Foreign proteins. When examined by precipitation tests with specific antisera against plasma proteins of a suitable range of species, only protein from the declared animal species is shown to be present.

Albumin. Purified immunosera comply with a test for albumin. Unless otherwise prescribed in the monograph, when examined electrophoretically, purified immunosera show not more than a trace of albumin, and the content of albumin is in any case not greater than 30 g/l of the reconstituted preparation, where applicable.

Total protein. Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride R* to obtain a solution containing about 15 mg of protein in 2 ml. To 2 ml of this solution in a round-bottomed centrifuge tube add 2 ml of a 75 g/l solution of *sodium molybdate R* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid R* and 30 volumes of *water R*. Shake, centrifuge for 5 min, discard the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion (*2.5.9*) and calculate the content of protein by multiplying by 6.25. The results obtained are not greater than the upper limit stated on the label.

Antimicrobial preservative. Determine the amount of antimicrobial preservative by a suitable physicochemical method. The amount is not less than the minimum amount shown to be effective and is not greater than 115 per cent of that stated on the label.

Formaldehyde (*2.4.18*). Where formaldehyde has been used in the preparation, the concentration of free formaldehyde is not greater than 0.5 g/l, unless a higher amount has been shown to be safe.

Sterility (*2.6.1*). Immunosera for veterinary use comply with the test for sterility. When the volume of liquid in a container is greater than 100 ml, the method of membrane filtration is used wherever possible. If this method is used, incubate the media for not less than 14 days. Where the method of membrane filtration cannot be employed, the method of direct inoculation may be used. Where the volume of liquid in each container is at least 20 ml, the minimum volume to be used for each culture medium is 10 per cent of the contents of the container or 5 ml, whichever is the least. The appropriate number of items to be tested (*2.6.1*) is 1 per cent of the batch with a minimum of 4 and a maximum of 10.

Mycoplasmas (*2.6.7*). Immunosera for veterinary use comply with the test for mycoplasmas.

Safety. A test is conducted in one of the species for which the product is recommended. Unless an overdose is specifically contraindicated on the label, twice the maximum recommended dose for the species used is administered by a recommended route. If there is a warning against administration of an overdose, a single dose is administered. For products to be used in mammals, use 2 animals of the minimum age for which the product is recommended. For avian products, use not fewer than 10 birds of the minimum age recommended. The birds are observed for 21 days. The other species are observed for 14 days. No abnormal local or systemic reaction occurs.

Extraneous agents. A test for extraneous agents is conducted by inoculation of cell cultures sensitive to pathogens of the species of the donor animal and into cells sensitive to pathogens of each of the recipient target species stated on the label (*2.6.25*). Observe the cells for 14 days. During this time, carry out at least one passage. The cells are checked daily for cytopathic effect and are checked at the end of 14 days for the presence of a haemadsorbing agent. The batch complies with the test if there is no evidence of the presence of an extraneous agent.

For immunosera of avian origin, if a test in cell culture is insufficient to detect potential extraneous agents, a test is conducted by inoculation of embryonated eggs from flocks free from specified pathogens (*5.2.2*) or by some other suitable method (polymerase chain reaction (PCR) for example).

POTENCY

Carry out a suitable test for potency.

Where a specific monograph exists, carry out the biological assay prescribed in the monograph and express the result in International Units per millilitre when such exist.

STORAGE

Protected from light, at a temperature of 5 ± 3 °C. Liquid immunosera must not be allowed to freeze.

LABELLING

The label states:

- that the preparation is for veterinary use;
- whether or not the preparation is purified;
- the minimum number of International Units per millilitre, where such exist;
- the volume of the preparation in the container;
- the indications for the product;
- the instructions for use including the interval between any repeat administrations and the maximum number of administrations that is recommended;
- the recipient target species for the immunoserum;
- the dose recommended for different species;
- the route(s) of administration;
- the name of the species of the donor animal;
- the maximum quantity of total protein;
- the name and amount of any antimicrobial preservative or other substance added to the immunoserum;
- any contra-indications to the use of the product including any required warning on the dangers of administration of an overdose;
- for freeze-dried immunosera:
  - the name or composition and the volume of the reconstituting liquid to be added;
  - the period within which the immunoserum is to be used after reconstitution.

01/2008:2031

MONOCLONAL ANTIBODIES FOR HUMAN USE

Anticorpora monoclonalia ad usum humanum

DEFINITION

Monoclonal antibodies for human use are preparations of an immunoglobulin or a fragment of an immunoglobulin, for example, F(ab')2, with defined specificity, produced by a single clone of cells. They may be conjugated to other substances, including for radiolabelling.

They can be obtained from immortalised B lymphocytes that are cloned and expanded as continuous cell lines or from rDNA-engineered cell lines.

Currently available rDNA-engineered antibodies include the following antibodies.

Chimeric monoclonal antibodies: 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: the 3 short hypervariable sequences (the complementarity determining regions) of non-human variable domains for each chain are engineered into the variable domain framework of a human antibody; other sequence changes may be made to improve antigen binding.

Recombinant human monoclonal antibodies: the variable heavy and light chain domain of a human antibody are combined with the constant region of a human antibody.

Monoclonal antibodies obtained from cell lines modified by recombinant DNA technology also comply with the requirements of the monograph *Products of recombinant DNA technology (0784)*.

This monograph applies to monoclonal antibodies for therapeutic and prophylactic use and for use as *in vivo* diagnostics. It does not apply to monoclonal antibodies used as reagents in the manufacture of medicinal products. Nor does it apply to monoclonal antibodies produced in ascites, for which requirements are decided by the competent authority.

PRODUCTION

GENERAL PROVISIONS

Production is based on a seed-lot system using a master cell bank and, if applicable, a working cell bank derived from the cloned cells. The production method is validated during development studies in order to prevent transmission of infectious agents by the final product. All biological materials and cells used in the production are characterised and are in compliance with chapter *5.2.8. Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products*. Where monoclonal antibodies for human use are manufactured using materials of human or animal origin, the requirements of chapter *5.1.7. Viral safety* also apply. Where an immunogen is used it is characterised and the method of immunisation is documented.

Process validation. During development studies, the production method is validated for the following aspects:

- consistency of the production process including fermentation, purification and, where applicable, fragmentation method;
- removal or inactivation of infectious agents;
- adequate removal of product- and process-related impurities (for example, host-cell protein and DNA, protein A, antibiotics, cell-culture components);
- specificity and specific activity of the monoclonal antibody;
- absence of non-endotoxin pyrogens;
- reusability of purification components (for example, column material), limits or acceptance criteria being set as a function of the validation;
- methods used for conjugation, where applicable.

Product characterisation. The product is characterised to obtain adequate information including: structural integrity, isotype, amino-acid sequence, secondary structure, carbohydrate moiety, disulphide bridges, conformation, specificity, affinity, specific biological activity and heterogeneity (characterisation of isoforms).

A battery of suitable analytical techniques is used including chemical, physical, immunochemical and biological tests (for example, peptide mapping, *N*- and *C*-terminal amino-acid sequencing, mass spectrometry, chromatographic, electrophoretic and spectroscopic techniques). Additional tests are performed to obtain information on cross-reactivity with human tissues.

For those products that are modified by fragmentation or conjugation, the influence of the methods used on the antibody is characterised.

Process intermediates. Where process intermediates are stored, an expiry date or a storage period justified by stability data is established for each.

Biological assay. The biological assay is chosen in terms of its correlation with the intended mode of action of the monoclonal antibody.

Reference preparation. A batch shown to be stable and shown to be suitable in clinical trials, or a batch representative thereof, is used as a reference preparation for the identification, tests and assay. The reference preparation is appropriately characterised as defined under Product characterisation, except that it is not necessary to examine cross-reactivity for each batch of reference preparation.

Definition of a batch. Definition of a batch (including batch size) is required throughout the process.

SOURCE CELLS

Source cells include fusion partners, lymphocytes, myeloma cells, feeder cells and host cells for the expression of the recombinant monoclonal antibody.

The origin and characteristics of the parental cell are documented, including information on the health of the donors, and on the fusion partner used (for example, myeloma cell line, human lymphoblastoid B-cell line).

Wherever possible, source cells 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.

CELL LINE PRODUCING THE MONOCLONAL ANTIBODY

The suitability of the cell line producing the monoclonal antibody is demonstrated by:

- documentation on the history of the cell line including description of the cell fusion, immortalisation or transfection and cloning procedure;
- characterisation of the cell line (for example, phenotype, isoenzyme analysis, immunochemical markers and cytogenetic markers);
- characterisation of relevant features of the antibody;
- stability of antibody secretion with respect to the characteristics of the antibody and level of expression and glycosylation up to or beyond the population doubling level or generation number used for routine production;
- for recombinant DNA products, stability of the host/vector genetic and phenotypic characteristics up to or beyond the population doubling level or generation number used for routine production.

CELL BANKS

The master cell bank is a homogeneous suspension of the cell line producing the monoclonal antibody, distributed in equal volumes in a single operation into individual containers for storage.

A working cell bank is a homogeneous suspension of the cell material derived from the master cell bank at a finite passage level, distributed in equal volumes in a single operation into individual containers for storage.

Post-production cells are cells cultured up to or beyond the population doubling level or generation number used for routine production.

The following tests are performed on the master cell bank: viability, identity, sterility (bacteria, fungi, mycoplasmas), characterisation of the monoclonal antibody produced. Non-endogenous viral contamination is tested with a suitable range of *in vivo* and *in vitro* tests. Retrovirus and other endogenous viral contamination is tested using a suitable range of *in vitro* tests.

The following tests are performed on the working cell bank: viability, identity, sterility (bacteria, fungi, mycoplasmas). Adventitious viral contamination is tested with a suitable range of *in vivo* and *in vitro* tests. For the first working cell bank, these tests are performed on post-production cells, generated from that working cell bank; for working cell banks subsequent to the first working cell bank, a single *in vitro* and *in vivo* test can be done either directly on the working cell bank or on post-production cells.

For the master cell bank and working cell bank, tests for specific viruses are carried out when potentially contaminated biological material has been used during preparation of the cell banks, taking into account the species of origin of this material. This may not be necessary when this material is inactivated using validated procedures.

The following tests are performed on the post-production cells: sterility (bacteria, fungi, mycoplasmas). Virus tests are performed on cells or cell culture supernatants. For this, non-endogenous viral contamination is tested with a suitable range of *in vivo* and *in vitro* tests. Retrovirus and other endogenous viral contamination is tested using a suitable range of *in vitro* tests.

CULTURE AND HARVEST

Production at finite passage level (single harvest). Cells are cultivated up to a defined maximum number of passages or population doublings (in accordance with the stability of the cell line). Product is harvested in a single operation.

Continuous-culture production (multiple harvest). Cells are continuously cultivated for a defined period (in accordance with the stability of the system and production consistency). Monitoring is necessary throughout the life of the culture; the required frequency and type of monitoring will depend on the nature of the production system.

Each harvest is tested for antibody content, bioburden, endotoxin and mycoplasmas. Routine general or specific tests for adventitious viruses are carried out at a suitable stage depending on the nature of the manufacturing process and the materials used. For processes using production at finite passage level (single harvest), at least 3 harvests are tested for adventitious viruses using a suitable range of *in vitro* methods.

The acceptance criteria for harvests for further processing are clearly defined and linked to the schedule of monitoring applied. If any adventitious viruses are detected, the process is carefully investigated to determine the cause of the contamination and the harvest is not further processed. Harvests in which an endogenous virus has been detected are not used for purification unless an appropriate action plan has been defined to prevent transmission of infectious agents by the final product.

PURIFICATION

Harvests may be pooled before further processing. The purification process includes steps that remove and/or inactivate non-enveloped and enveloped viruses. A validated purification process, for which removal and/or inactivation of infectious agents and removal of product- and process-related impurities has been demonstrated is used. Defined steps of the process lead to a purified antibody of constant quality and biological activity.

The test programme on the purified monoclonal antibody depends on the validation of the process, on demonstration of consistency and on the expected level of product- and process-related impurities. The purified monoclonal antibody is tested for bioburden and bacterial endotoxins, purity, integrity and potency by suitable analytical methods, comparing with the reference preparation where necessary.

If storage of intermediates is intended, adequate stability of these preparations and its impact on quality or shelf-life of the finished product are evaluated.

FINAL BULK

The final bulk is prepared from one or more batches of purified monoclonal antibody. Suitable stabilisers and other excipients may be added during preparation of the final bulk.

Only a final bulk that complies with the following requirements may be used in the preparation of the final lot.

Sterility (*2.6.1*). Carry out the test using 10 ml for each medium.

Bacterial endotoxins (*2.6.14*). It complies with the limit approved for the particular product.

Process-related impurities. Suitable tests for host-cell-derived proteins, host-cell- and vector-derived DNA and other process-related impurities are carried out on a suitable number of final bulks or batches of purified monoclonal antibodies. The final bulk complies with the limits approved for the particular product. When consistency of the purification process has been demonstrated, the tests may subsequently be omitted.

FINAL LOT

The final bulk is distributed aseptically into sterile containers, which are then closed so as to prevent contamination.

CHARACTERS

Liquid preparations are clear or slightly opalescent, colourless or slightly yellow liquids, without visible particles. Freeze-dried products are white or slightly yellow powders or solid friable masses. After reconstitution they show the same characteristics as liquid preparations.

IDENTIFICATION

The identity is established by suitable validated methods comparing the product with the reference preparation. The assay also contributes to identification.

TESTS

Appearance. Liquid or reconstituted freeze-dried preparations are clear or slightly opalescent and colourless or slightly yellow, without visible particles.

Solubility. Freeze-dried preparations dissolve completely in the prescribed volume of reconstituting liquid, within a defined time, giving a clear or slightly opalescent solution without visible particles.

pH (*2.2.3*). It complies with the limits approved for the particular product.

Osmolality (*2.2.35*): minimum 240 mosmol/kg, diluted for use where applicable.

Extractable volume (*2.9.17*). It complies with the test for extractable volume.

Total protein (*2.5.33*). It complies with the limits approved for the particular product.

Molecular-size distribution. Molecular-size distribution is determined by a suitable method, for example, size-exclusion chromatography (*2.2.30*). It complies with the limits approved for the particular product.

Molecular identity and structural integrity. Depending on the nature of the monoclonal antibody, its microheterogeneity and isoforms, a number of different tests can be used to demonstrate molecular identity and structural integrity. These tests may include peptide mapping, isoelectric focusing, ion-exchange chromatography, hydrophobic interaction chromatography, oligosaccharide mapping, monosaccharide content and mass spectrometry.

Purity. Examine by a suitable validated method, such as SDS-polyacrylamide gel electrophoresis (*2.2.31*) under non-reducing and reducing conditions or capillary electrophoresis (*2.2.47*). Suitable tests for process-related and product-related impurities are carried out.

Stabiliser. Where applicable, it complies with the limits approved for the particular product.

Water (*2.5.12*). Freeze-dried products comply with the limits approved for the particular product.

Sterility (*2.6.1*). It complies with the test for sterility.

Bacterial endotoxins (*2.6.14*). It complies with the limits approved for the particular product.

Tests applied to modified antibodies. Suitable tests are carried out depending on the type of modification.

ASSAY

Carry out a suitable assay against the reference preparation. Design of the assay and calculation of the results are made according to the usual principles (for example, *5.3*).

STORAGE

As stated on the label.

Expiry date. The expiry date is calculated from the date of sterile filtration, the date of filling (for liquid preparations) or the date of freeze-drying (where applicable).

LABEL

The label states:

- the number of International Units per millilitre, where applicable;
- the quantity of protein per container;
- the quantity of monoclonal antibody in the container;
- for liquid preparations, the volume of the preparation in the container;
- for freeze-dried preparations:
  - the name and the volume of the reconstitution liquid to be added;
  - the period of time within which the monoclonal antibody is to be used after reconstitution;
- the dilution to be made before use of the product, where applicable.

01/2008:1468

PRODUCTS OF FERMENTATION

Producta ab fermentatione

This monograph applies to indirect gene products obtained by fermentation. It is not applicable to:

- *monographs in the Pharmacopoeia concerning vaccines for human or veterinary use*;
- *products derived from continuous cell lines of human or animal origin*;
- *direct gene products that result from the transcription and translation from nucleic acid to protein, whether or not subject to post-translational modification*;
- *products obtained by semi-synthesis from a product of fermentation and those obtained by biocatalytic transformation*;
- *whole broth concentrates or raw fermentation products.*

This monograph provides general requirements for the development and manufacture of products of fermentation. These requirements are not necessarily comprehensive in a given case and requirements complementary or additional to those prescribed in this monograph may be imposed in an individual monograph or by the competent authority.

DEFINITION

For the purposes of this monograph, products of fermentation are active or inactive pharmaceutical substances produced by controlled fermentation as indirect gene products. They are primary or secondary metabolites of micro-organisms such as bacteria, yeasts, fungi and micro-algae, whether or not modified by traditional procedures or recombinant DNA (rDNA) technology. Such metabolites include vitamins, amino acids, antibiotics, alkaloids and polysaccharides.

They may be obtained by batch or continuous fermentation processes followed by procedures such as extraction, concentration, purification and isolation.

PRODUCTION

Production is based on a process that has been validated and shown to be suitable. The extent of validation depends on the critical nature of the respective process step.

CHARACTERISATION OF THE PRODUCER MICRO-ORGANISM

The history of the micro-organism used for production is documented. The micro-organism is adequately characterised. This may include determination of the phenotype of the micro-organism, macroscopic and microscopic methods and biochemical tests and, if appropriate, determination of the genotype of the micro-organism and molecular genetic tests.

PROCESSES USING A SEED-LOT SYSTEM

The *master cell bank* is a homogeneous suspension or lyophilisate of the original cells distributed into individual containers for storage. The viability and productivity of the cells under the selected storage conditions and their suitability for initiating a satisfactory production process after storage must be demonstrated.

Propagation of the master cell bank may take place through a seed-lot system that uses a working cell bank.