity

The degree of purity desirable and attainable will depend on several factors; these include the nature and intended use of the product, method of its production and purification and also the degree of consistency of the production process. The purity of each batch should be established and be within specified limits. For engineered monoclonal antibodies the analysis should include sensitive and reliable assays for DNA of host cell origin and the vectors applied to each batch of product. Strict upper limits should be set for DNA in the product.

The product should be shown to be free from microbial contamination. Evidence should be presented to show that any viral contaminant known to be present in the bulk harvest has been eliminated or inactivated (see Annex I). Pyrogenicity should be tested for.

Particular attention should be given to assessment of the degree of aggregation or molecular fragmentation of the immunoglobulin. All possible steps should be taken to prevent aggregation. Limits for the presence of oligomeric immunoglobulin molecules should be justified.

11.2.3 Test for potency

When appropriate, the biological activity of the monoclonal antibody should be established by a biological assay. In addition information on specific activity will be of considerable value

and should be reported. A fully characterised reference preparation is required to standardise measurements of specific activity (see section 13).

12. SPECIFICATIONS AND REFERENCE MATERIALS

The studies described in section 10 will contribute to a definitive specification for the product when justified by the information obtained from the examination of successive batches and results of batch analysis, as indicated in section 11.

The reference preparation should be produced from a suitable batch of the product, which has been clinically evaluated and fully characterised in terms of chemical composition, purity, potency and biological activity. Criteria for establishing the reference preparation and criteria for re-testing and prolongation of the shelf life should be stated.

13. MODIFIED MONOCLONAL ANTIBODIES

The preparation of sub-fragments of the antibody (Fab or $F(ab')_2$ fragments) may have advantages for some applications. Where such fragments are preferred for clinical use, their molecular and antigenic properties should be defined. Appropriate analytical tests should be performed. Specified limits for impurities such as fragments other than those desired or intact immunoglobulin, should be defined. Specifications, with limits, should be given for each contaminant (e.g. residual levels of enzymes used, such as pepsin or papain), specific activity, immunoreactivity, and antigen cross-reactivity. A reference batch should be prepared and all assays should be validated.

The therapeutic and diagnostic uses of monoclonal antibodies and antibody subfragments can sometimes be enhanced by chemical modifications (e.g. radiolabelling, conjugation with a toxin, attachment to specific substances for “targeting“ or chemically linking of two antibody molecules or their derivatives to generate a bispecific antibody). For these a detailed description of their preparation and purification should be supplied. Each relevant step in the production process requires validation and quality control covering source materials, limits for impurities arising from the production process, evidence for consistency etc. Modifications can change the properties of the monoclonal antibody and general requirements for such products must include information concerning the biological half-life of the antibody, of the medicinal product or toxin, and also of the conjugate after injection into a recipient. Information about the specificity, the toxicity and stability of the conjugate should also be supplied.

Criteria and specification limits for purity and potency of the final product should be applied and immunoreactivity and antigen cross-reactivity should be determined. Additional specific control procedures may be required, but these are dealt with best on a case by case basis.

The preparation of a reference batch is required and all assays should be validated.

Detailed information for the production of radiolabelled monoclonal antibodies can be found in the note for guidance *Radiopharmaceuticals based on Monoclonal Antibodies*.

14. FINISHED PRODUCT AND DEVELOPMENT PHARMACEUTICS

The development of the formulation should be described in detail and justified, particularly with regard to the presence and amount of stabilisers such as albumin and/ or detergents. The product in final containers should be shown to comply with the requirements of the European directives and pharmacopoeias. In circumstances where this is not possible the omission of tests should be justified by the manufacturer.

15. PRODUCT EQUIVALENCE

Some changes or adaptations in the production of a monoclonal antibody during clinical development or subsequent to product approval can lead to an altered form of the antibody with identical specificity. Examples of such changes are: transition of in vivo production to in vitro production, changes in culture procedures or culture conditions, changes in purification procedure, or additional modifications of the monoclonal antibody molecule. In these cases, studies to prove product equivalence should be performed to show that both forms of the antibody are essentially identical.

In all cases these studies should include a complete physico-chemical and biological characterisation of both antibodies.

15.1 In vitro studies on product equivalence

The physico-chemical characteristics of the monoclonal antibody, like isotype, subclass, microheterogeneity, molecular weight, primary structure, secondary structure, glycosylation pattern, structural integrity, should be determined. The biological characterisation should include immunoreactivity and crossreactivity, the determination of relevant functional characteristics and binding studies to determine affinity.

When there are changes in the cell culture procedure/conditions without changes in the MCB, relevant parameters such as morphology, cell growth, viability, isoenzymes, and stability of production should be analysed.

15.2 In vivo studies on product equivalence

The decision on the selection of in vivo tests depends on the results of the analytical characterisation. In the case of identical analytical results of both forms of antibody, at least the pharmacokinetic, biodistribution and half life should be determined.

15.3 Clinical studies

When both monoclonal antibodies are demonstrated to have identical physico-chemical, biological and pharmacological characteristics, clinical studies performed with the former monoclonal antibody can be accepted. However, an essential prerequisite is that the production is based on the same MCB. Otherwise, clinical trials have to be carried out with the second form of antibody.

15.4 Manufacturing procedure

Consistency of the manufacturing procedure of the monoclonal antibody, including validation of the production process and quality control in accordance to the requirements should be demonstrated.

ANNEX I

Testing for viruses should be performed in laboratories with experience in routine virus testing and should be performed in accordance with good laboratory practice.

Table 1 lists the tests for viruses to be performed at the different stages of production.

Table 2 lists viruses which should be considered as potential contaminants in the manufacture of monoclonal antibodies produced by cell lines of murine origin.

Table 3 lists viruses which should be considered as potential contaminants in the manufacture of monoclonal antibodies produced by cell lines of human origin.

Testing for viral Contamination

a) Tests for detection of specific viruses

(i) Monoclonal antibodies produced by cell lines of murine origin

Tests for detection of specific viruses listed in table 2, for example Mouse Antibody Production (MAP) or Rat Antibody Production (RAP) tests or other tests of at least equivalent sensitivity and reliability. Additional specific tests may need to be carried out for lymphocytic choriomeningitis virus (LCMV), mouse cytomegalo virus, mouse rotavirus (EDIM), thymic virus and lactic dehydrogenase virus. Tests capable of detecting murine retrovirus should be included, for example the XC plaque assay or the S+ L- focus assay for the detection of ecotropic or xenotropic retrovirus respectively.

(ii) Monoclonal antibodies produced by cell lines of human origin

For human monoclonal antibodies the viruses which may be found in the cell line depend to some extent on the nature and health of the donor. They may be specifically able to infect B lymphocytes. As a minimum, the viruses which are known to persist in lymphocytes and are listed in table 3 should be tested for. Viruses should be sought by culture methods employing cell lines including virus free lymphoblastoid cells as well by examination of the lymphocyte line itself by use of immunochemical procedures, electron microscopy, Southern blotting, polymerase chain reaction or other sensitive techniques.

(iii) Engineered monoclonal antibodies produced by mammalian cell lines

For engineered monoclonal antibodies the viruses which may be found in the cell line depend on the origin of the cell line. Relevant viruses should be tested for.

b) Inoculation of cell cultures capable of detecting a wide range of murine, human, and, if relevant, bovine viruses. Examples of useful cell types (substrates) are: murine fibroblast cultures, e.g. mouse embryo cultures; human fibroblast cultures, e.g. human diploid cells such as MRC5; continuous cell lines of human, murine and bovine origin. The indicator cell lines should additionally be tested for haemadsorbing viruses (with erythrocytes from human blood group O, guinea pig, chicken) at the end of the observation time. Tests for retroviruses should be included.

c) Tests in animals for adventitious agents should include the inoculation by the intramuscular route of each of the following groups of animals with the test material or with disrupted cells from the seed lot propagated beyond the maximum level (or population doubling, as appropriate) used for production:

– 2 litters of suckling mice, comprising at least 10 animals less than 24 hours old

– 10 adult mice

– 5 guinea-pigs

Test material should be injected intracerebrally into each of 10 adult mice.

The animals should be observed for at least 4 weeks. Any animals that are sick or show any abnormality should be investigated to establish the cause of illness. Test material can be considered to be suitable for production if at least 80 % of the animals inoculated remain healthy and survive the observation period and none of the animals shows evidence of the presence in the tested material of any adventitious agent.

Fertilised eggs may also act as useful substrates. Test material should be injected into eggs by appropriate routes, the chorioallantoic membrane, amniotic cavity and yolk sack of each of 10 embryonated chicken eggs, 9-11 days old. The embryonated eggs should be examined after not less than 5 days incubation. The allantoic fluids should be tested with guinea-pig and chick or other avian red cells for the presence of haemaglutinins.

TABLE 1

TESTING SCHEME FOR VIRAL CONTAMINANTS

Annex I sections which are applicable

MCB or MWCB	(a)	(b)	(c)
Mouse breeding Colony	(a)		
Ascitic fluid harvest	(a)*	(b)	
In vitro bulk harvest		(b)	

Bulk final processed product	Specified tests of (b) if virus contamination was detected in the bulk harvest

* It is proposed that these tests should be carried out on at least the first five production runs.

TABLE 2

MURINE VIRUSES

Group	Virus	Species Affected
I	Hantavirus (Haemorrhagic fever with renal syndrome)*	M, R
	Lymphocytic choriomeningitis virus (LCMV) *	M
	Rat rotavirus *	R
	Reovirus type 3 (reo 3)*	M, R
	Sendai virus*	M, R
II	Ectromelia virus*	M
	K virus	M
	Kilham rat virus (KRV)	R
	Lactic dehydrogenase virus (LDH)	M
	Minute virus of mice (MVM)	M, R
	Mouse adenovirus (MAV)	M
	Mouse cytomegalovirus (MCMV)	M
	Mouse encephalomyelitis virus (MEV, Theiler's or GDVII)	M
	Mouse hepatitis virus (mhv)	M
	Mouse rotavirus (EDIM)	M
	Pneumonia virus of mice (PVM)	M, R
	Polyoma virus	M
	Rat cornavirus (RCV)	R
	Retrovirus*	M, R
	Sialodacryoadenitis virus (SDA)	R
	Thymic virus	M
	Toolan virus (HI)	R

M - mouse
R - rat

Viruses for which evidence exists of a capacity to infect man or primates are to be found in Group I. Those viruses for which there is no evidence of infection in man but which could nevertheless pose a potential danger, for example in immunocompromised individuals, are listed in Group II. Viruses which are known to be capable of replicating in vitro in cells of human and monkey origin are indicated by * in Table 2.

TABLE 3

HUMAN VIRUSES

Virus
Human Immunodeficiency Virus (Type 1, Type 2)
Human T cell Leukaemia Virus (Type I, Type II)
Cytomegalo virus
HHV6
Epstein - Barr virus
Hepatitis B virus
Hepatitis C virus

ANNEX II

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, intestine

Pancreas, paratid, thyroid, para-thyroid, adrenal, pituitary

Brain, peripheral nerve

Heart, striated muscle

Ovary, testis

Skin

Blood vessels

ANNEX III

Glossary

1. Murine

"Murine" means derived from an animal belonging to the Muridae family which includes mice and rats.

2. Cell Banks

a) Master cell bank (MCB)

A homogeneous suspension of the original cells on which production is based, aliquoted into individual containers for storage (e.g. in a liquid nitrogen refrigerator). The original cell line may not necessarily have been produced by the manufacturer.

For engineered products the cells in the master cell bank are already transformed by the expression vector containing the desired gene. In some cases it may be necessary to establish separate master cell banks for the expression vector and the host cells.

b) Manufacturers working cell bank (MWCB)

A homogeneous suspension of cells derived from the master cell bank(s) by a finite passage level, aliquoted into individual containers for storage (e.g. in a liquid nitrogen refrigerator).

In both cell banks, all containers are treated identically during storage, and once removed from storage, the containers are not returned to the cell bank stock.

c) Post production cells (PPC)

Post production cells are the cells cultured up to 10 or more population doublings beyond the maximum population doubling level used for routine production (single harvest production) or cells cultured for a period of time which exceeds the total length of the cultivation period by one third (multiple harvest production).

3. Production Method

a) Production at finite passage (single harvest)

This cultivation method is defined by a limited number of passages or population doublings which must not be exceeded during production.

b) Continuous culture production (multiple harvest)

The number of population doublings are specified based on information concerning the stability of the system and the consistency of the product criteria for the termination has to be defined by the manufacturer.

4. Bulk Harvest

This is a homogeneous pool of individual harvests, lysates or ascitic fluids which is processed in a single manufacturing run.

5. Bulk Final Active Substance

This is the final product, after completion of the production process, obtained from a bulk harvest. It is maintained in one or more containers and used in the preparation of the final dosage form. The generation of this final batch has to be clearly defined and unambiguously recorded by the producer.

6. Finished Product

The active substance is formulated and filled into final, sealed containers which hold the product in its final dosage form, i.e. the finished product. The containers of a filling lot are processed together and uniform in their contents and biological potency.

7. Engineered Monoclonal Antibody

A human monoclonal antibody in which critical amino acid residues are replaced by molecular technology.

8. Fusion Partner

A cell line fused with the antibody producing cell with the intention to immortalise this cell.

9. Feeder Cells

Cells or cell line co-cultivated with the antibody producing cell line to provide optimal growth conditions.