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.

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以降P.152−P.158は雑誌/図書等に掲載された論文となりますので、「資料」をご参照ください。

「資料」

Criteria for investigation of the product equivalence of monoclonal antibodies for therapeutic and in vivo−diagnostic use in case of introduction of changes in the manufacturing process.

Schaffner G, Haase M, Giess S.

Biologicals 1995 Sep ; 23(3) : 253−9

Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use

U. S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
February 28,1997

Date: February 27, 1997

From: Kathryn C. Zoon , Ph.D., Director
Center for Biologics Evaluation and Research
Food and Drug Administration

Subject: Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use

To: Manufacturers of Biological Products and Other Interested Persons

This Points to Consider (PTC) document has been developed for manufacturers of monoclonal antibody products for human use. These "Points" are not regulations nor are they guidelines, but represent the current thinking that the Center for Biologics Evaluation and Research (CBER) staff believe should be considered at this time. This 1997 PTC document supersedes the 1994 PTC document of the same title, announced in the Federal Register of August 3, 1994 (59 FR 39571).

It is our intention to continuously update and revise this document in order to improve its usefulness. We invite your review and comment on the "Points". Comments should be identified with the docket number 94D-0259. Two copies of any comments should be submitted except that individuals may submit one copy. All comments should be addressed to:

Dockets Management Branch (HFA-305)
Food and Drug Administration
12420 Parklawn Drive, Room 1-23
Rockville, MD 20857

_____-s-_____
Kathryn C. Zoon, Ph.D.

Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use

[Docket No. 94D-0259]

For further information regarding this document, contact:
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301-594-3074

Submit written comments on this document to:

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Food and Drug Administration
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Rockville, MD 20857

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Send one self-addressed adhesive label to assist that office in processing your request.

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Persons with access to the INTERNET may obtain these documents using, the World Wide Web (WWW), or bounce-back e-mail. For WWW access, connect to CBER at "http://www.fda.gov/cber/cberftp.html". To receive this document by bounce-back e-mail , send a message to "ptc__mab@A1.CBER.FDA.GOV".

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Note: Page numbering may vary for documents distributed electronically.

Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use
February 1997

I. INTRODUCTION

A. BACKGROUND

Points to Consider documents provide a flexible approach in which FDA provides and updates its guidance on regulatory issues in many areas of drug development. Such documents are particularly useful in the rapidly evolving field of biotechnology-derived drugs and other biologics. The Center for Biologics Evaluation and Research (CBER) set out to revise the "Points to Consider (PTC) in the Manufacture and Testing of Monoclonal Antibody Products for Human Use" with several objectives. An important goal was to facilitate initial development of monoclonal antibodies for serious or life threatening indications. Additionally, it was felt that some of the information in the 1994 document required updating and streamlining. Finally, it was necessary to review this document for consistency with current CBER policy and with International Conference on Harmonisation (ICH) documents dealing with this category of products. This updated document supersedes the 1994 version, and is designed to assist sponsors and investigators regarding monoclonal antibody (mAb) product development, including information to submit when filing Investigational New Drug Applications ("INDs") and License Applications. Although this document does not create or confer any rights for or on any person and does not operate to bind FDA or the public, it does represent the agency's current thinking on monoclonal antibody products for human use.

For mAb, as for other biologics, certain regulations contained in 21 CFR Parts 200-299 and 600-680 apply and should be consulted. In common with the other PTC, the mAb PTC are not intended to be all-inclusive. They represent recommendations on how to conduct the clinical development of a product up to and after licensure, not checklists of items to be provided before or after phase 1 trials are initiated. Specific products which raise issues that are not considered in these "Points" will be evaluated on a case-by-case basis. The discussion on abbreviated product testing for feasibility trials in serious and immediately life-threatening conditions and on generic and modular virus clearance studies does not apply to human products made in human cell substrates. Consultation with CBER is strongly advised for sponsors considering the application of abbreviated testing policies to products that have the potential to be contaminated by human pathogens. For aspects of manufacturing and of the production facility that are not included in this discussion or in applicable regulations, sponsors should consult with the Office of Therapeutics Research and Review and the Office of Establishment Licensing and Product Surveillance respectively.

B. DEFINITIONS

For the purpose of this document, the terms "**antibody**" and "**monoclonal antibody**" (mAb) may be used interchangeably and refer to intact immunoglobulins produced by hybridomas, immunoconjugates and, as appropriate, immunoglobulin fragments and recombinant proteins derived from immunoglobulins, such as chimeric and humanized immunoglobulins, F(ab') and $F(ab')_2$ fragments, single-chain antibodies, recombinant immunoglobulin variable regions (Fvs) etc. Recommendations on the manufacture of recombinant products are contained in other PTC documents from CBER (1,2). Some of these recommendations pertaining to recombinant mAb produced in cell substrates other than hybridomas are reiterated in this document for convenience of consultation. This document applies to mAb used as therapeutic or *in vivo* diagnostic agents, as well as to **ancillary products**, i.e. mAb used in the manufacture of other products for *in vivo* use. The latter include mAb that are used alone or in