

Annex 3

Guidelines for assuring the quality of pharmaceutical and biological products prepared by recombinant DNA technology

| | |
|---|----|
| 1. Introduction | 59 |
| 2. General considerations | 60 |
| 3. Scope of guidelines | 61 |
| 4. Control of source materials | 62 |
| 5. Control of production | 63 |
| 6. Characterization of bulk product | 65 |
| 7. Routine control of final dosage form | 67 |
| 8. Reference materials | 68 |
| 9. Preclinical safety evaluation | 69 |
| References | 69 |
| Appendix | |
| Explanations of terms | 70 |

1. Introduction

These guidelines are concerned with the quality assurance of pharmaceutical and biological products made using recombinant DNA (rDNA) techniques and intended for use in humans.

Individual countries may wish to use this document to develop their own national guidelines or requirements for rDNA-derived products. It is not intended to apply to the control of genetically modified live organisms designed to be used directly in humans, e. g., live vaccines.

The purpose of the document is to indicate:

- appropriate methods for the manufacture and testing of rDNA-derived products; and
- information specific to rDNA products that should be included in submissions by manufacturers to national control authorities in support of applications for the authorization of clinical trials and marketing.

It is recognized that rDNA technology is a rapidly evolving field and that 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 the further development of new technologies. The

guidelines presented here therefore supersede those published in 1983 (1), and the intention is to provide an updated and scientifically sound basis for the manufacture and control of medicinal products produced by new biotechnologies.

2. General considerations

Advances in molecular genetics and nucleic acid chemistry now enable genes coding for natural biologically active proteins to be identified, analysed in fine detail, transferred from one organism to another and expressed under controlled conditions so as to synthesize efficiently the polypeptides for which they code. A gene is characterized by a specific nucleotide sequence in each 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 reproductions of genes while at the same time conserving the linear sequence of the four mononucleotide building blocks. The process of decoding this information and synthesizing the gene product takes place in the following two stages: (i) transcription of the DNA coding strand in the form of messenger RNA (mRNA) and; (ii) translation of the information carried by the mRNA molecule into a polypeptide. Genes coding for modified products possessing enhanced biological activity and/or fewer undesirable characteristics, as well as for entirely novel substances, can now be constructed.

A naturally occurring gene or a synthetically derived nucleotide sequence that codes for a specific product can be propagated by inserting the DNA into a suitable vector. For this purpose, highly specific restriction endonuclease enzymes (which cleave the vector DNA at predetermined sites) and ligases (which join the gene insert to the vector) are used, after which the vector is introduced into a suitable host organism. Individual clones that 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 objective 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 used for production. The use of animal cells as hosts is considered by some to offer distinct advantages as compared with bacterial systems. They can, for example, effect modifications, such as the addition of carbohydrate groups, which may take place on mammalian proteins. Correct processing is also more likely and secretion of the product into the culture medium avoids the need to

disrupt the cells and thus reduces potential contamination with host-cell proteins. On the other hand, the use of animal cells as hosts does raise specific safety issues (see below).

Certain factors may compromise the quality, safety and efficacy of rDNA-derived products and these need special attention, as indicated in the following paragraphs.

Products from naturally occurring genes expressed in foreign hosts may differ structurally, biologically or immunologically from their natural counterparts. Such differences can arise either at the genetic, post-transcriptional or post-translational level, or during production and/or purification.

In addition, 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 capable of eliminating. Examples include endotoxins in products expressed in bacterial cells and contaminating cellular DNA and viruses in those derived from animal cells. Contamination with nucleic acid from transformed mammalian cells is a particular concern because of the possible presence of potentially oncogenic DNA. The choice of manufacturing procedure will, of course, influence the nature and range of possible contaminants.

The “scaling up” of laboratory techniques into processes suitable for large-scale production may significantly affect the quality of the product and thus have major implications for control and testing. Unintended variability in the culture during production may lead to changes that favour the expression of other genes in the host/vector system or that 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 the consistency both of production conditions and of the final product are essential.

3. **Scope of guidelines**

The guidelines cover the following three main areas:

1. Control of starting materials, including baseline data both on the host cell and on the source, nature and sequence of the gene used in production.
2. Control of the manufacturing process.
3. Control of the 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 of the starting materials and manufacturing

procedure is just as necessary as that of the product. The guidelines therefore 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 materials, e.g., DNA, is also considered to be essential.

Requirements relating to establishments in which biological products are manufactured (e.g., the revised Requirements for Biological Substances No.1, (2) apply to rDNA-derived products, as do the general requirements for the quality control of biological products. Appropriate attention therefore 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 β -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, also apply to products made by rDNA techniques.

While the guidelines set out below should be considered as 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, any special features being taken fully into account. Furthermore, the guidelines for a product must reflect its intended clinical use. Thus, a preparation that is to be administered repeatedly over a protracted period of time, or in large doses, is likely to need careful testing for traces of antigenic contaminants. Different criteria might justifiably apply, however, to a product to be used only once but in a life-threatening condition.

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

4. Control of source materials

4.1 *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 identity of the gene 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 the 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, i.e., whether integrated or extrachromosomal, and copy number, should be provided. The genetic stability of the host-vector combination should be documented.

4.2 ***Sequence of cloned gene***

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

4.3 ***Expression***

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

5. **Control of production**

5.1 ***Manufacturer's working cell bank***

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

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

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

The DNA sequence of the cloned gene should normally be confirmed at the stage of the master seed lot. However, in certain cases, e.g., 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 that 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 infective bacterial, mycoplasmal, fungal, viral and, where appropriate, potentially oncogenic adventitious

agents should be provided. Special attention should be given to viruses that commonly contaminate the animal species from which cell lines are derived. Seed lots should preferably be free from all adventitious agents. However, certain cell lines contain endogenous viruses, e.g., retroviruses. Tests capable of detecting such organisms should be carried out under a variety of conditions known to cause their induction, and the results reported. Specific contaminants identified as endogenous agents in the master seed lot, or as part of the vector, should be shown to be inactivated and/or removed by the purification procedures used in production.

5.2 *Production at finite passage*

Procedures and materials used both for cell growth and for the induction of the product should be described in detail. For each production run, data on the extent and nature of any microbial contamination of the culture vessels immediately before harvesting should be provided. Acceptable limits for such contamination should be set and the sensitivity of the methods used to detect it indicated.

Data on the consistency of fermentation conditions and culture growth, and on the maintenance of product yield 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 specified, based on information on the stability of the host-cell/vector system on serial subculture up to and beyond the level used in production.

Host-cell/vector characteristics at the end of production cycles should be monitored, for which purpose detailed information on plasmid copy number and degree of retention of the expression vector within the host cell may be of value, as may restriction-enzyme mapping of the vector containing the gene insert. The nucleotide sequence of the insert encoding the rDNA product should be determined, where appropriate (see section 5.1), at least once after full-scale culture for each master seed lot. 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 total DNA should be considered.

5.3 *Continuous culture production*

As recommended in section 5.2, all procedures and materials used for cell culture and induction of the product should be described in detail. In addition, particular consideration should be given to the procedures used in production control. Monitoring is necessary throughout the life of the culture, although the frequency and type of monitoring required depend on the nature of both the production system and the product.

The molecular integrity of the gene being expressed and the phenotypic

and genotypic characteristics of the host cell after long-term cultivation should be established. Evidence should also be produced to show that variations in yield do not exceed the specified limits. The acceptance of harvests for further processing should be clearly linked to the monitoring schedule in use, and a clear definition of a “batch” of product for further processing will be required. Criteria for the rejection of harvests or termination of the culture should also be established. Regular tests for microbial contamination should be performed as appropriate to the harvesting strategy.

The maximum period of continuous culture should be specified, based on information on the stability of the system and consistency of the product during and after this period. In long-term continuous culture, the cell line and product should be fully re-evaluated at intervals determined by information on the stability of the host-vector system and the characteristics of the product.

5.4 **Purification**

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

In procedures involving affinity chromatography using biological substances, such as monoclonal antibodies, appropriate measures should be taken to ensure that these substances, or any other potential contaminants arising from their use, such as adventitious viruses, do not compromise the safety of the final product.

The ability 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, as should the reproducibility of the process. Data from validation studies on the purification procedures may be required to demonstrate clearance of DNA or viruses, both at each purification step and overall. In such pilot-scale studies, tests should be carried out with a carefully selected group of viruses exhibiting a range of physicochemical characteristics representative of potential contaminants, or with radiolabelled DNA, deliberately added to the crude preparation (“spiking”). The results will indicate the extent to which these contaminants can theoretically be removed during purification. Any virus-inactivation process used should be shown to be effective and not to compromise the quality of the product.

6. **Characterization of bulk product**

The identity, purity, potency and stability of the bulk product should be established. The type of testing necessary and the degree of purity

expected will depend on several factors, including the nature and intended use of the product, the method of production and purification, and experience with the production of several batches of the product.

6.1 **Characterization of 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, isoelectric 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. Techniques suitable for such purposes include polyacrylamide gel electrophoresis; isoelectric focusing; size-exclusion, reversed-phase, ion-exchange, hydrophobic-interaction or affinity chromatography; peptide mapping; amino-acid analysis; light scattering; and ultraviolet spectroscopy. Circular dichroism and other spectroscopic techniques can also provide valuable information.

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

The product should be shown to possess the expected biological activity; this should be of the expected magnitude and the potency of the product in appropriate units should be determined. In addition, the determination of the specific activity (units of activity/weight of product) of highly purified material is of particular value.

Sufficient sequence information to characterize the product should be obtained. The degree of sequence verification required will depend on the scope 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.

6.2 **Purity**

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

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 materials that may have been added during the production or purification processes. Limits should be specified for all impurities detected, and these should be identified and characterized as appropriate.

Substances that 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 specified. 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 antigens will be limited by the specificity and sensitivity of the antisera used, these immunoassays will complement, but not replace, other techniques, such as staining of gels used in sodium dodecyl sulfate/polyacrylamide gel electrophoresis. Patients given large or repeated doses of a product during clinical trials should be monitored for the production of antibodies both to contaminating antigens and to the product.

7. Routine control of final dosage form

It will be apparent that not all the tests described above need to be carried out on each batch of final dosage form. Some tests are required only to establish the validity or acceptability of a procedure, while others might be performed on a limited series of batches in order to establish consistency of production. Thus, a comprehensive analysis of the initial production batches should be undertaken to establish consistency with regard to identity, purity and potency; after the stability of the final dosage form has been established, a more limited series of tests may be appropriate, as outlined below.

7.1 Consistency

An acceptable number, e.g., five, successive batches of final dosage form should be characterized as fully as possible to determine consistency of composition. Any differences between one batch and another should be noted. The data obtained from such studies should be used as the basis for the product specification.

7.2 Identity

Each batch of final dosage form should be subjected to a selection of the tests used to characterize the purified active substance in order to confirm product identity. The specific tests that adequately characterize any particular product on a lot-to-lot basis, however, depend on both the nature of the product and the method of production. Depending on the

scope of other identification tests, sequence verification of a number of amino acids at the N- and C-termini, or the use of other methods, such as peptide mapping, will be necessary.

7.3 **Purity**

The purity of each batch of final dosage form should be determined and be within specified limits. The analysis should include sensitive and reliable assays for DNA of host-cell origin (e.g., hybridization 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 specified for the DNA content of the product. Theoretical concerns regarding transforming DNA derived from the cell substrates can be minimized by the general reduction in contaminating nucleic acid (3). DNA analyses should also be performed on each batch of product obtained from other eukaryotic cells, and limits specified for DNA content, until such time as further information on safety is obtained. Wherever appropriate from the point of view of the quality and safety of the product, tests for DNA of prokaryotic expression systems should be carried out.

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 of appropriate sensitivity and strict upper limits specified.

7.4 **Potency**

The potency of each batch of the final dosage form should be established using, wherever possible, an appropriate national or international reference material 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 physicochemical or *in vitro* bioassays and *in vivo* biological assays have been carried out showing that estimates based on *in vitro* tests are sufficiently precise and accurate, the requirement for an *in vivo* bioassay may be relaxed.

8. **Reference materials**

The studies described in section 6 together with those in section 7 will contribute to a definitive specification for the product.

A suitable batch of the product, preferably one that 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.

9. Preclinical safety evaluation

The general aim of preclinical safety evaluation 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 a large number of factors into account. Thus, certain proteins, e.g. interferons, are highly species-specific, so that the human protein is much more pharmacologically active in humans than in any other animal species. Furthermore, the amino-acid sequences of human proteins will often differ significantly from those of their natural counterparts in other species, as will the carbohydrate groups. Thus human proteins frequently produce immunological responses in foreign hosts which may ultimately modify their biological effects and may result in toxicity due to immune complex formation. Such toxicity would, of course, have little bearing on the safety of the product in the intended human host.

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

References

1. Quality control of biologicals produced by recombinant DNA techniques — WHO Consultation. *Bulletin of the World Health Organization*, 61: 897–911 (1983).
2. WHO Technical Report Series, No. 323, 1966.
3. WHO Technical Report Series, No. 747, 1987.

Appendix

Explanations of terms

Bulk harvest: A homogeneous pool of individual harvests or lysates processed in a single manufacturing run.

Bulk product: The product following purification, but before final formulation. It is obtained from a bulk harvest, and is kept in a single container and used in the preparation of the final dosage form.

Continuous culture production: A system in which the number of passages or population doublings after production has been started is not restricted. Strict criteria for terminating production must be specified by the manufacturer.

Final dosage form: The finished formulated product; it may be freeze-dried and contain excipients, which should have been shown not to affect stability adversely.

Manufacturer's working cell bank: A homogeneous suspension of the seed material derived from the master seed bank(s) at a finite passage level, dispensed in aliquots into individual containers for storage. All containers are treated identically and, once removed from storage, are not returned to the seed stock.

Master seed: A homogeneous suspension of the original cells, already transformed by the expression vector containing the desired gene, dispensed in aliquots into individual containers for storage. All containers are treated identically during storage and once removed from it are not returned to the seed stock.

Plasmid: 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 between organisms that contain the plasmid and those that do not.

Production at finite passage: A cultivation method involving a limited number of passages or population doublings which must not be exceeded during production.

Vector: 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 a vector may be integrated 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.