

Annex 3

REQUIREMENTS FOR HUMAN INTERFERONS PREPARED FROM LYMPHOBLASTOID CELLS

(Requirements for Biological Substances No. 42)

	Page
General considerations.....	72
Part A. Manufacturing requirements.....	76
1. Definitions	76
2. General manufacturing requirements	79
3. Validation and control of manufacturing procedures	80
4. Manufacturer's working cell bank (MWCB)	83
5. Controls for mammalian cell cultures	83
6. Controls for additives	84
7. Production precautions.....	84
8. Single harvests	84
9. Purification	85
10. Final bulk	88
11. Filling and containers.....	88
12. Final lot.....	88
13. Records.....	90
14. Samples.....	90
15. Labelling	90
16. Distribution and shipping.....	91
17. Storage and expiry date.....	91
Part B. National control requirements.....	91
1. General	91
2. Release and certification.....	92
Authors	93
Acknowledgements.....	93
References	93

GENERAL CONSIDERATIONS

The interferons (IFNs) are a family of proteins initially thought to be of interest as inhibitors of virus growth. They are now known

to have many other biological effects *in vivo*, including effects on cell growth and on many functions of the immune system. These probably reflect metabolic changes produced after an interferon is bound to a specific receptor on the cell surface.

Interferons are formed by cells in response to stimuli of various types, collectively known as inducers, which may be viruses, chemical substances, or immunological interactions. They are classified on the basis of antigenicity into three types: IFN- α , IFN- β , and IFN- γ . In humans, many individual subtypes of IFN- α are formed, and at least 23 are now known. These are termed HuIFN- α_1 , - α_2 , - α_3 , etc. (HuIFN- α_1 was previously known as HuIFN- α D and HuIFN- α_{2a} as HuIFN- α A). They are potent antiviral agents and in pure form have specific activities generally of the order of 2×10^8 units/mg of protein. Most of these subtypes of HuIFN- α contain 166 amino acids, of which about 65% are the same in all the subtypes, but there are reports that some shorter forms have been isolated from human leukocyte interferon preparations. Their chemical composition suggests that their relative molecular masses are in the region 18 000–20 000, but when analysed by gel filtration, some appear to have higher relative molecular masses of about 26 000–28 000. Few if any of the HuIFN- α subtypes contain carbohydrate in an N-glycosylated form, but some may be O-glycosylated. Individual subtypes differ not only in their chemical composition, but also in physical and antigenic characteristics. Furthermore, when tested *in vitro*, each has a unique spectrum of biological effects; differences in *in vivo* effects have also been seen in very limited clinical studies with subtypes α_1 and α_{2a} .

One major species of human IFN- β has been identified, composed of 166 amino acids; it shows 34% sequence similarity to HuIFN- α_{2b} . It is also a potent antiviral agent with a specific activity in the range of $1\text{--}5 \times 10^8$ units/mg of protein. HuIFN- γ has little or no sequence similarity to HuIFN- α or HuIFN- β and is a more potent modulator of the immune response. The mature gene codes for a protein of 143 amino acids. Natural HuIFN- β and HuIFN- γ are glycosylated.

In general, an interferon derived from the cells of one animal species is most active when it is used to treat cells from the same species, but being a protein, it may be immunogenic when administered to an animal of 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 humans.

The antiviral and antitumour activity of some human interferon preparations in human subjects is now well established.

Preparations of HuIFN- α for clinical use can be obtained in several quite different ways. Originally, so-called "leukocyte" interferon was obtained from human peripheral blood cells, with Sendai virus as the inducer. However, only small amounts of this interferon are obtained from each blood donation so that a very large number of donors are needed to produce it in amounts sufficient for large-scale clinical use. For this reason HuIFN- α preparations are now generally obtained by two other routes. One involves recombinant DNA procedures; in particular, HuIFN- α_2 has been expressed by cultures of *Escherichia coli* which have been transformed by a vector carrying the cloned DNA sequence specifying the mature protein. In the second approach, cultured human lymphoblastoid cells are treated with an inducer.

Cells of lymphoblastoid lines have the characteristics of transformed cells. For the most part, they grow readily *in vitro* and can be subcultured indefinitely. They are usually aneuploid and have chromosomal markers which may be characteristic for the particular line. Some lines, particularly those derived from Burkitt's lymphoma tissue, are tumorigenic.

The use of a transformed cell line, particularly one of neoplastic origin, as the substrate for the manufacture of a human pharmaceutical product raises questions of safety, which were first posed in connection with the production of alpha-interferons from cells of the Namalwa human lymphoblastoid line, which originated from Burkitt's lymphoma tissue. Over a period of some ten years, these questions were debated at a number of international and national scientific meetings. There is now a consensus that continuous cell lines can be used for pharmaceutical manufacture, provided that the final product is adequately purified, and subject to certain safeguards (1).

The requirements which follow take account of these points and should accordingly be met in the production control and testing of any human lymphoblastoid interferon preparation. These requirements have been formulated in relation to large-scale production, with particular reference to IFN- α but, with appropriate changes, are likely to be applicable to preparations of IFN- β or IFN- γ similarly derived from lymphoblastoid cells. They apply to the cells used as the substrate, the final purified product, and the method used for purification, which must be shown to inactivate or eliminate any

actual or potential noxious contaminants present in the crude product. 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 reliance 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 prepared from lymphoblastoid cells as to those made from other cell substrates or by recombinant DNA techniques. Certain tests will be required on every production batch of interferon. Others will be required only to establish the validity and acceptability of a particular part of the proposed manufacturing process. Any such tests must form part of or be in addition to those so far applied during the manufacture of conventional biologicals, such as vaccines, derived from cell cultures.

Special attention should be given to the potential presence in the final product of contaminants such as:

- (a) biologically active extraneous components (e.g., DNA, proteins, adventitious infectious agents, and endogenous retroviruses derived from the host cells), which may be present in the crude product but should be excluded from the final product;
- (b) materials derived from the culture medium, substances used to enhance interferon production, the interferon inducer used, and chemical substances derived from those used during purification (e.g., column matrices and antibodies).

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

The manufacturer should give a value for the specific activity of the purified human lymphoblastoid interferon in each lot of the final product, i.e., the biological potency (in International Units, whenever possible) per milligram of total protein (before the addition of any proteinaceous stabilizer) and its confidence limits. A minimum acceptable specific activity for each lot of final product should be proposed by the manufacturer. Tests that merely measure interferon protein, whether radioimmunometric or other, should not be used to control the potency of the final product, since evidence is required that the protein is biologically active.

Each of the following sections constitutes a recommendation. The parts printed in large type have been written in the form of requirements so that, if a health administration so desires, they may

be included in definitive national requirement as they stand. The parts printed in small type are comments or recommendations for guidance.

Should individual countries wish to adopt these requirements as the basis of their national regulations on human lymphoblastoid interferon, it is recommended that a clause should be included permitting modifications of 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 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 and/or used.

PART A. MANUFACTURING REQUIREMENTS

1. Definitions

1.1 International name and proper name

The international name shall be *Interferonum lymphoblastoidis humanum*, *alpha* or *beta* or *gamma*, followed in parenthesis by the name or designation of the cell line used as the source, e.g., "(*Namalwa*)". 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 preparations that satisfy the requirements given below.

1.2 Descriptive definition

Human lymphoblastoid alpha-interferon is a preparation containing a number of individual alpha-interferon subtypes produced by the induction of a human lymphoblastoid cell line; these interferons are harvested from the supernatant fluids, purified, and prepared in a form suitable for injection and that satisfies all the requirements formulated below.

1.3 International reference materials

The International Standard for Human Lymphoblastoid Interferon (Namalwa) (HuIFN- α (Ly)) was established in 1984. Each ampoule contains, by definition, 25 000 IU. The interferon included in the standard preparation was derived from Namalwa cells and induced with Sendai virus. It will be necessary to establish that this International Standard is appropriate for the standardization of other preparations of lymphoblastoid interferon derived from other lines or lymphoblastoid cells or with the use of inducers other than Sendai virus.

A number of other standard preparations for human interferons are available.

These include: the Second International Standard for Interferon, Human, Fibroblast, β , containing 15 000 IU/ampoule; the International Standard for Interferon, Human, Recombinant, $\alpha_1(\alpha D)$, containing 8000 IU/ampoule; the International Standard for Interferon, Human, Recombinant, $\alpha_2(\alpha_2b)$, containing 17 000 IU/ampoule; and the International Standard for Interferon, Human, Recombinant, β_{ser17} , containing 6000 IU/ampoule.

In addition, three standard preparations for murine interferons are available: the International Standard for Interferon, Murine, α , containing 16 000 IU/ampoule; the International Standard for Interferon, Murine, β , containing 15 000 IU/ampoule; and the International Standard for Interferon, Murine, γ , containing 1000 IU/ampoule.

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.

1.4 Terminology

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

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

Several national control authorities have drafted documents relating to cell substrates used in the manufacture of biologicals for human use.

Manufacturer's working cell bank (MWCB): a single uniform suspension of lymphoblastoid cells which have been dispensed in a

single working session into a number of containers which are stored at -70°C or below. Cells revived from one or more of these containers are used as a source of production cell cultures.

In normal practice a cell seed is expanded by serial sub-culture up to a passage number (or population doubling, as appropriate) selected by the manufacturer, at which point the cells are combined to give a single pool and preserved cryogenically to form the MWCB.

Production cell culture: a lymphoblastoid cell culture derived from one or more containers of MWCB that, in the production process, is induced to form interferon.

Inducer: a substance added to a culture of lymphoblastoid cells which leads to or stimulates the production of interferon.

Enhancer: any substance added to cultures of lymphoblastoid cells during the process of manufacture in order to increase the production of interferon when the cells are treated with an inducer.

Single harvest: the cell-free fluid containing unpurified interferon harvested from an individual production culture of induced lymphoblastoid cells.

Purified interferon solution: lymphoblastoid interferon, from a single harvest, that has been subjected to a designated purification process.

Purified interferon bulk solution: the result of blending two or more batches of purified interferon solution.

Stabilized interferon: a purified interferon bulk solution to which a stabilizer has been added.

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

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

Manufacturer's reference material: a large number of samples from one or more final lots of material that has been shown to be active in clinical use (or from lots directly related to such material) shall be fully characterized in ways to be specified by the national control authority and be suitably stored to serve as the manufacturer's reference material. For certain tests, a sample of such reference material shall be included in parallel with each lot of

production material, which must match the specification of the reference batch within limits to be agreed by the national control authority.

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, pp. 11–22) shall apply to establishments manufacturing human interferon, with the addition of the following directives.

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

Human interferon shall be produced by staff who have not handled animals or infectious microorganisms, other than those microorganisms directly required in the process, 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 microorganisms or eukaryotic cells, other than those required for the process and approved by the national control authority, shall be introduced or handled at any time during the manufacture of the human interferon in the areas used for cell culture and product purification.

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

Particular attention shall be given to the recommendations contained in Part A, section 1 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (2, p. 13) 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.

There shall be strict adherence to the use of a seed lot system. A description of the system used should be provided, including the number of vials of seed available and details of their storage. It must be possible to trace every culture back to the MWCB.

Full details of the production and control procedures used in manufacture shall be provided to the national control authority. Information on the sensitivity of test methods and the frequency of the tests shall be provided, together with criteria for the rejection of material. All test methods shall be validated to the satisfaction of the national control authority.

The yield of human lymphoblastoid interferon shall be monitored during the course of manufacture. Criteria for the acceptance of single harvests and intermediate products for further processing shall be defined. Consistency of production shall be established by testing a number of consecutive lots prepared by the same procedure, 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, pp. 15–16) shall apply to the manufacture of human lymphoblastoid interferon.

3.1 Production strategy

A full description shall be given of the biological characteristics of the lymphoblastoid cells and any additives, for example the inducer and any enhancer or other substances (e.g., antibiotics), used in production. The information should include:

- (a) documentation concerning the origin of the cell line, and the nature of any known relevant condition in the donor, such as Burkitt's lymphoma, active infectious mononucleosis, or infection with any other virus;
- (b) data that can be used to establish the identity of the cell line, for example the karyology and, in particular, any constant chromosomal markers which may be used to characterize the cells;
- (c) the growth characteristics of the cell line; and
- (d) data that document the stability of the cell line under the culture conditions used, if cells are to be subcultured for an extended or indefinite period (if stability is checked only at intervals, the lymphoblastoid interferon made between such checks must not

be approved for issue until the appropriate analysis has been made).

3.2 Purification procedures

The methods used to purify the lymphoblastoid interferon from the culture harvests should be fully described. The capacity of the purification procedure to remove and/or inactivate substances derived from the host cells or culture medium other than interferon, including, in particular, viruses, proteins, and nucleic acids, must be evaluated.

The results of pilot-scale studies which monitor the removal of individual marker substances deliberately added at appropriate stages in the purification procedure will provide valuable information in this respect.

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

If antibodies are used in the purification procedures, their origins and characteristics should be fully described. The degree of purity and freedom from viruses or cell-derived DNA of any monoclonal antibodies produced from hybridoma cell lines should conform to the regulations laid down by the national control authority.

3.3 Characterization of lymphoblastoid interferon

3.3.1 *Biological and antigenic characterization*

Tests shall be performed on at least two successive filling lots to show that the product has the biological characteristics expected for the relevant type of interferon.

These tests should be of at least two quite different types. One test might be to determine whether the preparation reduces the growth of at least two unrelated viruses in human cells (for HuIFN- α , appropriate animal cells such as bovine or porcine cells may be more convenient). Other tests might determine the inhibitory effect of the preparation on the growth of sensitive cells, for example the Daudi line of human lymphoblastoid cells, or measure the induction of histocompatibility antigens or the formation of the enzyme 2',5'-oligoadenylate synthetase in treated cells.

The antigenic type of interferon present in the preparation shall be established by neutralization of the antiviral activity of the preparation by an appropriate reference antiserum (for details of the reference antisera available, see WHO Technical Report Series, No. 687, 1983, p. 39).

3.3.2 *Chemical characterization*

Interferons should be characterized by means of techniques approved by the national control authority, which will specify those characterization tests to be applied only to one or more reference batches of purified interferon bulk solution and those to be applied to each bulk solution.

The protein composition should be analysed by techniques such as sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) performed under reducing and non-reducing conditions, or high-performance liquid chromatography. With SDS–PAGE, the stained gels should be analysed by an appropriate method, such as scanning densitometry, to determine the percentage purity of the interferon preparation. If bands other than the interferon monomers are observed on the gel, Western blot analysis using antibodies should 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 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.

3.3.3 *Consistency of production*

Data to show the consistency of the production process shall be provided in terms of the specific activity of the interferon (i.e., the number of units of biological activity per mg of total protein present) at different stages in the production process, and 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. Manufacturer's working cell bank (MWCB)

Only cells approved by the national control authority shall be used to produce human interferon. The continuous cell lines used shall have been 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.

The manufacturer shall show to the satisfaction of the national control authority that the MWCB satisfies the requirements outlined in this section for freedom from bacteria, fungi, and adventitious viral agents.

Preserved cell cultures must be maintained in a state that allows recovery of viable cells without alteration of genotype. The identity of the revived cells must be determined by appropriate tests.

In one country, cells recovered from the preserved state are checked to confirm that they have retained the karyological characteristics of the lymphoblastoid cell line concerned.

5. Controls for mammalian cell cultures

If serum is included in the medium for the production cell cultures, it shall be tested to determine whether it is free from bacteria, fungi, viruses, and mycoplasmas, according to the requirements in Part A, sections 5.2 and 5.3 of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (4, pp. 49–52) and by methods approved by the national control authority.

Alternatively, the serum may be processed in various ways, e.g., 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 shall be presented to the national control authority and, if accepted, it may be agreed by that authority that tests on each batch of serum used for production are not needed.

Antibiotics of the β -lactam type 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, it shall be obtained from a seed lot system under approved manufacturing conditions.

Additives shall be used at a concentration within a range previously shown to yield a satisfactory product from production cell cultures incubated at a given temperature for a specified period. 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 a single harvest does not adversely affect the stability of the product.

7. Production precautions

7.1 Production cell cultures

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

7.2 Culture conditions for production cell cultures

Production cell cultures shall be grown under conditions agreed with the national control authority. Information to be provided should include 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

Each single harvest shall be processed to remove cells and cellular debris.

8.1 Assays for pyrogens and interferon activity

These shall be carried out by methods approved by the national control authority.

8.2 Sterility

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

8.3 Consistency of yield

The yield of human interferon in a single harvest shall be shown to be within the limits approved by the national control authority.

9. Purification

9.1 The purification procedure

The purification procedure to be applied at any stage in the manufacturing process shall be approved by the national control authority.

Several purification steps are likely to be required, yielding progressively purer intermediate products. Details of any control procedures to be applied shall be agreed with the national control authority.

For manufacturing convenience, the intermediate products derived from two or more single harvests that have passed any applicable control tests may be combined. If required, the bulk may be subjected to further processing. The product after all purification procedures have been applied is a purified interferon solution or purified interferon bulk solution, which may be stabilized by the addition of protein or other substances of a nature and at a concentration approved by the national control authority. A stabilizing substance of human origin must have been manufactured in such a way as to ensure its freedom from contamination with adventitious agents. Any substances added must not impair the safety and efficacy of the product. Before any proteinaceous

stabilizer is added, all the samples required for certain tests, including those for protein content, purity, content of specific extraneous proteins, e.g., bovine proteins derived from the medium, chemical characterization, and DNA content, must be taken. All such tests, as well as tests for materials of animal origin (e.g., serum protein) or plant origin (e.g., lectins) used at any stage in production or purification, shall be carried out using methods approved by the national control authority.

9.2 Control of purified interferon solutions or bulk solutions

9.2.1 *Assay for protein content*

The total protein content of the purified solution shall be determined.

9.2.2 *Assay for interferon activity*

The interferon content of the purified solution shall be determined by a method which measures antiviral activity or some other biological activity, as agreed with the national control authority.

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

9.2.3 *Test for additives used during purification or other phases of manufacture*

A test shall be made for the presence of any potentially hazardous agent, including monoclonal antibodies, antibiotics, and other additives used at any stage in manufacture. The method used in the test and the permitted residual concentration in the final product shall be approved by the national control authority.

9.2.4 *Test for identity*

The human interferon product shall be identified by means of SDS-PAGE and/or by neutralization of biological activity, as required by the national control authority.

9.2.5 *Protein purity*

Before any stabilizing substance is added, the purity of each purified interferon solution or bulk solution shall be established, as approved by the national control authority.

One manufacturer has used for this purpose SDS-PAGE of a sample containing at least 10 µg of total protein. The protein peaks obtained are scanned in a densitometer and stained with specific antisera to interferon protein and to potential protein contaminants derived from the cells, the medium, or the inducer used in the production process. Identification of the protein bands and their relative amounts enables the purity of the preparation to be assessed.

A purity of at least 95% has been achieved by one manufacturer.

9.2.6 *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 immunoabsorption chromatography, tests shall be made to determine whether serum remains in the purified interferon solution or bulk solution.

Animal serum at 1 µl per litre of interferon solution is approximately equivalent to 50 ng/ml albumin. Methods such as ELISA and immunoprecipitation can detect as little as 1 ng of albumin.

9.2.7 *Test for DNA*

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

The WHO Study Group on Biologicals 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 (3).

10. Final bulk

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

10.1 Tests for sterility

The final bulk shall be tested for bacterial and mycotic sterility in accordance with the requirements given in Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (4, pp. 48–53).

10.2 Tests for pyrogenic substances

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

11. Filling and containers

The requirements covering filling and containers given in Part A, section 4, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (2, pp. 16–17) shall apply.

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

12. Final lot

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

12.1 Sterility test

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, pp. 48–53).

12.2 Identity test

Samples from each final lot shall be identified as human interferon by a method approved by the national control authority.

12.3 Potency test

Potency should be determined by means of a test measuring a biological activity. Statistical analysis of the data should show that the mean potency value obtained has confidence limits within a range accepted by the national control authority.

The detailed procedures for carrying out this test shall be approved by the national control authority and an appropriate interferon standard preparation homologous with the product should be tested in parallel. For interferon preparations made from Namalwa cells induced with Sendai virus, the International Standard for Human Lymphoblastoid Interferon (Namalwa) (HuIFN- α (Ly)) is appropriate.

12.4 Innocuity test

The innocuity of the final product shall be tested parenterally in guinea-pigs and mice by a method 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 any preservative added. The test used and the permitted concentration shall be approved by the national control authority.

12.7 Determination of moisture content

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

12.8 Determination of hydrogen ion concentration and degree of clarity

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

13. Records

The requirements given in Part A, section 6 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (2, p. 17) shall apply.

14. Samples

The requirements given in Part A, section 7 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (2, p. 18) shall apply.

15. Labelling

The requirements given in Part A, section 8 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (2, p. 18) shall apply, with the addition of the following directive.

The leaflet accompanying the package should include the following information, together with any other items required by the national control authority:

- description of the product,
- clinical pharmacology,
- indications and usage,
- contraindications,
- warnings,
- precautions,
- use during pregnancy,

- adverse reactions,
- dosage and route(s) of administration,
- directions for use,
- how supplied,
- storage conditions, and
- references.

16. Distribution and shipping

The requirements given in Part A, section 9 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (2, pp. 18–19) shall apply.

17. Storage and expiry date

The requirements given in Part A, section 10 of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (2, p. 19) shall apply.

17.1 Storage conditions

Filled containers of human lymphoblastoid 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 for each preparation of human lymphoblastoid interferon shall be that agreed by the national control authority on the basis of evidence for stability supplied by the manufacturer.

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, pp. 19–22) shall apply.

The national control authority shall:

- approve the methods for producing human interferon from lymphoblastoid cells;
- approve the tests for interferon concentration and specify the statistical precision required for a claimed value;
- approve the purity of the final product;
- approve the tests for extraneous substances and total protein;
- approve the tests for preservatives and the agents used for purification and in other aspects of manufacture;
- approve the tests used for freedom from abnormal toxicity in the final product;
- approve the tests used in the assay of potency; and
- approve the data from which it has been concluded that therapeutic responses are adequate and that the product is safe 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

Interferon shall be released only if it fulfils the above requirements.

A statement signed by the appropriate official of the national control authority shall certify whether or not the final lot of interferon in question meets all national requirements as well as the above requirements. The certificate shall state the date of the last satisfactory 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 proposed Requirements for Human Interferons Prepared from Lymphoblastoid Cells was prepared by Dr N.B. Finter, Wellcome Biotech, Beckenham, Kent, England.

The second draft of these Requirements was formulated in March 1987, contributions being made by Dr V. Grachev, Scientist, and Dr J.C. Petriccioni, Chief, Biologicals, World Health Organization, Geneva, Switzerland.

The third draft of these Requirements was formulated at the WHO Informal Consultation on the Standardization of Interferons, Geneva, 23–25 March 1987; for a list of the participants, please see WHO Technical Report Series, No. 771, 1988, Annex 1.

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