



GUIDELINES FOR ASSURING THE QUALITY OF PHARMACEUTICAL AND  
BIOLOGICAL PRODUCTS MADE BY RECOMBINANT DNA TECHNOLOGY

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## 1. Introduction

These guidelines are concerned with the quality assurance of pharmaceutical and biological products made using recombinant DNA (rDNA) techniques and which are intended for use in humans. The purpose of the document is to indicate:

- (a) appropriate methods for the manufacture and testing of rDNA derived products, and
- (b) the information specific to rDNA products which should be submitted to national control authorities in support of clinical trial and marketing authorization applications.

It is recognized that rDNA technology is a rapidly evolving field and it is important that a flexible approach to the control of these products be adopted so that requirements can be modified in the light of experience of production and use, and with the further development of new technologies. The guidelines presented here therefore supersede those published in 1983 (Bulletin of the World Health Organization, 61 (3) (1983), 897-911) and the intention is to provide an updated and scientifically sound basis for the manufacture and control of medicinal products produced by new biotechnologies. Individual countries may wish to use these guidelines to develop their own national requirements for rDNA derived products. The document is not intended to apply to the control of genetically modified live organisms designed to be used directly in man, like live vaccines.

## 2. General Considerations

Advances in molecular genetics and nucleic acid chemistry now enable genes coding for natural biologically active proteins to be identified, analyzed in fine detail, transferred from one organism to another and expressed under controlled conditions so as to obtain efficient synthesis of the polypeptides for which they code. A gene is characterized by a specific nucleotide sequence in one strand of the double-stranded DNA molecule. When the strands are separated, each forms a template for the synthesis of a complementary copy, thus providing a mechanism for the faithful reproduction of genes with conservation of the linear sequence of the four mononucleotide building blocks. The process of decoding this information and the synthesis of the gene product occurs in two stages: first, transcription of the DNA coding strand in the form of a messenger RNA (mRNA) and, second, translation of the information carried by the mRNA molecule into a polypeptide. Genes coding for modified products, possessing enhanced biological activity and/or diminished undesirable characteristics, as well as for entirely novel substances can now be constructed.

A naturally occurring gene, or a synthetically derived nucleotide sequence, which codes for a specific product, can be propagated by inserting the DNA into a suitable vector, using highly specific restriction endonuclease enzymes (which cleave the vector DNA at predetermined sites) and ligases (which join the gene insert to the vector), and introducing the vector into a suitable host organism. Individual clones which carry the desired gene can then be selected and grown in mass culture so as to ensure the efficient expression of the desired gene product. The factors affecting the expression of foreign genes introduced into a new host are, however, complex and the efficient, controlled and faithful expression of stable, cloned DNA sequences is an important aspect of current research.

Many vectors in use at present are bacterial plasmids and much gene cloning has been carried out in prokaryotes. However, other vector-host cell systems involving eukaryotes, including yeasts or continuously growing (transformed) cell lines of mammalian or insect origin, have been developed and are, in some cases, already employed for production. The use of animal cells as hosts is considered by some to offer distinct advantages over bacterial systems. They can, for example, effect many of the post-translational modifications which normally occur to mammalian proteins, such as the addition of carbohydrate groups, and mammalian cells are thought more likely than bacteria to produce correctly folded and processed 'human' proteins. On the other hand, the use of animal cells as hosts does raise specific safety issues (see (ii) below).

Certain factors may compromise the safety and efficacy of rDNA derived products and these need special attention:

- (i) Products from naturally occurring genes expressed in foreign hosts may deviate structurally, biologically or immunologically from their natural counterparts. Such alterations can arise either at the genetic, post-transcriptional or post translational level, or during production and/or purification.
- (ii) rDNA-derived products may contain potentially hazardous contaminants not normally present in their equivalents prepared by conventional means and which the purification process must be shown capable of eliminating. Examples of these are endotoxins in products expressed in bacterial cells and contaminating cellular DNA and viruses in products derived from animal cells. Nucleic acid contamination from transformed mammalian cells is a particular concern because of the possible presence of DNA of oncogenic potential. The choice of manufacturing procedure will of course influence the nature and the range of possible contaminants.
- (iii) Extensive 'scale-up' is often required as laboratory developments progress to full scale production, and this may have considerable consequences for the quality of the product and thus implications for control testing. 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 alterations in the polypeptide product. Such variations might result in decreased yield of the product and/or quantitative and qualitative differences in the impurities present. Similar considerations apply to the use of continuous culture production. Consequently, procedures to ensure consistency of production conditions as well as final product are imperative.

### 3. Scope of Guidelines

The guidelines set out below are placed under three broad headings:

- (i) Control of starting materials, which includes base-line data on the host cell and on the source, nature and sequence of the gene used in production.
- (ii) Control of the manufacturing process.
- (iii) Control of final product.

In this respect, rDNA products are considered to be similar to biologicals produced by traditional methods, such as bacterial and viral vaccines, where adequate control is seen to relate to the starting materials and manufacturing procedure as much as to the product. Thus, the guidelines place considerable emphasis on 'in-process' controls for ensuring the safety and effectiveness of the product, as well as on the comprehensive characterization of the final product itself. The validation of certain aspects of the manufacturing process, such as the ability of the purification procedure to remove unwanted material like DNA, is also considered to be essential.

Requirements relating to establishments in which biological products are manufactured (e.g. Revised Requirements for Biological Substances No. 1: WHO Technical Report Series 323), will apply to products derived by rDNA methodology as will the general requirements 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. If animal-derived additives are used (e.g. calf serum) they should be shown to be free from adventitious agents. It is undesirable to use in production any agent known to provoke sensitivity reactions in certain individuals, such as penicillin or other B-lactam antibiotics. Many of the general requirements for the quality control of biological products, such as tests for potency, abnormal toxicity, pyrogenicity, stability and sterility, will also apply to products made by rDNA techniques.

Whilst the requirements set out below should be considered to be generally applicable, individual products may present particular quality control problems. The production and quality control of each product must therefore be given careful individual consideration, taking fully into account any special features. Furthermore, the implementation of requirements for a product must reflect its intended clinical use. Thus, a preparation which is to be administered repeatedly over a protracted period of time, or in large doses, is likely to need vigorous examination for traces of antigenic contaminants. A product to be used once in a life-threatening condition, however, might justifiably require different criteria.

When the term 'final product' or 'bulk final product' is used in these guidelines, it refers to the substance in question following purification but before final formulation.

#### 4. Requirements

##### 4.1 Control of Source Materials

###### 4.1.1 Expression Vector and Host Cell

A description of the host cell, its source and history, and of the expression vector used in production should be given. This should include details of the origin and identification of the gene which is being cloned as well as the construction, genetics and structure of the expression vector. An explanation of the source and function of component parts of the vector, such as origins of replication, promoters or antibiotic resistance markers, should be provided, as should a restriction enzyme digestion map indicating at least those sites used in construction.

Details of the method by which the vector is introduced into the host cell and the state of the vector within the cell, integrated or extrachromosomal, and copy number, should be provided. The genetic stability of the host vector combination should be documented.

#### 4.1.2 Sequence of cloned gene

The nucleotide sequence of the gene insert and of the flanking control regions of the expression vector should be provided. All relevant expressed sequences should be clearly delineated.

#### 4.1.3 Expression

Measures used to promote and control the expression of the cloned gene in the host cell during production should be described in detail.

### 4.2 Control of Production

#### 4.2.1 Master Cell Bank

The production of a rDNA product should be based on a seed lot system involving a master seed bank, or a manufacturer's working seed bank derived from the master seed lot. A host cell containing the expression vector should be cloned and used to establish a master cell bank. During the establishment of the seed no other cell lines should be handled simultaneously in the same laboratory suite or by the same persons.

The origin, form, storage and life expectancy at the anticipated rate of use of seed material should be described in full. Evidence for the stability of the host-vector expression system in the seed stock under conditions of storage and recovery should also be provided. New seed lots should be fully characterized and acceptance criteria established.

Where higher eukaryotic cells are used for production, distinguishing markers of the cell, such as specific isoenzyme or immunological features, will be useful in establishing the identity of the seed. Details of the tumourigenicity of continuous cell lines should be obtained and reported. Where microbial cultures are used, specific phenotypic features which can form a basis for identification should be described.

The DNA sequence of the cloned gene should normally be confirmed at the master seed stage. However, in certain cases, for example where multiple copies of the gene are inserted into the genome of a continuous cell line, it may be inappropriate to sequence the cloned gene at this stage. In such circumstances, southern blot analysis of the total cellular DNA, northern blot analysis of transcripts which contain the product sequence or sequence analysis of product related mRNA may be informative and particular attention should be paid to the characterization of the final product.

Evidence that the seed lot is free from potentially oncogenic, where appropriate, or infective adventitious agents, bacterial, mycoplasmal, fungal and viral, should be provided. Special attention should be given to viruses which commonly contaminate the animal species from which the cell line has been derived. Seed lots should preferably be free from all adventitious agents. However, certain cell lines contain endogenous viruses, e.g. retroviruses, which may not readily be avoided. The presence of these organisms should be sought under a variety of conditions known to cause their induction and the results reported. Specific contaminants identified as endogenous agents in the master cell bank, or as part of the vector, should be shown to be inactivated and/or removed by the purification procedures used in production.

#### 4.2.2 Production at Finite Passage

Procedures and materials used for cell growth and induction of the product should be described in detail. For each production run, the extent and nature of any microbial contamination in the culture vessels immediately prior to harvesting should be provided. The sensitivity of the methods used to detect such contamination should be described and acceptable limits of contamination set.

Data on the consistency of fermentation conditions, of growth of the culture and on the maintenance of yield of the product should be presented. Criteria for the rejection of culture lots should be established. The maximum number of cell doublings, or passage levels, to be permitted during production should be defined and based on information concerning the stability of the host cell/vector system upon serial sub-culture up to and beyond the level used in production.

A means of monitoring host cell/vector characteristics at the end of production cycles should be employed. For example, detailed information on plasmid copy number and degree of retention of the expression vector within the host cell, as well as restriction enzyme mapping of the vector containing the gene insert may be of value. The nucleotide sequence of the insert encoding the rDNA product should be determined, where appropriate (see 4.2.1), at least once after full-scale culture for each master cell bank. If the vector is present in multiple copies integrated into the host cell genome, confirming the rDNA sequence directly may be difficult. In such cases the isolation and determination of the nucleotide sequence of the product related mRNA, northern blot analysis of product related transcripts or southern blot analysis of the total DNA should be considered.

#### 4.2.3 Continuous Culture Production

As before (4.2.2), procedures and materials used for cell culture and induction of the product should be described in detail. In addition, very special consideration has to be given to the procedures used to control production. Monitoring will be necessary throughout the life of the culture, although the required frequency and type of monitoring will depend on the nature of the production system and the product.

Information will be required on the molecular integrity of the gene being expressed and on the phenotypic and genotypic characteristics of the host cell after long term cultivation. Evidence should also be produced to show that the yield does not vary beyond defined limits. The acceptance of harvests for further processing should be clearly linked to the schedule of monitoring applied and a clear definition of a 'batch' of product for further processing will be required. Criteria for rejection of harvests or termination of the culture should also be established. Regular tests for microbial contamination should be performed in relation to the strategy for harvesting.

The period of continuous culture 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. In cases of long term continuous culture, the cell line and product should be fully re-evaluated at intervals based on information concerning the stability of the host-vector system and the characteristics of the product.

#### 4.2.4 Purification

The methods used for harvesting, extraction and purification should be described in detail. Special attention should be given to the elimination of virus and nucleic acid contamination, and of undesirable antigenic materials.

Procedures which make use of affinity chromatography employing biological substances such as monoclonal antibodies should include appropriate measures to ensure that these substances, or any additional potential contaminants arising from their use, such as adventitious viruses, do not compromise the safety of the final product.

The capacity of the purification procedure to remove unwanted product-related or host cell-derived proteins, nucleic acid, carbohydrate, viruses or other impurities, including media-derived components and undesirable chemicals introduced by the purification process itself, should be investigated thoroughly. So also should the reproducibility of the process. Data from validation studies on the purification procedures may be required to demonstrate clearance of DNA or viruses for each purification step, and overall. Such studies, using pilot scale experiments, should be based on results with a carefully selected group of viruses which exhibit a range of physico-chemical features representative of potential contaminants, or radioactivity-labelled DNA, intentionally mixed with the crude preparation ("spiking"). The results would provide the theoretical extent of the removal of these contaminants during purification. Any virus inactivation process used should be shown to be effective and not to compromise the quality of the product.

#### 4.3 Control of Final Product

The identity, purity and stability of the ~~bulk~~ final product should be established. The type of testing necessary and the level of purity expected will depend on several factors, including the nature and intended use of the product, the method of its production and purification, and experience of the production process.

##### 4.3.1 Characterization of the Purified Active Substance

Rigorous characterization of the active substance by chemical, physical and biological methods will be essential. Particular attention should be given to using a wide range of analytical techniques exploiting different physicochemical properties of the molecule: size, charge, iso-electric point, amino acid composition and hydrophobicity. It may also be necessary to include suitable tests to establish that the product has the desired conformation 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; amino acid analysis; light scattering; UV spectroscopy; circular dichroism and other spectroscopic techniques. Additional characterization of the product using immunochemical techniques may also provide valuable information.

Where relevant and possible, properties of the product should be compared with those of the naturally occurring molecule.

The product should be shown to possess the anticipated biological activity of expected magnitude and its potency (units of biological activity/ml) established. In addition the determination of the specific activity of highly purified material will be of particular value (units of activity/weight of product).

Sufficient sequence information to adequately characterize the product should be obtained. The degree of sequence verification required will depend on the extent of other characterization tests. For some purposes partial sequence determination and peptide mapping may suffice, for others full sequence determination may be necessary. Attention should be paid to the possible presence of N-terminal methionine, signal or leader sequences and other possible N- and C-terminal modifications (such as acetylation, amidation or partial degradation by exopeptidases). Other post-translational modifications, such as glycosylation, should be identified and adequately characterized. Special consideration should be given to the possibility that such modifications are likely to differ from those found in a natural counterpart and may influence the biological, pharmacological and immunological properties of the product.

#### 4.3.2 Purity

Data should be provided concerning the contaminants present in the product, including estimates of their maximum levels. The degree of contamination considered as acceptable and criteria for the rejection of a production batch should be set.

It is important that the techniques used to demonstrate purity be based on as wide a range of physicochemical properties as possible. Attention should be given to tests for viral and nucleic acid contamination and for other unwanted materials of host or product origin, as well as to materials which may have been added during the production or purification processes. Limits should be set for all detected impurities and these should be identified and characterized as appropriate.

Substances which are to be administered repeatedly or in large doses should be assayed for trace antigenic constituents and product-related impurities, such as aggregates or degradation products likely to contaminate the final product, and strict upper limits set. Tests such as immunoblotting, radioimmunoassays and enzyme-linked immunosorbent assays using high affinity antibodies raised against the product, host cell lysates, appropriate subcellular fractions and culture medium constituents can be used to detect contaminating antigens. Because the detection of antigen will be limited by the specificity and sensitivity of the antisera used, these immunoassays will complement, but not replace other techniques such as Coomassie Blue and Silver stain analysis of sodium dodecyl sulphate polyacrylamide gel electrophoresis gels. Patients given large or repeated doses of a product during clinical trials should be monitored for the production of antibodies to contaminating antigens, and to the product.

#### 4.4 Routine Batch Control

It will be apparent that not all tests described above need to be carried out on each batch of product. Some tests are required only to establish the validity or acceptance of a procedure, whilst others might be performed on a limited series of batches in order to establish consistency of production. Thus, it will be expected that a comprehensive analysis of the initial batches of a product would 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.

##### 4.4.1 Consistency

An acceptable number, for example five, of successive batches of the processed product should be characterized as fully as possible as above to determine consistency of composition. Any differences which occur between batches should be noted. The data obtained from such studies should be used as the basis for product specification.

##### 4.4.2 Identity

A selection of the tests used to characterize the purified active substance should be used to confirm the product identity for each batch. The specific tests that will adequately characterize any particular product on a lot-to-lot basis will, however, depend on the nature of the product and on the method of production. Depending on the extent of other identification tests, sequence verification of a number of amino acids at the N- and C-terminus or other methods such as peptide mapping should be performed.

##### 4.4.3 Purity

The purity of each batch should be established and be within specified limits. This analysis should include sensitive and reliable assays for DNA of host cell origin (such as hybridisation analysis of immobilized contaminating DNA using appropriate probes) for each batch of product prepared from continuous lines of mammalian cells (transformed cell lines); strict upper limits should be set for DNA in the product. Theoretical concerns regarding transforming DNA derived from the cell substrates will be minimized by the general reduction of contaminating nucleic acid (WHO Technical Report, Series 747 (1987)). It is recommended that DNA analyses are also performed on each batch of product obtained from other eukaryotic cells, and limits set for DNA content, until further information on safety is obtained. DNA of prokaryotic expression systems should be tested for wherever appropriate to considerations of the quality and safety of the product.

For products to be administered for an extended period of time, or in high doses, the residual cellular proteins should also be determined by an assay with appropriate sensitivity (e.g. parts per million) and strict upper limits set.

##### 4.4.4 Potency

The potency of each batch of the product should be established using, wherever possible, an appropriate national or international reference preparation calibrated in units of biological activity. In the absence of such preparations, an approved in-house reference preparation may be used for assay standardization.

When sufficient correlation studies between physico-chemical or in vitro bioassays and in vivo biological assays have been obtained to prove that estimates from in vitro tests are sufficiently precise and accurate, then a requirement for an in vivo bioassay may be relaxed.

#### 5. Reference Materials

The studies described in Section 4.3 together with those in 4.4 will contribute to a definitive specification for the product.

A suitable batch of the product, preferably one which has been clinically evaluated, should be fully characterized in terms of its chemical composition, purity and biological activity, including where possible full amino acid sequencing, and retained for use as a chemical and biological reference material. Where appropriate these properties should be compared with those of a highly purified preparation of the naturally occurring molecule.

#### 6. Pre-Clinical Safety Evaluation

The general aim of pre-clinical safety testing is to determine whether new medicinal products have the potential to cause unexpected and undesirable effects. However, classical safety or toxicological testing as recommended for chemical drugs may be of only limited relevance for rDNA derived products. These pose particular problems in relation to toxicity testing in animals and their safety evaluation will have to take into account many factors. For example, certain proteins, like interferons, are highly species-specific and thus the human protein is much more pharmacologically active in man than in any other animal species. Furthermore, the amino acid sequence of human proteins will often be significantly different from their natural counterparts in other species, as will the carbohydrate groups. Thus, these proteins frequently produce immunological responses in foreign hosts which may ultimately modify their biological effects and which may result in toxicity due to immune complex formation. Such toxicity would, of course, have little bearing on the safety of the product in man, the intended host.

For these and other reasons, it is likely that a more flexible approach is necessary for the pre-clinical safety evaluation of rDNA products. Although there can be no doubt that some safety testing will be required for most products, the range of tests which need be applied should be addressed on a case-by-case basis in consultation with the national control authority. A wide range of investigative techniques, pharmacological, biochemical, immunological, toxicological and histopathological, should be used, where appropriate, in the assessment of a product's effect, over an appropriate range of doses, during both acute and chronic exposure, but always taking into consideration the points made above concerning species specificity and antibody formation. Where studies are expected to exceed a duration of four weeks, the use of test species known to be low responders with regard to antibody production against the test substance should be considered.

ANNEX

GLOSSARY

Plasmid

A plasmid is an autonomously replicating, circular, extrachromosomal DNA element. It usually carries a few genes, some of which confer resistance to various antibiotics; such resistance is often used to discriminate organisms that contain the plasmid from those that do not.

Vector

A vector is a piece of DNA that can direct its own replication within a host cell and to which other DNA molecules can be attached and thus amplified. Many vectors are bacterial plasmids, but in other instances the vector may integrate into the host cell chromosome following its introduction into the cell and is maintained in this form during the growth and multiplication of the host organism.

Master Seed/Cell Bank

This is a homogenous suspension of the original cells, already transformed by the expression vector containing the desired gene, aliquoted into individual containers for storage. All containers are treated identically during storage and once removed from storage, the containers are not returned to the seed stock. In some cases it may be necessary to establish separate seed banks for the expression vectors and the host cells.

Manufacturers Working Seed/Cell Bank

This is a homogenous suspension of the seed material derived from the master seed bank(s) by a finite passage level, aliquoted into individual containers for storage. As before, all containers are treated identically and once removed from storage they are not returned to the seed stock.

Production at Finite Passage

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

Continuous Culture Production

In this system the number of passages or population doublings are not restricted from the beginning of production. Strict criteria for terminating production have to be defined by the manufacturer.

Bulk Harvest

This is a homogenous pool of individual harvests or lysates which is processed in a single manufacturing run.

Bulk Final Product

This is the finished product following purification, but before final formulation. It is obtained from a bulk harvest, is maintained in a single container and used in the preparation of the final dosage form. The generation of the bulk final product has to be clearly defined and unambiguously recorded by the manufacturer.

The final dosage form is the finished formulated product which may be freeze-dried and contain excipients.