World Health Organization Technical Report Series, No. 771, 1988

Annex 7

REQUIREMENTS FOR HUMAN INTERFERONS MADE BY RECOMBINANT DNA TECHNIQUES

(Requirements for Biological Substances No. 41)

GENERAL CONSIDERATIONS

Advances in molecular genetics and gene engineering have made it possible to identify genes coding for biologically active substances, to analyse them in detail, to transfer them within and between organisms, and to obtain gene expression under controlled

conditions with efficient synthesis of the product for which they code. A gene that codes for a specific product can be isolated and propagated by inserting its DNA into a suitable vector with the aid of highly specific restriction endonuclease enzymes (which cleave the vector DNA at predetermined sites) and ligases (which join the gene insert to the vector). The vector can then be introduced into host organisms, and individual clones that carry the desired gene can be selected and propagated in mass culture.

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 mononucleotides. The process of decoding this information and the synthesis of the gene product occurs in two phases: first, transcription of the DNA coding strand in the form of messenger RNA (mRNA), and, second, translation of the information carried by the mRNA molecule into an amino acid sequence. The factors affecting the expression of foreign genes introduced into prokaryotic and eukaryotic cells are complex; indeed, the efficient and controlled expression of stable, cloned DNA sequences is an important field of current research. Currently, recombinant DNA products are produced by the following systems: bacteria, yeast, insect cells, and mammalian cells.

Interferons are proteins with antiviral, antiproliferative, and immunomodulatory properties. There are three classes of interferon: alpha-interferon (α), beta-interferon (β), and gamma-interferon (γ). Human alpha-interferon (HuIFN-α) represents a family of more than 23 species of structurally similar proteins, many of which have been cloned. Within the HuIFN-α family there are two subclasses of genes, a major one encoding for interferons of 165 or 166 amino acids and a second which encodes for those of 172 amino acids. Both subclasses are found on chromosome 9. Many of the species of HuIFN-α are potent antiviral agents with specific activities of approximately 2×10^8 units/mg protein and apparent relative molecular masses in the range 17–28 000. One major species of HuIFN-β has been identified, and the gene is also located on chromosome 9. This interferon is composed of 166 amino acids, and shares 34% sequence similarity HuIFN-α_{2b}. It is also a potent antiviral agent with a specific activity in the range $1–5 \times 10^8$ units/mg protein. A single gene on chromosome 12 codes for HuIFN-γ.

The gene codes for a mature protein of 143 amino acids and has little or no sequence similarity to HuIFN-α or HuIFN-β. Compared with HuIFN-α and HuIFN-β, HuIFN-γ is a more potent modulator of the immune response. Natural human beta- and gamma-interferons are glycosylated; several of the natural human alpha-interferons examined also appear to be glycosylated.

The genes for HuIFN-α_2 have been cloned and their products isolated in pure form. HuIFN-α_{2a} and HuIFN-α_{2b} were shown to have antiviral activity *in vitro* similar to that of interferon preparations obtained from paramyxovirus-induced human leukocytes and lymphoblastoid cells.

In general, an interferon derived from the cells of a given animal species is most active when it is used to treat cells from the same species; and, being a protein, it may be antigenic when administered to an animal from another species. Therefore, although human interferons have been used in a number of animal studies, these have for the most part little relevance to their use in man. However, the antiviral and antitumour activity of human interferon preparations in man is now well established.

The Requirements which follow should be considered in the control and testing of recombinant human alpha-2-interferon made by recombinant DNA methods. They have been formulated bearing in mind the scale-up required for commercial production. Particular emphasis is placed on "in-process control" and consistency of the manufacturing process, a concept which has been highly effective in the control of other biological products, rather than on relying entirely on tests on the final product. General requirements, such as tests for potency, identity, purity, toxicity, pyrogenicity and sterility, will apply as much to interferon made by recombinant DNA methods as to those derived from lymphoblastoid cells and peripheral blood lymphocytes. Certain tests will be required on every production batch of interferon, whereas others will be required only to establish the validity, acceptability, and consistency of a given manufacturing process.

A detailed description of the strategy by which the product is manufactured should be given. Evidence should be presented to show that interferon made by recombinant DNA techniques possesses antiviral activity, plus any other biological activity expected of the product. Rigorous identification and characterization of the recombinant-DNA-derived interferon will be required since structural alterations can arise at the genetic or post-trans-

lational level during cultivation or at the protein level during purification. Therefore structural and biological characterization is necessary for each lot to ensure product consistency. In addition, microbial contamination may occur during fermentation, and testing for contaminants must therefore be thorough. Finally, whenever possible, information pertaining to the chemical, structural, biological and/or immunological properties of the naturally occurring interferon component or components should be provided and compared with the corresponding properties of the recombinant product.

Special attention should be given to the potential presence of contaminants in the final product. For instance:

(1) Unwanted gene products may be co-expressed unexpectedly with the interferon. Such gene products might arise because (*a*) mutations, insertions, deletions or rearrangements in the coding region of the product occur during fermentation; (*b*) transcription initiates at several sites; or (*c*) changes occur during culture that affect transcription, initiation or termination processes favouring the expression of other genes in the vector or the host cell.

(2) Biologically active extraneous components such as DNA, proteins and any adventitious agents, including retroviruses, derived from the host-cell system may be found in the final product.

(3) Agents used in the purification process (column matrices, column ligands, e.g., antibodies) may give rise to specific contaminants in the final product.

Therefore the methods used for the purification and to identify and characterize the product must be described.

The product arising from the recombinant system should have biological activity in a cell line sensitive to the given interferon in conjunction with the appropriate international standard.

Each of the following sections constitutes a recommendation. The parts of each section that are printed in large type have been written in the form of requirements, so that, if a health administration so desires, these parts as they appear may be included in definitive national requirements. The parts of each section that are printed in small type are comments and recommendations for guidance.

Should individual countries wish to adopt these requirements as the basis of their national regulations concerning interferon made by recombinant DNA techniques, it is recommended that a clause should be included that would permit modifications of the

manufacturing requirements on the condition that it can be demonstrated to the satisfaction of the national control authority that such modified requirements ensure that the degree of safety and the potency of the recombinant interferon product are at least equal to those provided by the requirements formulated below. The World Health Organization should then be informed of the action taken.

The terms "national control authority" and "national control laboratory" as used in these Requirements always refer to the country in which the interferon is manufactured.

PART A. MANUFACTURING REQUIREMENTS

1. Definitions

1.1 International name and proper name

The international name shall be *Interferon humanum recombinatum*. The proper name shall be the equivalent of the international name in the language of the country of origin.

The use of the international name should be limited to interferons that satisfy the requirements formulated below.

1.2 Descriptive definition

Interferon humanum recombinatum is a preparation of purified interferon that has been derived through recombinant DNA techniques. The preparation shall satisfy all the requirements formulated below.

1.3 International standards and reference preparations

International standards and reference preparations shall be used for the control of interferon for use in the determination of potency.

For example, the International Standard for Interferon, Human, rDNA (HuIFN-α_2(αA)) is intended for comparison of the sensitivity of the bioassays in different laboratories for the measurement of the antiviral activity of interferon made by recombinant DNA techniques. This preparation was established in 1984 (*1*).

This standard should be used for the calibration of only those national preparations of HuIFN-α_2 that have dose–response curves parallel to the dose–response curve of this preparation.

Interferon standards and reference reagents, are held and distributed by the National Institutes of Health, Bethesda, MD, USA, and the National Institute for Biological Standards and Control, Potters Bar, England.

Samples from one or more final lots of material that has been shown to be active in clinical use, or samples directly related to such material, shall be fully characterized in ways to be specified by the national control authority and suitably stored to serve as manufacturer's reference material. For certain critical tests, such reference material shall be included in parallel with each lot of production material, which must match the specification of the reference batch with limits to be agreed by the national control authority.

1.4 Terminology

The following definitions are given for the purposes of these Requirements only.

Cell seed: a quantity of cells of uniform composition, stored frozen at −70 °C or below in aliquots, one or more of which would be used for the production of a manufacturer's working cell bank.

Several national control authorities have drafted documents on cell substrates used in the manufacture in their countries of biological products for human use.

Manufacturer's working cell bank (MWCB): a quantity of cells derived from one or more aliquots of the cell seed, that are of uniform composition and have been dispensed in a single working session into a number of ampoules, one or more of which would be used for the production of each single harvest.

In normal practice a cell seed is expanded by serial subculture up to a passage number (or population doubling, as appropriate) selected by the manufacturer and preserved cryogenically to form the MWCB.

Production cell culture: a collection of cell cultures being used for biological production that has been derived from one or more ampoules of the MWCB.

Single-harvest: the biological material prepared from a single production run.

Purified interferon bulk solution: interferon purified from one or more single harvests.

Final bulk: The finished biological material prepared from the purified interferon bulk solution present in the container from which the final containers are filled.

Final lot: a collection of sealed final containers that derive from the same final bulk and are homogeneous with respect to the risk of contamination during filling or preparation of the finished product. A final lot consists therefore of finished material dispensed into containers in one working session and processed as a single lot.

2. General Manufacturing Requirements

The general manufacturing requirements contained in the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*2*) shall apply to establishments manufacturing interferon, with the addition of the following directives.

Production areas shall be decontaminated before they are used for the manufacture of interferon.

The production of human interferon shall be conducted by staff who have not handled animals or infectious microorganisms in the same working day. The staff shall consist of persons whose state of health does not compromise the quality of the product.

No culture of any microorganism or eukaryotic cells, other than those required for the manufacturing process and approved by the national control authority, shall be introduced or handled in the production area at any time during the manufacture of the interferon including the establishment of the cell seed.

Persons not directly concerned with the production processes, other than official inspectors, shall not be permitted to enter the production area without valid reason and specific authorization.

Particular attention shall be given to the recommendation in Part A, section 1, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*2*) regarding the training and experience of persons in charge of production and testing and of those assigned to various areas of responsibility in the manufacturing establishment, as well as to the registration of such personnel with the national control authority.

The use of the seed lot system shall be strictly adhered to, and a description of the system used shall be provided, including the

number of vials of seed available and details of their storage. Particular attention shall be paid to the stability of the expression vector and to the plasmid copy number in the seed stock under conditions of storage and recovery.

Full details of the cell culture process used in manufacture shall be provided to the national control authority, with particular reference to tests to monitor the presence of microbial contamination. Information on the sensitivity of methods to detect such contamination and the frequency of the tests shall be provided, together with information on the criteria for the rejection of contaminated materials. All tests shall be validated to the satisfaction of the national control authority.

The yield of interferon shall be monitored during the course of individual production runs. Criteria, based on yield, for the acceptance of single harvests for further processing into a final lot shall be defined; and consistency of production shall be established by testing a number of consecutive lots prepared by the same procedures, which shall be determined by the national control authority.

3. Validation and Control of Manufacturing Procedures

The general production precautions formulated in Part A, section 3, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*2*) shall apply to the manufacture of interferon.

3.1 Strategy for cloning and expressing the gene

A full description of the biological characteristics of the host cell and expression vectors used in production shall be given. This shall include details of: (*a*) phenotypic and genotypic markers of the host cell; (*b*) the construction, genetics, and structure of the expression vector; (*c*) the analysis of host cell for viral particles and viral nucleic acid, where appropriate; and (*d*) the origin, identification and sequencing of the gene that is being cloned, including its flanking regions.

The association of the vector and host cell may be permanent, allowing continuous expression of the product, or self-limiting

—for example, where the vector is an acceptable cytopathic virus.

The physiological measures used to promote and control the expression of the cloned gene in the host cell shall be described in detail.

Data that establish the stability of the expression system during storage of the MWCB and beyond the maximum level used for production shall be provided. The stability of the expression system shall be monitored at intervals to be established by the national control authority. Any instability of the expression system that occurs in the seed culture or after a production-scale run, for example involving rearrangements, deletions or insertions of nucleotides, must be documented. Unstable preparations must *not* be used until approval to continue use has been obtained from the national control authority.

3.2 Biochemical characterization of recombinant vector

The nucleotide sequence of the gene insert and of adjacent segments of the vector and restriction enzyme mapping of the vector containing the gene inserts shall be provided in respect of the cell seed, the manufacturer's working cell bank, and the cells at the end of fermentation, as required by the national control authority.

3.3 Purification procedures

The methods used to purify the interferon from culture harvests shall be fully described. The capacity of each step of the purification procedure to remove substances other than interferon that may be derived from the host cell or culture medium, including in particular virus particles, proteins, and nucleic acids, shall be evaluated.

If individual contaminants are difficult to monitor, the results of pilot-scale studies to follow the removal of individual, deliberately added contaminants at appropriate stages of purification will provide valuable information in this respect.

If any substance is added during purification, it shall be reduced to an insignificant concentration during further purification or shown not to affect the safety and efficacy of the final product to the satisfaction of the national control authority.

If antibodies are used in the purification procedures, their origins and characteristics shall be fully described. The degree of purity of

monoclonal antibodies produced from hybridoma cell lines and the criteria for freedom from cell-derived or virus-derived DNA and from murine viruses shall conform to the regulations set by the national control authority.

3.4 Characterization of the gene product (interferon)

3.4.1 *Protein quantification*

The protein content of purified interferon bulk solution shall be established by quantitative amino acid analysis or by another accurate method in comparison to a reference reagent.

3.4.2 *Protein characterization*

The characterization of interferons shall be established by techniques approved by the national control authority, which will specify the procedures to be applied to one or more reference batches of purified interferon bulk solution and those to be applied to each purified interferon bulk solution.

The protein composition shall be analysed by techniques such as sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) performed under both reducing and non-reducing conditions and/or high-performance liquid chromatography (HPLC). In the case of SDS–PAGE, the stained gels shall be analysed by an appropriate method, such as scanning densitometry, to quantify the percentage purity of the interferon preparation. If bands other than the interferon monomers are observed on the gel, Western blot analysis using antibodies shall be used to identify which species are the interferon products (e.g., oligomers, fragments) and which are non-interferon contaminants.

> The following tests have also been found useful in characterizing the protein product: isoelectric focusing, size-exclusion chromatography, amino acid analysis, ultraviolet spectroscopy, affinity chromatography, circular dichroism, and neutralization by anti-interferon antibody.

Peptide mapping under reducing and non-reducing conditions shall be performed to provide confirmatory evidence that the structure of the product has not been altered.

The amino acid sequence of the amino terminal shall be analysed to confirm product identity and purity.

In addition, carboxy-terminal amino acid sequencing may be required by the national control authority.

3.4.3 *Consistency of production*

Data on the consistency of the production process shall be provided in terms of the specific activity (units of biological activity per mg of protein) at different stages in the production process and in terms of the purity (percentage content of extraneous protein) of each lot of purified interferon bulk solution. The national control authority shall approve the criteria for an acceptable production run.

4. Cell Seed and Manufacturer's Working Cell Bank (MWCB)

4.1 Origin of cell seed

Only cells approved by and registered with the national control authority shall be used to produce human interferon. If continuous cell lines are used, they shall be characterized as specified in the Requirements for Biological Substances No. 37 (Requirements for Continuous Cell Lines) (*3*). The national control authority shall have responsibility for approving the cell seed.

4.2 Characterization of cell seed and MWCB

The characteristics of the cell seed and manufacturer's working cell bank (host cell in combination with the expression vector system) shall be fully described, and information given on the absence of adventitious agents, and on genetic homogeneity. The nucleotide sequence of the human interferon gene insert and its flanking regions and the restriction mapping of the vector shall be given.

4.3 Phenotypic indicators of purity and genetic consistency of the recombinant cultures

Cells must be maintained in a frozen state that allows recovery of viable cells without alteration of genotype. Recovery of the cells from the frozen state shall be accomplished, if necessary, in selective media such that the genotype and phenotype are consistent with the characteristics of the original host-vector system. The identity of the cells shall be determined by the use of appropriate tests.

5. Controls for Mammalian Cell Cultures

If serum is included in the medium for the production cell cultures, it shall be tested to demonstrate freedom from bacteria, fungi, and mycoplasmas according the requirements in Part A, section 5.2 and 5.3 of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*4*) and to demonstrate freedom from viruses. The methods used shall be approved by the national control authority.

> Suitable tests for the detection of bovine viruses in serum are given in Appendix 3 of the revised Requirements for Biological Substances No. 7 (Requirements for Poliomyelitis Vaccine (Oral)) (*5*).
>
> Alternatively, the serum may be processed in various ways, such as by filtration together with irradiation or chemical treatment(s), which eliminate or inactivate any bacteria, fungi, viruses, or mycoplasmas that might be present in the untreated serum. The supporting data should be presented to the national control authority, and, if accepted, it may be agreed by the national control authority that tests on each batch of serum for production are not needed.

Beta-lactam antibiotics shall not be used at any stage in the production process.

6. Controls for Additives

Any additives, including any inducers and enhancers, shall be defined and approved by the national control authority. If the inducer is a virus, this shall be derived according to a seed lot system under approved manufacturing conditions.

Any additives used shall be added to the production cell cultures at a concentration within a range previously shown to yield a satisfactory crude product from cultures incubated at a temperature and for a period specified. These details shall be agreed with the national control authority. The manufacturer shall demonstrate to the satisfaction of the national control authority that the presence of inducers or enhancers in the crude product does not adversely affect its stability.

7. Production Precautions

7.1 Production cell cultures

Only cell cultures derived from the MWCB shall be used for production. All processing of cells shall be done in an area in which no other cells or organisms are handled, other than those directly required for the process.

7.2 Cultural conditions for production cell cultures

Production cell cultures shall be grown under conditions agreed with the national control authority. These conditions shall include details of the culture system used, the cell doubling time, the number of subcultures or the duration of the period of subcultivation permitted, and the incubation temperature.

Cell cultures shall be monitored for freedom from microbial contamination as required by the national control authority.

8. Single Harvests

The single harvests shall have been processed to remove cells and cell debris.

8.1 Sterility

The degree and nature of any microbial contamination shall be monitored during and at the end of the production runs by methods approved by the national control authority. The sensitivity of the test methods and criteria for the rejection of harvests shall be approved by the national control authority.

8.2 Consistency of yield

The yield of human interferon following production shall be shown to be within the limits approved by the national control authority (see Part A, section 3.4.3, of these Requirements).

8.3 Stability of the expression system

The method to assess the stability of the expression system used shall be approved by the national control authority.

8.4 Cell identification

Samples from each production run shall be tested to confirm the identity of the cells by a method and at intervals specified by the national control authority.

9. Purification

The purification procedure to be applied at any stage of the manufacturing process shall be approved by the national control authority. Human interferon shall be purified prior to formulation. Adequate purification may require several purification steps based on different principles; this will minimize the possibility of co-purification of extraneous cellular materials. The methods used for purification of the human interferon shall be appropriately validated (see Part A, section 3.3, of these Requirements) and approved by the national control authority. The purified bulk solution may be stabilized by the addition of protein or other substances of a nature and at a concentration approved by the national control authority. If the stabilizing substance is of human origin, it shall be manufactured in such a way as to ensure its freedom from adventitious agents. Any substances added shall not impair the safety and efficacy of the product.

The samples required for certain tests such as those for protein content, purity and composition analysis (e.g., SDS–PAGE), residual cellular DNA, peptide mapping, HPLC, and amino acid sequencing must be taken before any proteinaceous stabilizer is added. The test methods used shall be approved by the national control authority. Tests, to be approved by the national control authority, shall be made for any materials of animal origin (e.g., serum protein) or plant origin (e.g., lectin) used at any stage of production and purification; and the national control authority shall determine the acceptable levels of such materials in the interferon preparation.

In batches of HuIFN-α_2 produced by manufacturers, the purity of HuIFN-α_2 was greater than 95% as determined by photometric scanning of gels following reducing and non-reducing SDS–PAGE, the relative molecular mass was estimated to be 19 000, and the specific activity was approximately 2×10^8 International Units per mg of protein, as determined by protection against the cytopathic effect of virus.

Cell debris and nucleic acids were flocculated and removed by centrifugation. HuIFN-α_{2a} in the supernatant fluid was purified by a series of procedures involving affinity chromatography on immobilized anti-interferon monoclonal antibodies, ion-exchange chromatography, and molecular-exclusion chromatography.

9.1 Assay for protein content

The total protein content of the human interferon shall be quantified (see Part A, section 3.4.1, of these Requirements).

9.2 Test for human interferon content

The human interferon content of the purified preparation shall be determined by an appropriate biological method.

Tests that have been found suitable include SDS–PAGE, radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), and single radial immunodiffusion with comparison to a known standard. Analysis of the data by the parallel-line method has been found suitable for most of these techniques.

9.3 Test for additives used during purification or other phases of manufacture

A test shall be made for the presence of any potentially hazardous additives used in manufacture. The method used and the permitted concentration shall be approved by the national control authority.

9.3.1 *Monoclonal antibody*

When a monoclonal antibody is used in the preparation procedure (e.g., for use in immunosorbent affinity chromatography to purify HuIFN-α_2) the product shall be tested for residual antibody. The method used and the permitted concentration of antibody shall be approved by the national control authority.

Several national control authorities have drafted monographs on the control of monoclonal antibody preparations used for the manufacture of biological products for human use.

9.3.2 *Antibiotics*

A test shall be made as required by the national control authority for the presence in the interferon preparation of any antibiotics used

in the manufacturing process. The assay methods shall be described, and the permitted concentration in the final product shall be approved by the national control authority.

9.4 Test for identity (molecular and immunochemical identity)

Tests shall be made for the identity of the human interferon product by SDS–PAGE and/or neutralization of biological activity, as required by the national control authority.

9.5 Protein purity

The purity of each purified interferon bulk solution before the addition of any stabilizing solutions shall be established by methods approved by the national control authority.

Techniques that are useful include SDS–PAGE under reducing and non-reducing conditions, HPLC, Western blot analysis, isoelectric focusing, size-exclusion chromatography, peptide mapping, amino acid composition and sequence analysis, ultraviolet spectroscopy, and circular dichroism.

Procedures used for HuIFN-α_{2a} and HuIFN-α_{2b} have included SDS–PAGE, amino acid analysis and sequencing, peptide mapping, and isoelectric focusing. Purity of greater than 95% has been established for these preparations.

9.6 Test for serum proteins

If serum is used in the medium for the production cell cultures, or at any stage in the purification process, for example, as a reagent in immunosorption chromatography, tests shall be made for any residual serum in the purified interferon bulk blend by radio-immunoassay, ELISA, or another test agreed with the national control authority.

9.7 Test for residual DNA

The amount of residual DNA in each batch of product shall be determined by sensitive methods, which must be validated and approved by the national control authority. The acceptable level of DNA per human dose shall be determined by the national control authority.

A WHO Study Group on Biologicals (*6*) concluded that the probability of risk associated with heterogeneous contaminating

DNA in a product derived from a continuous cell line is negligible when the amount of such DNA is 100 pg or less in a single dose given parenterally.

10. Final Bulk

Substances such as diluent, stabilizers and/or preservatives added to the purified interferon bulk solution shall be approved by the national control authority.

10.1 Test for sterility

The final bulk shall be tested for bacterial and mycotic sterility according to the requirements in Part A, section 5.2, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*4*).

10.2 Test for pyrogenic substances

The pyrogen content shall be determined by a method agreed with the national control authority.

11. Filling and Containers

The requirements concerning filling and containers in Part A, section 4, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*2*) shall apply.

Care shall be taken to ensure that the materials of which the container and closure are made do not react with interferon.

12. Control of the Final Lot

Samples shall be taken from each final lot for the following tests.

12.1 Tests for sterility

Each final lot shall be tested for sterility according to the requirements in Part A, section 5, of the revised Requirements for

Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*4*).

12.2 Test for identity

Human interferon made by recombinant DNA techniques shall be identified as human interferon by appropriate methods approved by the national control authority.

Radioimmunoassay, ELISA, and neutralization assay are useful methods.

12.3 Test for potency

The test for potency, which shall be based on a biological activity and approved by the national control authority, shall be performed on samples representative of the final filling lots. The essential information to be provided shall be that indicated in WHO Technical Report Series, No. 687, Annex 1 (*7*). An appropriate reference preparation shall be tested in parallel. Statistical analysis of the data must show that the mean potency value obtained has confidence limits within a range accepted by the national control authority.

12.4 Tests for innocuity

Each final lot shall be tested for innocuity in mice and guinea-pigs by methods approved by the national control authority.

12.5 Test for pyrogenic substances

Each final lot shall be tested for pyrogenic substances by a method approved by the national control authority.

12.6 Test for preservative

Each final lot shall be tested for the presence of preservative. The test used and the permitted concentration shall be approved by the national control authority.

12.7 Tests for additives

Each final lot shall be tested for the presence of additives. The tests used and the permitted concentrations shall be approved by the national control authority.

12.8 Moisture content

For lyophilized products. the moisture content per vial shall not exceed a level approved by the national control authority.

12.9 pH and clarity

The pH and degree of clarity of the interferon solution in the final container or in the reconstituted final container shall be within the limits approved by the national control authority.

13. Records

The requirements in Part A, section 6, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*2*) shall apply.

14. Samples

The requirements in Part A, section 7, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*2*) shall apply.

15. Labelling

The requirements in Part A, section 8, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*2*) shall apply, with the addition of the following directive.

The leaflet accompanying the package shall include the following information:

(*a*) description of the product,
(*b*) clinical pharmacology,
(*c*) indications and usage,
(*d*) contraindications,
(*e*) warnings,
(*f*) precautions,
(g) use during pregnancy,
(*h*) adverse reactions,
(*i*) dosage and administration,
(*j*) directions for use,
(*k*) how supplied,
(*l*) storage conditions,
(*m*) references.

16. Distribution and Shipping

The requirements in Part A, section 9, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories (*2*) shall apply.

17. Storage and Expiry Date

The requirements in Part A, section 10, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*2*) shall apply.

17.1 Storage conditions

Filled containers of human interferon intended for clinical use shall be stored under conditions such that the product conforms with the specification agreed with the national control authority during the claimed shelf life.

17.2 Expiry date

The expiry date shall be fixed with the approval of the national control authority on the basis of evidence for stability supplied by the manufacturer.

The stability of freeze-dried preparations may be determined by the methods indicated in WHO Technical Report Series, No. 687, Annex 1, Appendix 3 (*7*).

PART B. NATIONAL CONTROL REQUIREMENTS

1. General

The general requirements for control laboratories contained in Part B of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*2*) shall apply. The national control authority shall:

(*a*) approve the methods for producing human interferon by recombinant DNA techniques;
(*b*) approve the tests for human interferon concentration and define the minimum acceptable value of that concentration;
(*c*) approve the cell seed and manufacturer's working cell bank (MWCB);
(*d*) approve the methods for purification;
(*e*) approve the tests for the purity of the product;
(*f*) approve the tests for extraneous substances and total protein;
(*g*) approve the tests for preservative and for the agents used for purification or other aspects of manufacture;
(*h*) approve the tests for freedom from abnormal toxicity in the final product;
(*i*) approve the types of tests used in the assay of potency; and
(*j*) approve the data to establish clinical activity and safety in humans.

The national control authority shall be satisfied that the results of all tests, including those done on individual batches during the process of manufacture, are satisfactory and that consistency has been established.

2. Release and Certification

Human interferon made by recombinant DNA techniques shall be released only if it fulfils the above requirements.

A statement signed by the appropriate official of the national control authority shall be provided at the request of the manufacturing establishment and shall certify whether or not the final lot of human interferon in question meets all national requirements as well as the above requirements. The certificate shall state the date of the last satisfactory human interferon potency test, the lot number, the number under which the lot was released, and the number appearing on the labels of the containers. In addition, a copy of the official national release document shall be attached.

The purpose of the certificate is to facilitate the exchange of human interferon between countries.

AUTHORS

The first draft of the Requirements for Human Interferons made by Recombinant DNA Techniques was prepared in 1986 by:

Dr V. Grachev, Biologicals, World Health Organization, Geneva, Switzerland
Dr J. Petricciani, Chief, Biologicals, World Health Organization, Geneva, Switzerland

The second draft of these Requirements was formulated in March 1987 by WHO staff together with:

Dr K. Zoon, Chief, Laboratory of Immunology, Center for Drugs and Biologics, Food and Drug Administration, Bethesda, MD, USA (*Consultant*)

The third draft of these Requirements was formulated by the following participants in a WHO Informal Consultation on the Standardization of Interferons, Geneva, 23–25 March 1987:

Dr O.G. Andzhaparidze, Director, Moscow Research Institute for Viral Preparations, Moscow, USSR
Dr T.T. Ayres, Quality Assurance Division, Wellcome Research Laboratories, Beckenham, Kent, England
Dr E. De Maeyer, CNRS Director of Research, Biology Section, Curie Institute, Orsay, France
Dr N. Finter, Development Division, Wellcome Research Laboratories, Beckenham, Kent, England
Professor R. Friedman, Chairman, Department of Pathology, Uniformed Services University of the Health Services, Bethesda, MD, USA
Professor S.E. Grossberg, Department of Microbiology, Medical College of Wisconsin, Milwaukee, WI, USA
Dr S.M. Kramer, Genentech Inc., San Francisco, CA, USA
Dr C.A. Laughlin, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA
Dr P.J. Leibowitz, Director, Molecular and Analytical Biology, Schering-Plough, Bloomfield, NJ, USA

Dr A. Meager, Division of Immunobiology, National Institute for Biological Standards and Control, London, England
Mr M. Narachi, Amgen, Thousand Oaks, CA, USA
Dr M. Truehaft, Section Head, Biological Assay Research, Schering-Plough, Bloomfield, NJ, USA
Professor J. Vilcek, Department of Microbiology, New York University Medical Center, New York, NY, USA
Dr S. Yamazaki, Director, Central Virus Diagnostic Laboratory, National Institute of Health, Tokyo, Japan
Dr K. Zoon, Chief, Laboratory of Immunology, Center for Drugs and Biologics, Food and Drug Administration, Bethesda, MD, USA

WHO secretariat

Dr J. Esparza, Microbiology and Immunology Support Services, World Health Organization, Geneva, Switzerland
Dr V. Grachev, Biologicals, World Health Organization, Geneva, Switzerland
Dr P. Sizaret, Biologicals, World Health Organization, Geneva, Switzerland

REFERENCES

1. WHO Technical Report Series, No. 725, 1985, p. 24.
2. WHO Technical Report Series, No. 323, 1966, Annex 1.
3. WHO Technical Report Series, No. 745, 1987, Annex 3.
4. WHO Technical Report Series, No. 530, 1973, Annex 4.
5. WHO Technical Report Series, No. 687, 1983, Annex 4.
6. WHO Technical Report Series, No. 747, 1987.
7. WHO Technical Report Series, No. 687, 1983, Annex 1.