

PRODUCTION AND QUALITY CONTROL OF MEDICINAL PRODUCTS DERIVED BY RECOMBINANT DNA TECHNOLOGY

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Additional Notes	This note for guidance is intended to facilitate the collection and submission of data to support applications for marketing authorisation within the EEC for polypeptide based products derived by rDNA technology and intended for medicinal use in man. It concerns the application of Part 2, sections A-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 derived by rDNA technology.

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PRODUCTION AND QUALITY CONTROL OF MEDICINAL PRODUCTS DERIVED BY RECOMBINANT DNA TECHNOLOGY

1. INTRODUCTION

Developments in molecular genetics and nucleic acid chemistry enable the genes coding for natural, biologically active proteins to be identified, analysed in fine detail, transferred between organisms, and expressed under controlled conditions so as to obtain synthesis of the polypeptide for which they code.

Sufficient quantities of medicinal products which were previously difficult to prepare from natural sources can now be produced using such recombinant DNA (rDNA) technology. In addition, the ability to synthesise and manipulate nucleic acids allows the construction of genes coding for modified products possessing different properties from their natural counterpart, or even entirely novel products.

A common strategy in the development of rDNA derived products is the insertion of naturally occurring or intentionally modified natural sequences or novel nucleotide sequences into a vector which is introduced into a suitable host organism so as to ensure the efficient expression of the desired gene product. Both prokaryotic and eukaryotic vector/host cell expression systems have been developed and are in use for production. The factors affecting the expression of foreign genes introduced into a new host using a suitable vector are complex and the efficient, controlled expression of stable, cloned DNA sequences is an important aspect of product development.

A flexible approach to the control of these products should be adopted so that recommendations can be modified in the light of experience of production and use, and with the further development of new technologies. Implementation of these recommendations for an individual product should reflect its intended clinical use.

This note for guidance is intended to facilitate the collection and submission of data to support applications for marketing authorisation within the European Union for polypeptide based products derived by rDNA technology and intended for medicinal use in man. It should be read in conjunction with the European Directives and other specialised guidelines where appropriate.

2. POINTS TO CONSIDER IN PRODUCTION

Requirements relating to establishments in which biological products are produced (e.g. GMP Directive 91/356/EEC and Directive 90/219/EEC on the contained use of genetically modified micro-organisms) will apply to the production of products derived by rDNA methodology as will several of the general recommendations for the quality control of biological products.

Thus, appropriate attention needs to be given to the quality of all reagents used in production, including components of fermentation media; specifications for these are to be included in documentation and they must comply with any relevant European recommendations (e.g.

note for guidance on *Minimising the Risk of Transmitting Agents causing Spongiform Encephalopathy via Medicinal Products*).

Tests for potency, abnormal toxicity, pyrogenicity and sterility etc., which apply to products made by conventional methods, will also apply to products made by rDNA technology. It is undesirable to use in production agents which are known to provoke sensitivity in certain individuals, such as, for example, penicillin or other β -lactam antibiotics.

Although comprehensive characterisation of the final product is essential, considerable emphasis must also be placed on “in-process” control, a concept which has been highly effective in the quality control of bacterial and viral vaccines prepared by conventional methods.

Certain factors may compromise the consistency, safety and efficacy of rDNA-derived products; these should be given special attention and are outlined below:

- a) All biological systems are inherently subject to genetic alteration through mutation and selection and foreign genes inserted into new host cells may exhibit increased genetic instability. The purpose of molecular genetic studies is to establish that the correct sequence has been made and incorporated in the host cell and that both the structure and the number of copies of the inserted sequence are maintained within the cell during culture to the end of production. Such studies can provide valuable information which should be considered in conjunction with tests performed at the protein level for assuring the quality and consistency of the product.
- b) Products expressed in foreign hosts may deviate structurally, biologically or immunologically from their natural counterparts. Such alterations can arise at post-translational level or during production or purification and may lead to undesirable clinical effects. Therefore, their presence must be justified and shown to be consistently controlled.
- c) The choice of manufacturing procedure will influence the nature, range and amount of potential impurities in the final product and which the purification processes must be shown to be capable of removing. Examples of these are endotoxins in products expressed in bacterial cells, and adventitious agents and DNA in products expressed in mammalian cells.
- d) Unintended variability in the culture during production may lead to changes which favour the expression of other genes in the host/vector system or which cause alteration in the product. Such variation might result in differing yield, in change to the product itself (e.g. in the nature and degree of glycosylation) and/or in quantitative and qualitative differences in the impurities present. Consequently, procedures to ensure consistency of production conditions as well as the final product are imperative.
- e) Extensive “scale-up” at the level of fermentation and/or purification occurs as laboratory developments progress to full scale commercial production, and this may have considerable consequences for the quality of the product including effects on its conformational structure, yield and/or in quantitative and qualitative differences in impurities. Therefore, sufficient in-process controls and quality control tests during each production run to show equivalency are required.

Whilst the recommendations set out below should be considered to be generally applicable, individual products may present particular quality control issues. Thus, the production and control of each product must be given careful individual consideration taking fully into account any special features.

3. DEVELOPMENT GENETICS

3.1 Gene of interest, Vector and Host Cell

A detailed description of the cloned gene should be given. This should include details of its origin, identification and isolation, as well as the details of the origin and structure of the expression vector. A description of the 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 cross-contamination with other cells or viruses.

3.2 Expression construct

Full details of the nucleotide sequence of the gene of interest and of the flanking control regions of the expression vector should be provided to confirm that the construction is identical to that desired. The steps in the assembly of the expression construct should be described in detail. A detailed map and a complete annotated sequence of functionally relevant regions of the vector should be given, indicating the regions which have been sequenced during the construction and those deduced from the literature. All the junctions created by ligation during construction directly impinging on the expression of the inserted gene should be confirmed by sequencing. All known expressed sequences should be clearly identified.

3.3 Status of the rDNA within the host cell

The method by which the vector is introduced into the host cell and the status of the rDNA within the host (integrated or extrachromosomal, copy number, etc.) should be described. For extrachromosomal expression systems, the percent of host cells retaining the expression construct should be determined. The coding sequence for the recombinant product of the expression construct should be verified at the cell bank stage. In systems where multiple integrated copies of the gene exist, which may or may not be the result of amplification, a detailed study using various restriction enzymes and Southern blot analysis should be used, in addition to sequence analysis of mRNA or cDNA molecules in order to provide convincing data on the integrity of the expressed gene(s).

3.4 Expression

The strategy by which the expression of the relevant gene is promoted and controlled during production should be described in detail.

3.5 Stability of the expression system

The stability of host/vector genetic and phenotypic characteristics should be investigated up to and beyond the population doubling level or generation number used for routine

production (End of Production Cells). The expression construct should be analysed in the End of Production Cells, as described above, at least once for each MCB.

Stability studies should also provide detailed information on:

- i) gene copy number in relation to productivity of the culture,
- ii) deletions and/or insertions affecting any part of the expression vector
- iii) the protein produced.

For this purpose, analysis should be performed in such a way that the results can confirm that the number of variants is below an acceptable limit to be established on a case by case basis depending on the nature and proposed use of the product. Analysis at the protein and/or at the DNA level can be envisaged. Whichever method is used, it should be validated and the detection limit given.

4. CONTROL OF CELL BANKS

It is essential that production is based on a well defined master and working cell bank system. During the establishment of the banks no other cell lines should be handled simultaneously in the same laboratory suite or by the same persons. The origin, form, storage, use, and expected duration at the anticipated rate of use must be described in full for all cell banks. New working cell banks should be fully characterised.

A critical part of quality control will involve the full characterisation of cells. Where eukaryotic cells are used for production, distinguishing genetic, phenotypic and immunological markers of the cell will be useful in establishing the identity of the cells. Likewise, where microbial cultures are used, specific phenotypic features which form a basis for identification should be described.

The cell banks should be examined for adventitious agents (viral, bacterial, fungal and mycoplasmal). Special attention should be given to viruses which can commonly contaminate the animal species from which the cell line has been derived. Certain cell lines contain endogenous viruses, e.g. retroviruses, which may not readily be eliminated. The possibility of mutations of endogenous viruses during prolonged culture should be considered. Furthermore, the purification process should be shown to be capable of removing and/or inactivating any such virus which may inevitably be present in the cells as an endogenous agent.

Cell banks should be periodically tested for cell viability, genetic and phenotypic stability and any other relevant parameters.

5. FERMENTATION OR CELL CULTURE

A clear definition of a "batch" of product for further processing should be provided.

Whatever the production process, details of the fermentation or culture with the in-process controls should be provided. Criteria for rejection of harvests and premature termination of the culture should be defined.

The presence, extent and nature of any microbial contamination in the culture vessels must be thoroughly examined at a suitable stage at the end of each production run. Detailed

information to confirm the adequate sensitivity of the methods used to detect contamination should be provided and acceptable limits of contamination set.

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 validation data presented for the absence of cross-contamination between them.

5.1 Single harvest production

The maximum permitted generation number or population doubling level for production should be defined and should be based on information concerning the stability of the host cell/vector system 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 host cell/vector 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.

5.2 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 duration of the culture. The required frequency and type of monitoring will depend upon several factors including the nature of the expression system and product, 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 product does not change with respect to specific parameters.

6. PURIFICATION OF THE PRODUCT

6.1 Methods

Methods used to purify the product and their in-process controls including their specification limits should be described in detail, justified and validated. 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. Attention is drawn to the notes for guidance “Production and Quality Control of Monoclonal Antibodies” and “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.

6.2 Validation of the purification procedure

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.

Studies using a carefully selected group of viruses which exhibit a range of physico-chemical features relevant to their behaviour on purification (see note for guidance *Virus Validation Studies: The Design, Contribution and Interpretation of Studies Validating the Inactivation and Removal of Viruses*) intentionally mixed with the crude preparation (spiking) should be undertaken. The ability of the purification process to remove other specific contaminants such as host-cell proteins, other potential impurities derived from the production process and DNA should also be demonstrated using, where necessary, concentrations of those contaminants in excess of that expected during normal production (spiking). A reduction factor for such contaminants at each stage of purification, and overall, should be established.

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. Columns should also be validated regarding leaching of ligands (e.g. dye, affinity ligand, etc.) and/or chromatographic material, throughout the expected life span of the column.

7. ACTIVE SUBSTANCE

7.1 Characterisation of the active substance

7.1.1 Physico-chemical characterisation, relative molecular mass, pI value

Rigorous characterisation of the active substance by chemical and biological methods will be essential. Particular attention should be given to using a wide range of analytical techniques exploiting different physico-chemical properties of the molecule; for instance, size, charge, isoelectric point and hydrophobicity. A list of the analytical possibilities is beyond the scope of this guideline. In the following there are only examples of the type of analysis.

7.1.2 Structural evidence for the active substance (including comparison with reference or natural product)

Sufficient sequence information to characterise the gene product adequately should be obtained. The degree of sequence verification required will depend on the size and complexity of the molecule, considering the extent of other characterisation tests. In most instances, determination of the entire sequence can be obtained after HPLC separation and sequencing of the peptides released by enzymatic digestion. Attention should be paid to the possible presence of N-terminal methionine and N-formyl methionine, signal or leader sequences, other possible N- and C-terminal modifications (proteolytical processing). It should be considered integrating modern mass spectrometry techniques in characterising the primary structure.

7.1.3 Post-translational modifications

Apart from the proteolytical processing, potential types of post-translational modifications are N- and O- glycosylation and for instance acetylation, hydroxylation and gamma-carboxylation. In addition, there are post-translational modifications that occur as degradation products such as deamidation and oxidation.

Some rDNA products are glycoproteins. There is a large range of oligosaccharide structures and these substances are characterised by glycoform heterogeneity both in their natural forms and those resulting from rDNA technology. The detail of this heterogeneity can be affected by many factors and the glycosylation pattern may have an important role in determining activity, particularly in vivo. The extent of analysis undertaken should depend on the role played by the carbohydrate moiety where this is known. A range of different analytical techniques should be explored, for instance quantitative isoelectric focusing or capillary electrophoresis, anion exchange chromatography for monosaccharide component analysis and oligosaccharide determination, lectin affinity chromatography, mass spectrometry.

7.1.4 Conformational data for macromolecules

It is desirable to include suitable tests to establish that the product has the desired conformational structure and state of aggregation. Examples of techniques suitable for such purposes are: polyacrylamide gel electrophoresis; isoelectric focusing; size exclusion, reversed phase ion exchange, hydrophobic interaction or affinity chromatography; peptide mapping and subsequent amino acid sequencing; light scattering; UV spectroscopy; circular dichroism and mass spectrometry. Additional characterisation of the product using for example NMR spectra, X-ray crystallography or relevant immunochemical techniques may provide valuable information.

7.1.5 Biological, immunological characterisation, expression of strength

Biological and immunological characterisation should include as wide a range of techniques as necessary. The specific activity of highly purified material should be determined (units of activity/weight of product).

When appropriate the biological activity of the product and its physical characteristics, including the amino acid sequence, should be compared with that of a highly purified preparation of the naturally occurring molecule.

7.2 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 for 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. Unwanted materials of host origin, as well as materials which may have been added during the production or purification processes, and where appropriate, viral and nucleic acid contamination should be tested.

8. CONSISTENCY AND ROUTINE BATCH CONTROL OF BULK FINAL ACTIVE SUBSTANCE

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. A clear difference should be made between the analytical tests performed during the development, in order to fully characterise the active substance and tests performed routinely on each production batch of purified bulk product.

8.1. Consistency

An acceptable number, for example 5 (smaller numbers could be acceptable where justified), of successive batches of the bulk processed product should be characterised as fully as possible to determine consistency of composition. In the case of a production where multiple harvests are applied, batches from different fermentation runs should normally be studied. The studies should include biological, chemical and immunological methods to characterise and assay the active substance (including methods showing the consistency of the glycosylation pattern for glycoproteins) and methods to detect and identify impurities. Any differences which occur between batches should be noted.

8.2. Routine batch control analysis

8.2.1 Identity

A selection of the tests used to characterise the purified active substance (see 7.1) should be used to confirm the product identity for each batch. The methods employed should include tests for the physico-chemical and immunological characteristics, together with test for the anticipated biological activity. Depending on the extent of other identification tests, sequence verification of a number of amino acids at the N- or C-terminus or other methods such as peptide mapping should be performed.

8.2.2 Purity

The degree of purity desirable and attainable will depend on several factors; these include the nature and intended use of the product, the method of its production and purification and also the degree of consistency of the production process. In general, a very high degree of purity can be achieved for most products by modern manufacturing procedures.

The purity of each batch should be established and be within specified limits. The analysis should include sensitive and reliable assays for DNA of host cell origin and/or of the vector applied to each batch of product prepared from cell lines of mammalian origin, in which case upper limits should be set. It is recommended that DNA analyses are also performed on each batch of bulk product obtained from other eukaryotic cell systems and limits set for DNA content. DNA of prokaryotic expression systems should be tested for wherever appropriate to consideration of the quality of the product. The residual cellular proteins should also be determined by an assay with appropriate sensitivity (e.g. ppm) and strict upper limits set. In some instances, potential impurities such as DNA can only be determined on intermediates of purification, at an earlier step.

8.2.3. Test for potency

The potency of each batch of the product should be established (e.g. units of biological activity per ml) using, wherever possible, an appropriate national or international reference preparation calibrated in units of biological activity (see section 9).

In addition, information on specific activity (units of biological activity per unit weight of product) will be of considerable value and should be reported. A highly purified reference preparation is required to standardise measurements of specific activity (see section 9).

It is recommended that correlation between potency measurements, involving biological tests, and the results of physico-chemical methods of assay are made and the information reported. If possible, batches should be calibrated using accurate physico-chemical tests, and the biological assays used to confirm -within stated limits- that the product is biologically potent.

9. SPECIFICATION AND REFERENCE MATERIALS

The studies described in section 7 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 8.

A suitable batch of the product, preferably one which has been clinically evaluated, should be fully characterised in terms of its chemical composition, purity, potency and biological activity, including where possible full amino acid sequencing, and retained for use as a chemical and biological reference material.

Criteria for expiration and possible re-testing and re-qualification of reference standards should be established.

10. 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 stabiliser 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.

GLOSSARY

1. Cell Banks

a) Master cell bank (MCB)

A homogeneous suspension of the original cells already transformed by the expression vector containing the desired gene, aliquoted into individual containers for storage (e.g. in a liquid nitrogen refrigerator). In some cases it may be necessary to establish separate master cell banks for the expression vector and the host cells.

b) Working cell bank (WCB)

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.

2. 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 (or duration of culture for certain production systems) 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.

3. Bulk harvest

This is a homogeneous pool of individual harvests or lysates which is processed in a single purification run.

4. Bulk final active substance

This is the final product, after completion of the production process, obtained from a bulk harvest. It is maintained in a single container or multiple identical containers where necessary 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.

5. 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.