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WHO Expert Committee on Biological Standardization

Twenty-ninth Report

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Geneva, 6–12 December 1977

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WHO EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION

Twenty-ninth Report

GENERAL

The WHO Expert Committee on Biological Standardization met in Geneva from 6 to 12 December 1977. The meeting was opened on behalf of the Director-General by Dr Ch'en Wen-chieh, Assistant Director-General.

The Committee considered that one of the most useful documents made available at the meeting was Guidelines for the Preparation and Establishment of Reference Materials and Reference Reagents for Biological Substances. It was formulated for the guidance of international associations that were helping in setting up international standards as well as for the guidance of national authorities that were faced with the task of establishing national standards. The guidelines had been amended in the light of comments received from a number of scientists, and further amendments were made by the Expert Committee. The final version was annexed to the report (see Annex 4).

Another important matter was the Requirements for the Collection, Processing and Quality Control of Human Blood and Blood Products (see Annex 1). It was agreed that it would be most useful to have a single set of requirements applicable to all organizations and laboratories involved in the collection or fractionation of blood and blood products.

There is a need for better understanding of the distinction between estimates of hormone concentration using bioassays, which are based on the biological activity or function of a hormone, and estimates using binding assays (particularly radioimmunoassays), which are based on aspects of hormone structure that may not be related to biological activity or function. Failure to understand this distinction had led to considerable confusion in the literature and in clinical practice, and the confusion is even worse when the results of structure-based assays are misinterpreted as measures of biological activity. Measures recommended by the Expert Committee in its twentieth, twenty-first, and twenty-sixth reports had proved insufficient to maintain a clear distinction between the results of these two types of assay.

Experience gained in various assay performance studies, including the WHO human reproduction matched reagent programme, shows that the results of radioimmunoassay determinations cannot always be reproduced reliably with different sets of reagents. In addition, preparations containing heterogeneous forms of a hormone can yield markedly different potency ratios when tested in different *in vivo* and *in vitro* comparative bioassay systems.

It is recommended, therefore, that a report of an assay shall always be accompanied by a statement of the assay method employed, the standard used and its stated unitage, and the calibration method used (bioassay or binding assay). Unless this is done, confusing and possibly dangerous misinterpretations of potency estimates may be made. The Committee requested WHO to investigate this further in order to formulate guidance on the use of hormone preparations.

The Committee observed that until now the international unit of activity of all international reference materials has been expressed as that contained in a given weight or volume of the preparation contained in a sealed ampoule. The Committee has been aware for some time that the activity of some reference materials may not be distributed evenly throughout the freeze-dried preparation and that it may therefore be misleading to give the impression that the unit of activity is contained in a given weight of the preparation. In practice, irrespective of the definition of the unit by weight or by volume, for those preparations that have been filled as accurately reproduced volumes, instructions are given to the user to treat the contents of an ampoule as containing a defined number of units. The Committee was informed that there appears to be no legal objection to assigning a finite number of international units to the contents of an ampoule of material. Such a policy, however, calls for great accuracy in filling and care in freeze-drying in order to ensure that the variation of fill is not greater than 1%.

The Committee was informed of the increased use of certain antitumour antibiotics for the treatment of human tumours and leukaemias in a number of countries, some of which have national reference materials for control purposes. The measurement of activity, however, causes problems, and although an antitumour activity test in animals can be included in the original characterization of the preparation such tests cannot be used for the quantitative assessment of individual batches. For routine batch control, reliance must be placed partly on quantitative antimicrobial activity tests. The Committee noted that some of these antibiotics are pure crystalline materials, for which a chemical reference preparation would be suitable, while others consist of a mixture of several closely

related components requiring a biological test for the measurement of activity; for these products biological reference materials are needed.

The Committee agreed that guidelines on the quality control of antitumour antibiotics would be useful to many countries and requested WHO to formulate such guidelines.

The Committee was informed of new developments in the determination of the haemagglutinin content of influenza vaccines. Recent methods in which the quantitative measurement of haemagglutinin is determined by an antigen/antibody reaction have shown that haemagglutinin content correlates closely with the ability of the virus haemagglutinin to stimulate the production of antihaemagglutinin. It appears that the only effective means of controlling the potency of the vaccines is to provide each year a reference material in which the haemagglutinin content has been calibrated in micrograms of haemagglutinin per millilitre together with a specific antiserum. The Committee agreed to accept the generous offer of the National Institute for Biological Standards and Control, London, to provide haemagglutinin from current infectious strains annually, but it pointed out that such preparations should not be referred to as international reference materials but described as WHO Influenza Virus Reference Haemagglutinin, each preparation being identified by the year in which it was prepared.

The Committee was informed that there has been considerable progress in the standardization of allergens and that three international organizations [1] had formed an International Joint Steering Committee on Allergen Standardization. Several allergen extracts, with their matching antisera, had been prepared and a study on the standardization of an anti-IgE preparation was about to start. This progress had been made possible by the use of radioimmunoassay techniques and other *in vitro* methods. The Committee looked forward to seeing the reports on the preparations.

The Committee noted that many of the sets of requirements for biological substances were formulated more than 10 years ago and now need revision to take account of the developments that have since taken place in technology. Revision of the Requirements for Rabies Vaccine for Human Use is particularly needed, and the spread of rabies in many countries gives the matter greater urgency. The newer rabies vaccines are not only safe to be given prophylactically but are more potent, so that only 14–21 doses are needed after known exposure to street virus.

[1] The International Association of Allergology, the International Association of Biological Standardization, and the International Union of Immunological Societies.

In discussions on the progress being made in the establishment of reference materials it became apparent that the analysis of the results, for which statistical services are required, is a lengthy process and frequently delays the completion of the study. In an attempt to resolve this problem the Committee asked WHO to explore the possibility of obtaining additional biometric services. It was suggested that institutes specializing in the analysis of the results of biological assays might be able to help.

SUBSTANCES

ANTIBIOTICS

1. Erythromycin

The Committee noted that supplies of the International Standard for Erythromycin were becoming depleted and that the National Institute for Biological Standards and Control, London, had taken steps to replace it.[1] A suitable preparation of erythromycin had been obtained and dispensed into ampoules so that each contained approximately 75 mg of the dried powder.

Samples of the first international standard and the proposed second international standard had been sent to seven laboratories in six countries for comparison in a collaborative assay. The results had been received from all laboratories and were being analysed.

The Committee authorized the National Institute for Biological Standards and Control to establish the preparation as the second international standard for erythromycin and on the basis of the results of the collaborative assay and with the agreement of the participants, to define the international unit.

2. Streptomycin

The Committee noted that supplies of the second International Standard for Streptomycin were becoming depleted and that the National Institute for Biological Standards and Control, London, had taken steps to replace it.[2] A suitable preparation of streptomycin sulfate had been obtained as a dry powder but because of its hygroscopic nature it had been dissolved, dispensed into ampoules as an aqueous solution, and

[1] Unpublished working document WHO/BS/77.1170.
[2] Unpublished working document WHO/BS/77.1168.

freeze-dried. These ampoules of the proposed third international standard together with the second International Standard for Streptomycin had been sent to nine laboratories in eight countries for comparison in a collaborative assay. So far results had been received from seven laboratories.

The Committee authorized the National Institute for Biological Standards and Control to establish the preparation as the third international standard for streptomycin and, on the basis of the results of the collaborative assay and with the agreement of the participants, to define the international unit.

3. Candicidin

The Committee noted that the results of the collaborative assay of the proposed international reference preparation of candicidin referred to in its twenty-eighth report [1] had been received and were being analysed.[2]

The Committee authorized the National Institute for Biological Standards and Control, London, to establish the preparation as the international reference preparation of candicidin and, on the basis of the results of the collaborative assay and with the agreement of the participants, to define the international unit.

4. Bleomycin and Doxorubicin

The Committee noted that international specifications for the antitumour antibiotics bleomycin (complex of bleomycins A_2 and B_2) and doxorubicin referred to in its twenty-eighth report [1] were not yet complete.

The Committee was informed that there was a need for an international reference preparation of bleomycin complex (A_2/B_2) to be used in connexion with an international quality specification and that suitable material was available.

The Committee therefore requested the National Institute for Biological Standards and Control, London, to obtain the preparation of bleomycin complex (A_2/B_2) and arrange a collaborative assay in relation to existing national standard preparations.

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[1] WHO Technical Report Series, No. 610, 1977, p. 16.

[2] Unpublished working document WHO/BS/77.1171.

5. Other Antitumour Antibiotics

The Committee agreed that guidelines for quality assessment of other antitumour antibiotics would be useful. It was suggested that the guidelines should include published information on the composition and characteristics of different antitumour antibiotics and references to analytical methods used in deriving the data, as well as information on available national reference preparations. The guidelines would indicate the type of international reference material appropriate for particular antitumour antibiotics—e.g., chemical reference substances for those that can be characterized by chemical and physical means or biological reference preparations for those that cannot be so characterized and must therefore be characterized in part by the responses observed in a biological system. The Committee requested WHO to take the necessary steps to formulate such guidelines.

6. Amikacin

The Committee noted that there was a need for an international reference material for amikacin.[1] This antibiotic is produced by chemical modification of kanamycin and is active against a number of strains of Gram-negative organisms that are resistant to kanamycin, gentamicin, and tobramycin. As amikacin is in worldwide use and several national standards have been established, the Committee agreed that an international reference material is needed. It requested the National Institute for Biological Standards and Control, London, to obtain suitable material and arrange a collaborative assay.

7. Sisomicin

The Committee noted that sisomicin, an aminoglycoside antibiotic resembling gentamicin C_{1a}, is in clinical use in several countries.[2] Its properties differ sufficiently from those of gentamicin to require assays to be carried out in terms of a homologous standard. The Committee therefore agreed that an international reference material is needed and requested the National Institute for Biological Standards and Control, London, to obtain suitable material.

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[1] Unpublished working document WHO/BS/77.1173.

[2] Unpublished working document WHO/BS/77.1174.

8. Tobramycin

The Committee noted that a collaborative assay was being arranged [1] of the preparation of tobramycin referred to in its twenty-eighth report.[2]

The Committee authorized the National Institute for Biological Standards and Control, London, to establish the preparation as the international reference preparation of tobramycin, and, on the basis of the results of the collaborative assay and with the agreement of the participants, to define the international unit.

ANTIBODIES

9. Antipneumococcus Sera

The Committee noted that the International Standard for Antipneumococcus Serum (Type 1), Equine, and the International Standard for Antipneumococcus Serum (Type 2), Equine, both established in 1934 for the control of therapeutic antisera, had not been available for distribution for several years.[3] As there had been no recent requests for these standards, the Committee agreed to discontinue the International Standard for Antipneumococcus Serum (Type 1), Equine, and the International Standard for Antipneumococcus Serum (Type 2), Equine, and saw no reason for their replacement.

10. *Clostridium botulinum* Type B Antitoxin

The Committee noted that the State Serum Institute, Copenhagen, had obtained a sufficient quantity of the material referred to in its twenty-eighth report [4] for the replacement of the International Standard for *Clostridium botulinum* Type B Antitoxin.[5]

The preparation will be dispensed into ampoules in appropriate quantities, and the studies requested by the Committee in its twenty-seventh report [6] will be initiated.

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[1] Unpublished working document WHO/BS/77.1172.
[2] WHO Technical Report Series, No. 610, 1977, p. 17.
[3] Unpublished working document WHO/BS/77.1146.
[4] WHO Technical Report Series, No. 610, 1977, p. 19.
[5] Unpublished working document WHO/BS/77.1161.
[6] WHO Technical Report Series, No. 594, 1976, p. 16.

11. Influenza Vaccine

The Committee noted the progress that has been made in the control of influenza vaccines.[1] It has been shown that haemagglutinin activity, as measured by haemagglutination or chick cell agglutination techniques, does not give a reliable measure of the haemagglutinin content of the vaccines.

On the other hand, more recent developments have shown that single-radial-diffusion or immunoelectrophoresis (Laurell) techniques, which are based on the quantitative reaction between the haemagglutinin and the specific antihaemagglutinin antibody in gels, do give an accurate measure of antigen content.

It is known that both the haemagglutinin and neuraminidase components of viruses causing prevalent infections in man have undergone a number of changes during the past two decades and that the vaccine and the reference preparations used to control the potency of vaccines must therefore contain both the haemagglutinin and the neuraminidase representative of these viruses. Accordingly it is necessary to make an annual review of the reference materials appropriate to the control of potency of current vaccines. The Committee accepted the offer of the National Institute for Biological Standards and Control, London, to provide WHO annually with a WHO Influenza Virus Reference Haemagglutinin having a specified content of haemagglutinin (mg/l) prepared from recent infectious strains.

The Committee recognized that the annual provision of such material was a new development in the establishment of reference materials for the control of a vaccine but agreed that there was no alternative. Since it would not be possible for each preparation to be established officially as an international reference material, it should be referred to as a WHO Influenza Virus Reference Haemagglutinin, with the year of production given in brackets.

The Committee decided that the current International Reference Preparation of Influenza Virus Haemagglutinin (Type A) established in 1967 should not be used in the control of vaccines. It therefore discontinued the International Reference Preparation of Influenza Virus Haemagglutinin (Type A).

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[1] Unpublished working document WHO/BS/77.1147.

The Committee noted that the Requirements for Inactivated Influenza Vaccine,[1] which refer to the International Reference Preparation of Influenza Virus Haemagglutinin (Type A), would need amendment because of the discontinuation of this preparation (see page 25).

12. Tetanus Toxoid, Adsorbed

The Committee noted that the State Serum Institute, Copenhagen, had made a further preparation of tetanus toxoid, adsorbed,[2] to which reference was made in the Committee's twenty-eighth report.[3] This material (coded TEXA-55) has been found suitable in comparison with the International Standard for Tetanus Toxoid. A collaborative assay will be arranged.

13. Tetanus Toxin

The Committee noted the progress being made in the collaborative study referred to in its twenty-eighth report[4] of the suitability of a number of tetanus toxin preparations for use in the assay of tetanus antitoxin.[5] Twelve countries agreed to take part, and nine of them offered to provide samples of toxin. A preliminary study comparing these toxins is being made at the National Institute for Biological Standards and Control, London.

14. Pertussis Vaccine

The Committee noted that the collaborative assay to replace the International Standard for Pertussis Vaccine had been delayed because of the need for investigation of the mouse protection test used for the assay.[6]

A subsequent investigation, in which the proposed second international standard was compared with the existing International Standard, has shown that the proposed standard is suitable, and the National Institute for Biological Standards and Control, London, was requested to arrange a collaborative assay.

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[1] WHO Technical Report Series, No. 384, 1968.
[2] Unpublished working document WHO/BS/77.1160.
[3] WHO Technical Report Series, No. 610, 1977, p. 17.
[4] WHO Technical Report Series, No. 610, 1977, p. 18.
[5] Unpublished working document WHO/BS/77.1149.
[6] Unpublished working document WHO/BS/77.1148.

15. Purified Protein Derivative (PPD) of Mallein

The Committee noted that there is a need for an international reference material for mallein—an antigen analogous to tuberculin that is prepared from the heat-treated products of growth and lysis of *Pseudomonas mallei*.[1] Infection with this organism is enzootic in horses in certain parts of the world, and on those occasions when man is infected the outcome is usually fatal. Horses are frequently moved from country to country and many are tested using national preparations of mallein.

The Central Veterinary Laboratory, Weybridge, was requested to obtain a preparation of mallein PPD suitable to serve as an international reference material and to arrange a collaborative assay.

16. *Brucella abortus* Strain 19 Vaccine and *Brucella melitensis* Strain Rev. 1 Vaccine

The Committee noted that international reference materials may be needed for the two vaccines *Brucella abortus* strain 19 and *Brucella melitensis* strain Rev. 1.[2] The absence of these reference materials is referred to in the requirements for these vaccines.[3, 4] The Committee requested the Central Veterinary Laboratory, Weybridge, to determine whether such reference materials are needed and, if so, to obtain suitable materials.

17. Allergens

The Committee was informed that until recently little progress had been made in the standardization of allergens but that radioimmunoassay systems and other techniques now enable the concentration of allergens in extracts to be assessed. Much progress has been made in the past three years and several allergen extracts together with their specific antisera are now available. Work is in progress to standardize these extracts and to compare their activities as measured by tests *in vitro* with their activities as shown in the skin of sensitized patients.

A recently formed International Joint Steering Committee for Allergen Standardization is to carry out a collaborative study with a preparation of anti-IgE.

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1 Unpublished working document WHO/BS/77.1157.

2 Unpublished working document WHO/BS/77.1158.

3 WHO Technical Report Series, No. 444, 1970, p. 58.

4 WHO Technical Report Series, No. 610, 1977, p. 85.

18. Blood Coagulation Factor VIII, Human

The Committee noted that in accordance with the authorization in its twenty-eighth report [1] the National Institute for Biological Standards and Control, London, had established the second International Standard for Blood Coagulation Factor VIII, Human, and had defined its International Unit as the activity contained in 12.745 mg of the second International Standard for Blood Coagulation Factor VIII, Human.[2] There are 1.1 International Units per ampoule.

19. Antithrombin III, Human

The Committee noted that a quantity of highly purified antithrombin III, human, had been obtained by the National Institute for Biological Standards and Control, London, and that the material had been distributed into ampoules and freeze-dried.[3]

The Committee noted also that 14 laboratories in 8 countries were participating in a collaborative study. Preliminary results have shown that the material is stable.

20. Anti-A, Anti-B, Anti-(A+B), Anti-C and Anti-E Blood Typing Sera

The Committee noted that as requested in its twenty-eighth report [4] the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, had collected about 10 litres of anti-B serum.[5]

The Committee noted also that this material will be compared with the International Standard for Anti-B Blood Typing Serum, Human.

21. Blood Group Substances A and B

A WHO Working Group on the Standardization of Human Blood Products and Related Substances [6] concluded that there is no need for

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1 WHO Technical Report Series, No. 610, 1977, p. 12.

2 Unpublished working document WHO/BS/77.1150.

3 Unpublished working document WHO/BS/77.1151.

4 WHO Technical Report Series, No. 610, 1977, p. 10.

5 Unpublished working document WHO/BS/77.1175.

6 Unpublished working document WHO/BS/76.1130.

international specifications or reference materials for blood group substances A and B.

The Committee was informed, however, that the results of recent studies carried out under the aegis of the European Pharmacopoeia Commission suggest that a maximum limit for the presence of blood group substances A and B should be included in the European Pharmacopoeia monograph for human albumin. Tests of such limits would require reference materials.

The Committee requested the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, to investigate the need for international reference materials, to obtain, if necessary, a suitable preparation of blood group substances A and B, and to arrange a collaborative study.

22. Anti-Hepatitis A Immunoglobulin

The Committee considered that an international reference material was needed for the estimation of hepatitis A antibody immunoglobulin and requested the Bureau of Biologics, Food and Drug Administration, Bethesda, to obtain suitable material and arrange a collaborative study.

23. Anti-Hepatitis B Immunoglobulin

The Committee noted that a collaborative assay of the proposed reference preparation of anti-hepatitis B immunoglobulin referred to in its twenty-eighth report [1] had been carried out in 16 laboratories in 11 countries.[2] The study was coordinated by the Bureau of Biologics, Food and Drug Administration, Bethesda.

The preparation was diluted and freeze-dried as two lots (coded 26-1-77 and 17-2-77) and the collaborative assay showed that both lots were suitable to serve as an international reference preparation. The Committee established lot 26-1-77 of this preparation as the International Reference Preparation of Anti-Hepatitis B Immunoglobulin and, on the basis of the results of the collaborative assay and with the agreement of the participants, defined its International Unit as the activity contained in one fiftieth of the contents of an ampoule of the International Reference Preparation. Each ampoule therefore contains 50 International Units of Anti-Hepatitis B Immunoglobulin.

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[1] WHO Technical Report Series, No. 610, 1977, p. 12.

[2] Unpublished working document WHO/BS/77.1164.

24. Anti-Varicella Zoster Immunoglobulin

The Committee noted that plasma suitable for the preparation of anti-varicella zoster immunoglobulin is being collected at the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam.[1] After the isolation of the immunoglobulin a collaborative study will be organized.

25. Fluorescein-Isothiocyanate-Conjugated Sheep Anti-Human IgM (Anti-μ Chain)

The Committee noted the report of the collaborative assay of the proposed international standard for FITC-conjugated sheep anti-human IgM (anti-μ chain).[2] The report had been circulated to the Expert Advisory Panel on Biological Standardization and found to be satisfactory.

The Committee established the preparation as the International Standard for Fluorescein-Isothiocyanate-Conjugated Sheep Anti-Human IgM (Anti-μ Chain) and, on the basis of the results of the collaborative assay, defined its International Unit as the activity contained in 0.0447 mg of the International Standard. There are 100 International Units per ampoule.

26. Human Serum Proteins for Immunoassay

The Committee noted the report [3] on the international collaborative study of five freeze-dried human serum preparations to determine their suitability to serve as a reference preparation for immunoassay of the following six serum proteins: albumin, alpha-1-antitrypsin, alpha-2-macroglobulin, ceruloplasmin, complement C3, and transferrin. Although there appeared to be little to choose between the preparations, the Committee agreed with the conclusion in the report that the fourth preparation should be selected as the reference preparation.

The Committee therefore established this preparation as the International Reference Preparation of Albumin, Alpha-1-Antitrypsin, Alpha-2-Macroglobulin, Ceruloplasmin, Complement C3, and Transferrin, for Immunoassay, and defined its International Unit as the activities contained in one hundredth of the material in an ampoule of the International Reference Preparation. There are 100 International Units of each of of these six proteins per ampoule.

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[1] Unpublished working document WHO/BS/77.1176.
[2] Unpublished working document WHO/BS/77.1143.
[3] Unpublished working document WHO/BS/77.1155.

27. Human Placental Lactogen for Immunoassay

The Committee noted the results [1] of the collaborative assay of the preparation of human placental lactogen referred to in its twenty-sixth report.[2]

The Committee also noted that, in accordance with the authorization in its twenty-sixth report, the National Institute for Biological Standards and Control, London, had established the preparation as the International Reference Preparation of Human Placental Lactogen for Immunoassay and, on the basis of the results and with the agreement of the participants, had defined its International Unit as the activity contained in 6.705 g of the International Reference Preparation. There is 0.000850 of an International Unit in an ampoule.

28. Human Prolactin

The Committee noted that in accordance with the request in its twenty-sixth report [3] the National Institute for Biological Standards and Control, London, had obtained a purified preparation of human prolactin that could serve as an international reference preparation for the immunoassay of the human hormone.

The Committee noted also the interim report [4] on the radioimmunoassays of human prolactin in coded samples of serum and plasma carried out in 14 laboratories in 9 countries. The results showed good agreement in the ranking order of the estimated hormone content in these samples.

The Committee authorized the National Institute for Biological Standards and Control to establish the preparation as the international reference preparation of human prolactin for immunoassay and, on the basis of the results of the collaborative study and with the agreement of the participants, to define the international unit.

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[1] Unpublished working document WHO/BS/77.1141.

[2] WHO Technical Report Series, No. 565, 1975, p. 16.

[3] WHO Technical Report Series, No. 565, 1975, p. 20.

[4] Unpublished working document WHO/BS/77.1165.

29. Human Pituitary Luteinizing Hormone (LH)[1] for Immunoassay

The Committee noted that in accordance with the request in its twenty-sixth report [2] the National Institute for Biological Standards and Control, London, had organized a collaborative assay of the International Reference Preparation of Human Pituitary Luteinizing Hormone (LH) for Immunoassay in terms of the International Reference Preparation of Pituitary FSH and LH (ICSH), Human, for Bioassay. The collaborative assay was carried out in 12 laboratories in 7 countries using different bioassay methods.[3]

The Committee also noted that the first-mentioned preparation had been used in many laboratories for 6 years with a provisional potency of 77 units per ampoule and that in order to maintain the continuity of the unit, the National Institute for Biological Standards and Control, with the agreement of the participants, had defined the International Unit for Human Pituitary Luteinizing Hormone (LH) for Immunoassay as the activity contained in 0.09106 mg of the International Reference Preparation. Each ampoule contains 77 International Units.

30. Growth Hormone for Bioassay

The Committee noted that supplies of the first International Standard for Growth Hormone, Bovine, for Bioassay were nearly exhausted and that the National Institute for Biological Standards and Control, London, had taken steps to obtain material suitable to serve as an international standard for human growth hormone for bioassay.[4] This was needed urgently for the control of human growth hormone preparations used for therapy in man.

The Committee agreed that the preparation obtained should be calibrated in terms of the International Standard for Growth Hormone, Bovine, for Bioassay, and that the unitage assigned to it should be on the basis of bioassays.

The Committee requested the National Institute for Biological Standards and Control to arrange a collaborative assay. It also requested the Central Veterinary Laboratory, Weybridge, to investigate the need in the veterinary field for the replacement of the International Standard for Growth Hormone, Bovine, for Bioassay.

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[1] The name for this substance recommended by the IUPAC–IUB Commission on Biochemical Nomenclature is lutropin.

[2] WHO Technical Report Series, No. 565, 1975, p. 13.

[3] Unpublished working document WHO/BS/77.1152.

[4] Unpublished working document WHO/BS/77.1156.

31. Oxytocin

The Committee noted that supplies of the third International Standard for Oxytocin and Vasopressin, Bovine, for Bioassay were almost exhausted and that the National Institute for Biological Standards and Control, London, had obtained a quantity of purified synthetic oxytocin as a proposed international reference material.[1] A collaborative assay of the oxytocin had been carried out in 28 laboratories in 19 countries.

The Committee authorized the National Institute for Biological Standards and Control to establish the preparation of oxytocin as the international standard for oxytocin for bioassay and, on the basis of the results of the collaborative assay and with the agreement of the participants, to define the international unit. The international unit for oxytocin defined thereby would then replace the International Unit of Oxytocin at present defined by the third International Standard for Oxytocin and Vasopressin, Bovine, for Bioassay.

32. Lysine Vasopressin

The Committee noted that there was a need for international reference material of lysine vasopressin.[1] The Committee also noted that the National Institute for Biological Standards and Control, London, had obtained a preparation of synthetic lysine vasopressin suitable for this purpose and that a collaborative assay had been carried out in 25 laboratories in 18 countries.

The Committee established the preparation as the International Standard for Lysine Vasopressin and authorized the National Institute for Biological Standards and Control, on the basis of the results of the collaborative assay and with the agreement of the participants, to define the international unit.

33. Arginine Vasopressin

The Committee noted that the stock of the third International Standard for Oxytocin and Vasopressin, Bovine, for Bioassay was low and that the National Institute for Biological Standards and Control, London, had obtained a preparation of arginine vasopressin and had ascertained its suitability to serve as an international reference material.[1] A collaborative assay in terms of the third International Standard for Oxytocin and Vasopressin, Bovine, for Bioassay was in progress.

[1] Unpublished working document WHO/BS/77.1163.

In view of the urgent need for this reference material, the Committee authorized the National Institute for Biological Standards and Control to establish the material as the international standard for arginine vasopressin for bioassay and, on the basis of the results of the collaborative assay and with the agreement of the participants, to define the international unit. The international unit for arginine vasopressin defined thereby would replace the International Unit of Vasopressin at present defined by the third International Standard for Oxytocin and Vasopressin, Bovine, for Bioassay.

34. Desmopressin

The Committee noted that there was a need for an international reference material of desmopressin [1] and requested the National Institute for Biological Standards and Control, London, to obtain suitable material and arrange a collaborative assay.

35. Kininogenase

The Committee noted that preparations of porcine pancreas kininogenase (kininogenin (EC 3.4.21.8) ; kallidinogenase) were available from a number of manufacturers and that an international reference material was required.[2] The Committee requested the National Institute for Biological Standards and Control, London, to obtain suitable reference material and to arrange a collaborative assay.

The Committee was informed that reference materials of human plasma kininogenase and human urinary kininogenase might also be needed. The Committee requested the National Institute for Biological Standards and Control to investigate such needs.

36. Aprotinin

The Committee noted that preparations of the peptidase inhibitor aprotinin are manufactured and that an international reference material might be needed.[2] The Committee requested the National Institute for Biological Standards and Control, London, to investigate this possible need.

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[1] Unpublished working document WHO/BS/77.1163.

[2] Unpublished working document WHO/BS/77.1167.

37. Leptospira Reference Sera

The Committee noted that the importance of leptospirosis as a zoonosis is becoming more fully understood and that there is a need for reference sera against a greater number of leptospiral serotypes.[1] Only 58 of a possible 164 serotype antisera are available or in the course of preparation.

The Committee requested the Central Veterinary Laboratory, Weybridge, to find out whether additional sera exist in the reference centres and to determine whether additional materials could be prepared so that all such sera can be made available in view of their importance in human and veterinary medicine.

REQUIREMENTS FOR BIOLOGICAL SUBSTANCES

38. Requirements for the Collection, Processing and Quality Control of Human Blood and Blood Products

The Committee noted that in accordance with the request in its twenty-eighth report [2] proposed requirements for the collection, processing, and quality control of human blood and blood products had been prepared.[3] They contained five parts.

Part A. Requirements for the collection of source materials.
Part B. Requirements for single donor and small pool products.
Part C. Requirements for the manufacture of blood products and related substances.
Part D. Requirements for the control of plasma fractions.
Part E. National control requirements.

The Committee agreed that each part was important and must be considered in conjunction with the other parts of the document. In order to achieve common standards of good manufacturing practice the requirements applied to all establishments whether they were carrying out all or only part of the functions of collection and fractionation.

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[1] Unpublished working document WHO/BS/77.1177.

[2] WHO Technical Report Series, No. 610, 1977, p. 20.

[3] Unpublished working document WHO/BS/77/1144 Rev. 1.

After making a number of minor amendments to the document, the Committee adopted the Requirements for the Collection, Processing and Quality Control of Human Blood and Blood Products and agreed that they should be annexed to this report (Annex 1).

39. Requirements for Meningococcal Polysaccharide Vaccine

The Committee was informed that several technical developments had taken place in the production of meningococcal polysaccharides in the past year and that these had important implications for the Requirements for Meningococcal Polysaccharide Vaccine. The Committee noted that the main development was an improvement of the stability of the Group A polysaccharide brought about by the addition of lactose.[1] The Committee was informed that the Requirements, formulated in 1975 and revised in 1976, now required further revision. In order to avoid the need to refer to three documents the Committee agreed that the revisions included in the twenty-eighth report [2] should be included with the latest revisions to form a composite addendum. The Committee adopted the addendum and agreed that it should be annexed to this report (Annex 2).

40. Requirements for Inactivated Influenza Vaccine

The Committee noted that the International Reference Preparation of Influenza Virus Haemagglutinin (Type A) was not typical of the haemagglutinin in the current infectious strains of influenza virus.[3] The preparation was therefore inappropriate for use in the control of haemagglutinin content of inactivated influenza vaccines, and the Committee accordingly discontinued the International Reference Preparation of Influenza Virus Haemagglutinin (Type A).

The Committee agreed that the discontinuation of this reference preparation necessitated changes in the Requirements for Inactivated Influenza Vaccine.[4] The Committee considered the amendments to be made and agreed that they should be annexed to this report (Annex 3).

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[1] Unpublished working document WHO/BS/77.1166.

[2] WHO Technical Report Series, No. 610, 1977, p. 52.

[3] Unpublished working document WHO/BS/77.1147.

[4] WHO Technical Report Series, No. 384, 1968, p. 43.

41. Guidelines for the Preparation and Establishment of Reference Materials and Reference Reagents for Biological Substances

In accordance with a recommendation of a Working Group on the Standardization of Human Blood Products,[1] WHO had formulated Guidelines for the Preparation and Establishment of Reference Materials and Reference Reagents for Biological Substances, for distribution principally to scientific societies.

The Committee noted that the provision of WHO working standards based on the use of a master ampoule system had been suggested for some particular substances. The Committee accepted this proposal and agreed that it would bring well calibrated standards within the reach of many countries that might not otherwise be able to prepare them.

The Committee considered that the guidelines would be most useful to those intending to assist in the establishment of international and national reference materials. After making some minor amendments, it adopted the Guidelines for the Preparation and Establishment of Reference Materials and Reference Reagents for Biological Substances and agreed that they should be annexed to this report (Annex 4).

42. Requirements for Rabies Vaccine for Veterinary Use

The Committee noted that very few countries in the world were considered to be free of rabies.[2] The Committee was informed that vaccination of domestic animals in affected countries plays an essential part in the control of the disease in both animal and human populations.

Although Requirements for Rabies Vaccine for Human Use have been formulated [3] they are not entirely appropriate for vaccines used in animals. The Committee agreed that similar requirements for veterinary use were needed and asked WHO to take the necessary steps to draw them up.

43. The Need for Revision of Some Requirements for Biological Substances

The Committee noted that as a result of several advances in technology a number of tests in several requirements are no longer in current

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[1] WHO Technical Report Series, No. 610, 1977, Annex 1, pp. 40–41.

[2] Unpublished working document WHO/BS/77.1159.

[3] WHO Technical Report Series, No. 530, 1973, p. 22.

use.[1] Furthermore, some additional tests are now necessary, especially where new cell substrates are being used or where further potential contaminating organisms could now be detected. The Committee agreed that some of the requirements formulated more than 10 years ago should be revised and asked WHO to undertake this task. The requirements involved are :

Requirements for Diphtheria and Tetanus Toxoid
Requirements for Pertussis Vaccine
Requirements for Dried BCG Vaccine
Requirements for Rabies Vaccine for Human Use
Requirements for Poliomyelitis Vaccine (inactivated).

44. Requirements for Snake Antivenins

The Committee noted that the Requirements for Snake Antivenins were limited in their scope [2] and was informed that many developing countries had asked for requirements that are appropriate for venins from a greater variety of snakes as well as for those from scorpions and spiders.

The Committee agreed that a revision broadening the scope of these requirements was needed and asked WHO, in consultation with the recently established WHO Collaborating Centre for the Control of Antivenins at the School of Tropical Medicine, Liverpool, to take the necessary steps to draw up such requirements.

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[1] Unpublished working document WHO/BS/77.1153.
[2] Unpublished working document WHO/BS/77.1162.

Annex 1

REQUIREMENTS FOR THE COLLECTION, PROCESSING AND QUALITY CONTROL OF HUMAN BLOOD AND BLOOD PRODUCTS[1]

(Requirements for Biological Substances No. 27)

[1] Prepared by a team of WHO consultants and staff members whose names are given in Appendix 1.

INTRODUCTION

In the past a number of documents of the World Health Organization have been concerned with whole blood and its components, but each one has concentrated on guidelines mainly concerned with blood transfusion services and except for human immunoglobulin none has dealt with the requirements applicable to the quality control of whole blood and blood products.

A WHO Working Group on the Standardization of Human Blood Products and Related Substances [1] considered that there was an urgent need for international requirements for the processing and control of whole human blood and blood products. It emphasized that, as the quality of the source material played an important part in the quality of the final products, such requirements should cover all stages, from the collection of source materials to the quality control of the final product.

In the compilation of these international requirements for human blood products, advice and data from a number of experts have been taken into account. The names of these experts are given in Appendix 2.

GENERAL CONSIDERATIONS

The setting up of an organization for the collection and fractionation of human blood and blood components calls for a great deal of expertise

[1] WHO Technical Report Series, No. 610, 1977, p. 24 (Annex 1).

and considerable investment. Any country contemplating the establishment of such an organization should carry out a careful cost-benefit analysis to determine whether the investment is justified. The collection and distribution of whole blood, the separation of whole blood into components, and the fractionation of plasma batches is a logical developmental sequence for a comprehensive organization. It is not always possible to be specific about the details of the procedures employed, the in-process controls, or the tests applied at each stage of production. This is particularly the case with whole blood and component cells. Although the general principle of fractionation of plasma is well established, there are in practice numerous variations in the details of the various production steps. Therefore, any country wishing to begin the collection and fractionation of blood and blood components should send personnel for training to a plant that is operating successfully. WHO can help in arranging such training.

It would not be possible to rely on any product unless the relevant requirements for each step are complied with, and any attempt to reduce these requirements may have serious consequences for the safety of the final product. It is recommended, therefore, that these requirements be applied as a complete document.

One of the basic questions to be answered in considering whether the fractionation of plasma should be started is whether the country has a suitable donor population of sufficient size to guarantee an adequate supply of source material. It is not possible to set a lower limit for the quantity of source material that would be necessary to make such an operation economic because too many factors are involved. In order to maintain competence in production and to avoid certain contamination risks, it is important to have sufficient source material to maintain the fractionation facility in continual operation. In general it would be difficult to justify setting up a plant unless at least 250 litres or 1000 donor pools of plasma are available for fractionation at regular intervals. Even with this amount the fractionation plant would be working on a small scale, but it could serve as the basis for later expansion to a much larger project. Alternatively it could be used for specialized national services, or it could form part of an integrated service organized on a regional basis with neighbouring countries.

The greatest expense involved is in setting up the fractionation plant, but it would be possible to consider the collection of source material and the fractionation as quite separate operations. A country may wish to establish collection centres to separate the cell components and send the plasma to an established fractionation centre in another country. The

products would then be returned to the original country and the costs involved in this operation might be less than those needed to establish and operate a fractionation plant.

The general prevalence of viral diseases, such as various forms of hepatitis, and of parasitic diseases differs so markedly in different geographical regions that each national authority must decide for itself whether the application of the most sensitive test on each blood donation is cost-effective and whether it is feasible to collect suitable source material. A brief protocol of the collection of source material is in any case mandatory (see Appendix 3). In countries where the prevalence of hepatitis B virus (HBV) and parasitic diseases is so high that the supply of the most suitable source material is markedly restricted, greater emphasis should be placed on the production of fractions made by a process that experience has shown causes the least risk of contamination. For example, immunoglobulin prepared by the cold ethanol fractionation method of Cohn has a well established reputation of being free from contamination with HBV, as have albumin products prepared by the same method and heated for 10 hours at 60°C.[1] Nevertheless, the assurance of freedom of these products from infectious viruses requires extreme care in manufacture and cannot be assumed when new fractionation methods are introduced.

When source material with a high risk of contamination is handled, special care should be given to the protection of the health of the staff and appropriate protective measures approved by the national control authority insisted upon.

The transport of source materials from blood collecting centres and hospitals to the fractionation facilities requires special consideration. Thus refrigeration at the temperature range appropriate for the product must be efficient and reliable and proved to be so by monitoring. Thermal insulation must be adequate as a safeguard against a temporary failure of refrigeration. Containers of liquid source material should be filled so as to diminish frothing due to shaking. Because of the potentially infective nature of these biological materials, suitable safeguards should be taken in the event of breakage, spillage, or leakage of containers.

In this document the qualifying word " human " has been dropped from the names of the products derived from human blood. Blood products of animal origin are immunogenic, and their administration to man should be avoided wherever an equivalent product of human origin

[1] WHO Technical Report Series, No. 602, 1977, p. 45.

could be used in its place. Any such products of nonhuman origin should now carry the species of origin in its proper name. In order to avoid confusion while the recommendation is being implemented, it is advised that for an interim period national authorities introduce the animal species in the proper names of animal products before the qualification " human " is dropped.

These requirements have been formulated in the following five parts.

A. Requirements for the collection of source materials.
B. Requirements for single-donor and small-pool products.
C. Requirements for the manufacturing of human blood products.
D. Requirements for the control of plasma fractions.
E. National control requirements.

Each deals with a separate part of the whole process but all the parts taken together are intended to make a composite document.

The parts are divided into sections, each of which constitutes a recommendation. Text printed in type of normal size is 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. Paragraphs printed in small type are comments and recommendations for guidance.

Should individual countries wish to adopt these requirements as the basis for their national regulations concerning blood products and related substances, it is recommended that a clause be included that would permit manufacturing requirements to be modified on the condition that it be demonstrated, to the satisfaction of the national control authority, that such modified requirements ensure a degree of safety and efficacy of the products at least equal to that provided by the requirements formulated below. It is desirable that 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 product is collected, manufactured or used, as appropriate.

Rapid technological developments in the measurement of biological activity of blood products and related substances require the establishment of international biological reference materials. The first two international reference materials (for anti-A and anti-B blood typing sera) were established in 1950, and a further six reference materials have been established in the last seven years. There are a number of materials currently under investigation for the preparation of new standards.

Furthermore, the increased demand for the use of blood products is resulting in the extensive movement of such products between countries. Internationally accepted requirements are therefore necessary in order that countries without any regulations concerning blood products and related substances may refer to these requirements when importing such products.

International standards and international reference preparations

The activity of blood and blood products shall be expressed in international units where an international standard or international reference preparation exists.

A list of international standards and international reference preparations appropriate for the control of blood products and related substances is given in Appendix 4.

These standards are in the custody of the laboratories in Copenhagen, London, Amsterdam and Bilthoven mentioned in Appendix 4.

Samples are distributed free of charge on request to national control laboratories. The international standards are intended for the calibration of national standards for use in the manufacture and laboratory control of human blood and blood products.

PART A:

REQUIREMENTS FOR THE COLLECTION OF SOURCE MATERIALS

A.1. DEFINITION OF CENTRES, ACTIVITIES AND SOURCES

A.1.1 Centres for the collection of source material

The following definitions are intended for use in this document and are not necessarily valid for other purposes.

Blood donor centre : an establishment in which blood and/or blood components are obtained from donors.

Placenta collecting centre : an establishment in which placentas and/or retroplacental blood or parts of either are received from hospitals, accumulated and stored.

A.1.2 Activities of collection centres

Blood collection : a procedure by which a single donation of blood is collected either in an anticoagulant and stabilizing solution or in a container of a kind that permits the separation of serum from coagulated blood.

Processing : any procedure used after collection and before compatibility testing with a prospective recipient.

Plasmapheresis and cytopheresis : procedures by which whole blood is separated by physical means into components and one or more of them returned to the donor.

A.1.3 Donors

Blood donor : a suitable person who gives blood.

A.1.4 Single-donor materials

Whole blood (sometimes referred to as " blood ") : the blood collected in an anticoagulant solution with or without the addition of nutrients such as glucose or adenine.

Whole blood, plasma-reduced (sometimes referred to as " plasma-reduced blood ") : the whole blood in which the erythrocyte volume fraction (" packed cell volume ") has been elevated to approximately 0.6 by the removal of plasma.

Whole blood, modified : the whole blood from which plasma has been separated for the purpose of obtaining cryoprecipitate, platelets, or leukocytes and the plasma returned to the blood cells.

Blood component : any part of blood separated from the rest by physical procedures.

Plasma : the liquid part of blood collected in a receptacle containing an anticoagulant.

Plasma, fresh frozen : a plasma frozen within 6 hours of donation and stored below −20°C (and preferably below −30°C).

Plasma, frozen : a plasma obtained from whole blood within a specified short time (but longer than 6 hours) of collection and maintained in the frozen state below −20°C (and preferably below −30°C).

Plasma, platelet-poor : a plasma from which most platelets have been removed.

Plasma, specific immune : a plasma that can be used either for passive immunization or for the manufacture of specific immunoglobulins.

Plasma, freeze-dried : any of the above forms of plasma that have been freeze-dried for preservation.

Plasma, recovered : a plasma that does not meet the requirements of " plasma, fresh frozen " or " plasma, frozen " and is intended for further processing.

Plasma, platelet-rich : a plasma containing at least 70% of the platelets of the original whole blood.

Cryoprecipitated Factor VIII : a preparation of Factor VIII that is obtained either from plasma from whole blood or by plasmapheresis, through a process involving chilling and precipitation.

Serum : the liquid part of coagulated blood or plasma.

Specific immune serum or plasma : a serum that can be used either for passive immunization or for the manufacture of specific immunoglobulins.

Red cell concentrate : whole blood from which most of the plasma has been removed and having an erythrocyte volume fraction (" packed cell volume ") greater than 0.7.

Red cell concentrate, washed : a red cell concentrate from which most of the plasma, leukocytes and platelets have been removed by one or more stages of washing with an isotonic solution.

Red cell concentrate, leukocyte-poor : a red cell concentrate containing at least 80% of the red cells and less than 25% of the leukocytes of the original whole blood.

Red cell concentrate, frozen : a frozen red cell concentrate to which a cryoprotective agent such as glycerol has been added prior to freezing.

Red cell concentrate, deglycerolized : a red cell concentrate, frozen, that has been thawed and has had the glycerol removed by washing.

Platelet-concentrate : platelets obtained either by separation of whole blood or by pheresis and suspended in a small volume of autologous plasma.

Leukocyte concentrate : a concentrate of leukocytes obtained either by the separation of whole blood or by pheresis and suspended in autologous plasma.

A.1.5 Postpartum source materials

Placenta : the placenta with or without the retroplacental blood from a single delivery.

Placental blood : the blood expressed from the placenta.

Retroplacental blood : uterine blood collected during and after delivery.

Retroplacental serum : the liquid part of the coagulated retroplacental blood.

A.2. PREMISES

The premises shall be of suitable size, construction, and location to facilitate their proper operation, cleaning, and maintenance in accordance with accepted rules of hygiene. They shall comply with the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) [1] and in addition provide adequate space, lighting, and ventilation for the following activities where applicable :

(1) Medical examination of individuals to determine their fitness as donors of blood and/or blood components.

(2) Withdrawal of blood from donors and, where applicable, reinfusion of the components with minimum risk of contamination and errors.

(3) Care of donors, including the treatment of those who suffer reactions.

(4) Storage of whole blood and blood components in quarantine pending completion of processing and testing.

(5) Laboratory testing of blood and blood components.

(6) Processing and distribution of whole blood and blood components in a manner that prevents contamination, loss of potency, and errors.

(7) Performance of all steps in pheresis procedures.

(8) Labelling, packaging, and other finishing operations in a manner that prevents errors.

(9) Storage of equipment.

(10) Storage of finished products prior to distribution.

(11) Documentation and recording of data on the donor, the donated blood, and the ultimate recipient.

[1] WHO Technical Report Series, No. 323, 1966, p. 13.

The collection of blood can be achieved by mobile teams. Although the premises used by such teams may not comply with the more stringent requirements for centres built specially for the purpose, the facility must be adequate for the safety of both the donor and the collected blood or blood components.

A.3. EQUIPMENT

Equipment used in the collection, processing, storage, and distribution of blood and blood components shall be kept clean and shall be maintained and checked regularly. The revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) [1] shall apply in every particular.

Equipment employed to sterilize materials used in blood or blood component collection or for the disposal of contaminated products shall ensure the destruction of contaminating microorganisms. The effectiveness of the sterilization procedure shall be not less than that achieved by a temperature of 121.5°C maintained for 20 minutes by saturated steam at a pressure of 103 kPa (1.05 kgf/cm^2 or 15 lbf/in^2) or by a temperature of 170°C maintained for two hours with dry heat.

Tests for sterility are indicated in the International Pharmacopoeia.[2]

The disposal of contaminated material should comply with the local by-laws controlling such procedures.

A.4. PERSONNEL

A blood or blood component collection organization shall be under the direction of a designated qualified person who shall be responsible for ensuring that all operations are carried out properly and competently. The director shall have an adequate knowledge and experience of the scientific and medical principles involved in the procurement of blood and, if applicable, the separation of blood components and the collection of plasma by plasmapheresis.

The director shall be responsible for ensuring that employees are adequately trained and acquire practical experience and that they are

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[1] WHO Technical Report Series, No. 323, 1966, p. 13.

[2] *Specifications for the quality control of pharmaceutical preparations ; second edition of the International Pharmacopoeia*. Geneva, World Health Organization, 1967, p. 747.

aware of the application of accepted good practice to their respective functions.

The director should have the authority to enforce or to delegate the enforcement of discipline among employees.

The persons responsible for the collection of the blood and blood components shall be supervised by licensed physicians who shall be responsible for all medical decisions.

The personnel responsible for the processing, storage, distribution, and quality control of blood, blood components, and plasma shall be adequate in number, and each shall have a suitable educational background and training or experience to assure competent performance of assigned functions so that the final product has the required safety, purity, potency, and efficacy.

A.5. THE COLLECTION OF BLOOD AND BLOOD COMPONENTS

A.5.1 The selection of donors

Source materials for further processing are obtained from donations of blood or its components. The medical criteria for accepting donors—criteria relating to the safety, purity, potency, and efficacy of the final products—must be the same for donors of whole blood and of cellular components or blood components collected by pheresis.

Blood from donors with glucose-6-phosphate dehydrogenase deficiency, sickle-cell trait, or other inherited erythrocyte abnormalities may give rise to transfusion reactions under certain circumstances. Decisions regarding the suitability of such donors should be made by the national control authority.

A.5.2 Donors of whole blood

The physical fitness of a donor shall be determined by a licensed physician or a person under the direct supervision of a licensed physician. Donors shall be healthy persons of either sex between the ages of 18 and 65 years. The frequency of donations shall not exceed one every two months, with a maximum volume in any consecutive 12-month period of 2 litres.

The recruitment of volunteer (non-remunerated) donors should be the aim of any national blood programme. In some countries

the upper and lower age limits of the donors may differ from 18 and 65 years.

The frequency of donation may have to be modified on an individual basis, and, in general, premenopausal female donors may be bled less frequently than males. Donors should be within normal weight limits (see section A.5.4).

A.5.3 Medical history

A.5.3.1 *General*

Before each donation questions shall be asked to determine that the donor is in normal health and has not suffered, or is not suffering, from any serious illness, e.g., malignant disease, diabetes, epilepsy, hypertension, renal disease.

Any donor who appears to be suffering from symptoms of acute or chronic disease or who is receiving oral or parenteral medication, with the exception of vitamins or oral contraceptives, may not be accepted for donation unless approved by a physician.

Any donor who appears to be under the influence of alcohol or any drug or who does not appear to be providing reliable answers to medical history questions shall not be accepted.

A.5.3.2 *Infectious diseases*

Donors shall have a negative history of viral hepatitis, of close contact with an individual with hepatitis within the past six months, of receipt within six months of human blood or any blood component or fraction that might be a source of transmission of viral hepatitis, or of tattooing within six months.

Acupuncture within six months may also present a risk.

In some countries donors with a history of viral hepatitis or of a positive test for hepatitis B surface antigen are permanently excluded. In other countries such donors are accepted providing that recovery occurred longer than one year previously and that the reaction for hepatitis B surface antigen is negative when tested by a sensitive technique.

Any donor shall be permanently excluded if a previous blood donation given by him was the only unit of whole blood or of a blood component administered to a patient who developed hepatitis within six months and who received no other blood fractions capable of hepatitis transmission during this period.

Donor populations showing a prevalence of acute or chronic hepatitis higher than that found in the general population should be avoided for collection both of single donor products (whole blood and its components) and of plasma for pooling for the manufacture of plasma fractions known to be capable of transmitting hepatitis, such as clotting factor concentrates.

Countries with a low incidence of hepatitis should not use whole blood or blood products obtained from source material collected from an area in which there is a high incidence of hepatitis.

The testing of blood or plasma for the presence of hepatitis B surface antigen shall be done by methods described in section B.1.

National health authorities shall develop policies designed to prevent the transmission of other infectious diseases based on the prevalence of these diseases in the donor population and the susceptibility of recipients to the same diseases.

In countries where malaria is not endemic, donors should have a negative history of malaria exposure during the past six months and a negative history of clinical malaria or malaria prophylaxis while residing in an endemic area within three years of donation. Such restrictions may be less important in countries where a high level of endemic malaria is present in both donors and recipients, except when blood products are required by visitors from non-endemic areas.

Other diseases that can be transmitted by blood include syphilis, brucellosis, trypanosomiasis (Chagas' disease), infectious mononucleosis, and cytomegalovirus infection. Precautions should be taken to avoid blood collection from persons known to have suffered acute or chronic brucellosis or trypanosomiasis in areas where these diseases are prevalent. Spread of herpes viruses (Epstein-Barr virus and cytomegaloviruses) by blood transfusion is a hazard not easily avoided owing to the high prevalence of asymptomatic chronic infection with these agents in the general population.

A.5.3.3 *Minor surgery*

Donors shall have a negative history of tooth extraction or other minor surgery during a period of 72 hours prior to donation.

A.5.3.4 *Pregnancy*

Pregnant women shall be excluded from blood donation. In general, mothers shall also be excluded for the period of lactation and for at least six months after full-term delivery.

The interval following pregnancy may be shorter in some cases—e.g., six weeks following an abortion during the first trimester.

In some countries donors are accepted when pregnant or during the period of lactation when the blood contains rare blood group antibodies. The volume to be taken should be determined by the physician responsible.

A.5.3.5 *Immunization*

Symptom-free donors who have recently been immunized may be accepted with the following exceptions.

- Those receiving smallpox vaccine shall be excluded until the scab has fallen off or until two weeks after an immune reaction.
- Those receiving attenuated vaccines for measles (rubeola), mumps, yellow fever, or poliomyelitis shall be excluded until two weeks after the last immunization or injection.
- Those receiving attenuated rubella (German measles) vaccine shall be excluded until eight weeks after the last injection.
- Those receiving rabies (therapeutic) vaccine or immunoglobulin shall be excluded until one year after the last injection.
- Those receiving passive immunization using animal serum products shall be excluded until four weeks after the last injection.

A.5.4 Physical examination

Donors shall have a weight, blood pressure, pulse rate, and temperature within normal limits. Donors with any measurements outside the established normal limits of weight, blood pressure, and pulse rate may be accepted only if approved by the responsible licensed physician.

The following recommendations may be useful for guidance :

(1) Blood pressure. Systolic blood pressure between 12 and 24 kPa (90 and 180 mmHg) ; diastolic blood pressure between 6.67 and 13.3 kPa (50 and 100 mmHg).

(2) Pulse. Between 50 and 100 beats per minute and regular.

(3) Temperature. Oral temperature not exceeding 37.5°C.

(4) Weight. Donors weighing less than 50 kg may be bled proportionately less than 450 ml in an appropriate volume of anticoagulant, provided all other donor requirements are met.

In some countries it is not required to take the body temperature but such decisions should be made by the national control authority.

Donors shall be free from any infectious skin disease at the venepuncture site and of skin punctures or scars indicative of addiction to narcotics.

A.5.5 Haemoglobin or haematocrit determination

The haemoglobin shall be not less than 125 g/l of blood for women and 135 g/l of blood for men or the haematocrit, if substituted, shall be not less than 38% or 41% respectively.

These limits are not universally accepted, and national control authorities should raise or lower them when appropriate.

A.5.6 Donors for plasmapheresis

All phases of plasmapheresis, including explaining to donors what is involved in the process and obtaining their informed consent, shall be performed under the direct supervision of a licensed physician.

There are two groups of plasmapheresis donors : those who donate at a frequency comparable to that allowed for whole blood donations and those who donate more frequently. The former group shall be accepted on the basis of the above criteria for donors of whole blood.

In addition to these criteria, donors participating in a more frequent plasmapheresis programme shall be examined by a licensed physician on the day of the first donation, or no more than one week prior to the first donation. This examination shall include urine analysis and blood sampling for liver function tests, a serological test for syphilis, and determination of plasma proteins by electrophoresis or another suitable method.

On the day of each donation, in addition to meeting the requirements for whole blood donors, plasmapheresis donors shall be shown to have a total serum protein of no less than 60 g/l.

The medical evaluation of plasmapheresis donors shall be repeated at regular intervals, as specified by national control authorities. The interval between physical and laboratory examinations shall not exceed four months.

Whenever a laboratory value is found outside the established normal limits or a donor exhibits any important abnormalities of history or on physical examination, the donor shall be removed from the programme. The donor shall not return to the programme until the abnormal finding has returned to normal and the responsible physician has given approval.

In the event that a plasmapheresis donor donates a unit of whole blood or does not have the red blood cells returned from a unit taken

during the procedure, the donor shall be deferred for eight weeks unless special circumstances warrant approval by the responsible physician of earlier plasmapheresis.

In general, plasma collected by therapeutic plasmapheresis shall not be used for fractionation.

There may be individual exceptions to this last requirement—e.g., plasma collected by intensive plasmapheresis during pregnancy of patients with high levels of anti-Rh_0 (anti-D) immunoglobulin.

It is difficult to state the maximum volumes of plasma that can be safely collected from donors until more definitive data are available on the effects of plasmapheresis on donors. In 1967 the Subcommittee of Specialists on Blood Problems, of the Council of Europe, recommended that no more than 8 single units of plasma (each of approximately 300 ml) should be removed in one month, and not more than 50 single units should be removed in one year.[1] However, different limits are imposed in certain countries, e.g., USSR and France : 10 litres per year ; USA : 50 and 60 litres per year for donors weighing respectively below and above 80 kg.

A.5.7 Donors for plateletpheresis and leukopheresis

In general, plateletpheresis and leukopheresis donors shall meet the criteria for whole blood and plasmapheresis donors.

The optimum conditions for performing plateletpheresis and leukopheresis to assure donor safety and satisfactory quality of the products are under active investigation in many countries. The following recommendations may be useful for guidance.

On the day of each donation, plateletpheresis donors should have an absolute platelet number concentration (" count ") of not less than 100×10^9/l and leukopheresis donors should have an absolute granulocyte number concentration of not less than 1.5×10^9/l. Both types of donor should have a normal leukocyte type number fraction (" differential count ").

Recovery of circulating platelet and leukocyte levels occurs promptly in donors, but data are not at present available to define the maximum numbers of platelets and leukocytes that can be safely collected from donors.

Leukopheresis may entail the administration of drugs to donors and their exposure to colloidal agents in order to enhance the yield of granulocytes. Appropriate precautions should be taken to protect the donors, such as investigation for latent diabetes by a glucose tolerance test on those who are to be given corticosteroids.

[1] WHO Technical Report Series, No. 468, 1971, p. 11.

Where leukopheresis is carried out for the treatment of a patient with chronic myeloid leukaemia it should be done only if approved by his attending physician. It is generally considered inadvisable to use the leukocytes from such patients.

A.5.8 Donors for immunization

Immunization of donors shall be carried out only when sufficient supplies of material of suitable quality cannot be obtained by selection of appropriate donors or from donations selected by screening. Donors must be fully informed of the risk of any proposed immunization procedure, and pressure shall not be brought to bear on a donor to agree to immunization. Donors of blood and those undergoing plasmapheresis shall if necessary undergo investigations that may reveal hypersensitivity to a proposed antigen.

When immunization is intended, the donor should be :

(1) informed of the procedures by a licensed physician and encouraged to take part in a free discussion, which in some countries is achieved by informing potential donors initially in small groups of people ;

(2) encouraged to seek advice from his family doctor before agreeing to immunization ;

(3) informed that any licensed physician of his choice will be sent all information about the proposed immunization procedure ; and

(4) required to indicate his agreement by signing an informed-consent form.

A.6 COLLECTION OF BLOOD

In the collection of blood several precautions must be taken, as described in the following sections.

A.6.1 The taking of the blood

The skin of the donor at the site of venepuncture shall be prepared by a method that has been shown to give reasonable assurance that the blood collected will be sterile. The collection of blood into a container shall be done using an aseptic method. The equipment for collecting the sterile blood may be closed or vented provided that the vent is designed to protect the blood against microbial contamination (see section B.1.1).

A.6.2 The containers

The original blood container or a satellite attached in an integral manner shall be the final container for whole blood and red cell concentrates, with the exception of modified red cell concentrates. Containers shall be uncoloured and transparent and the labelling shall be placed to allow visual inspection of the contents. Closures shall maintain a hermetic seal and prevent contamination of the contents. The container material shall not interact with the contents under the prescribed conditions of storage and use since such interaction may have an adverse effect on the safety or efficacy of the products.

The specifications for containers should be approved by the national control authority.[1]

Wherever possible it is desirable to use sterilized non-detachable satellite containers to prepare components in a closed system in order to minimize the likelihood of microbial contamination.

A.6.3 Anticoagulants

The anticoagulant solution shall be sterile, pyrogen-free, and of a composition that will ensure satisfactory safety and efficacy of the whole blood and of the separate blood components.

Commonly used anticoagulant solutions are acid citrate dextrose and citrate phosphate dextrose.

For plasmapheresis, sodium citrate as a 40 g/l solution is widely used as an anticoagulant.

A.6.4 Volume of blood

The national control authority shall determine the quantity of anticoagulant to be used in each container of whole blood and the volume of blood to be collected. Provision shall be made to ensure that only those units meeting the requirements are issued for use.

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[1] Much useful information is contained in the following publications : COOPER, J. *Plastic containers for pharmaceuticals : testing and control.* Geneva, World Health Organization, 1974 (WHO Offset Publications, No. 4). *Specifications for the quality control of pharmaceutical preparations : second edition of the International Pharmacopoeia.* Geneva, World Health Organization, 1967, p. 869. WHO Technical Report Series, No. 614, 1977 (WHO Expert Committee on Pharmaceutical Preparations : twenty-sixth report), p. 25, Annex 3.

A.6.5 Pilot samples

Pilot samples are blood samples provided with each unit of whole blood or of red blood cells. They shall be collected at the time of donation by the person who collects the whole blood. They shall be marked before collection to be identical with that of the unit of whole blood.

Pilot samples should be attached to the final container in a manner that will indicate whether they have been removed and reattached.

Laboratory samples used in testing the blood may be collected in addition to the pilot samples. They should meet the above requirements.

A.6.6 Identification of samples

Each container of blood, blood components, and pilot and laboratory samples shall be identified by a unique number or symbol so that it can be traced back to the donor and from the donor to the recipient. The identity of each donor shall be established at the time of determination of donor fitness as well as at the time of blood collection.

When source material is transferred to a fractionation plant the appropriate details shall accompany such material.

An example of a protocol that may be useful for such purposes is included in Appendix 3.

PART B:
REQUIREMENTS FOR SINGLE-DONOR AND SMALL-POOL PRODUCTS

GENERAL CONSIDERATIONS

These requirements for single-donor and small-pool products cover the methods used to prepare products directly from units of whole blood or of components collected by pheresis, starting with the testing of the units and proceeding to the separation of the various cell and plasma protein components. Among the products are those that may be prepared in small pools (12 donors or fewer), such as cryoprecipitated Factor VIII and platelet concentrates. In addition to tests on the units of whole blood that provide information on the safety, efficacy, and labelling of the components, specific tests are included where applicable to ensure the quality of various components.

It is important to note that single-donor and small-pool products have certain specialized uses other than therapeutic application to replace deficits in patients. Although not dealt with further in these requirements, these uses include: (1) stimulation of plasma donors with red blood cells in order to raise antibody levels for the preparation of anti-D immunoglobulin [1] and special blood grouping reagents; and (2) preparation of *in vivo* diagnostic products such as radiolabelled fibrinogen for the diagnosis of deep vein thrombosis. It is of the utmost importance that the donors of cells and plasma for such purposes be carefully studied both initially as well as on a continuing basis to minimize the likelihood of transmitting viral hepatitis to recipients. Red cells, stored frozen, which have been demonstrated to be free from hepatitis are valuable for the immunization of volunteers to reduce the risk of transmitting viral hepatitis.

Plasma donors may be immunized also with viral or bacterial antigens for the preparation of specific immunoglobulin products. All donor immunization procedures must be planned and carried out under the supervision of a physician who is knowledgeable about the antigens being used and especially about reactions or complications that may arise. Donors being immunized shall have been fully informed of all known hazards and shall have given their written informed consent to the procedures.

B.1. TESTING OF WHOLE BLOOD

B.1.1 Sterility

Each donation of whole blood intended for transfusion and each preparation of component cells constitutes a single batch. It shall not be tested for sterility by a method that entails broaching the final container before the blood is transfused.

> National control authorities may require that tests for sterility shall be carried out at regular intervals on final containers, taken at random and at the end of the storage period. The purpose of this test is to check on the aseptic technique used for taking and processing the blood as well as on the conditions of storage.

Each donation of whole blood shall be visually inspected immediately before issue. It shall not be issued if there is any evidence of leakage or

[1] WHO Technical Report Series, No. 468, 1971, pp. 7–12.

suspicion of microbial contamination such as unusual turbidity, haemolysis, or change of colour.

B.1.2 Laboratory tests

Laboratory tests are made on laboratory samples taken at the time of collection or from the pilot samples accompanying the final container, labelled as required in section A.6.

> In some countries the national control authorities require that test reagents, particularly those used for blood grouping and detection of hepatitis B surface antigen, should be approved by the authorities.

Results of the tests are used for ensuring the safety and proper labelling of all components prepared from units of whole blood.

B.1.3 Tests for infectious agents

B.1.3.1 *Test for syphilis*

Each donation of whole blood shall, if required by the national authorities, be subjected to a serological test for syphilis. If so tested, only units giving negative results shall be used for transfusion or component preparation.

B.1.3.2 *Test for viral hepatitis*

A test for hepatitis B surface antigen shall be done on each unit of blood or plasma collected and only those giving a negative result shall be used.[1] Units giving a positive result shall be so marked, segregated, and disposed of by a method approved by the national control authorities, unless designated for reagent or experimental vaccine production in an area designed and segregated for such production.

> The test should preferably be done by a highly sensitive method such as radioimmunoassay, reversed passive haemagglutination, or enzyme-linked immunoassay.
>
> With respect to plasma intended for pooling, all donations entering the pool should be tested and found nonreactive for hepatitis B surface antigen. In some countries small subpools and the final products are tested by highly sensitive methods, but this is considered to be inferior to the testing of single donations.

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[1] WHO Technical Report Series, No. 602, 1977 (*Advances in viral hepatitis*), pp. 42–45 and 59.

As with other infectious diseases, there may be areas of such high levels of hepatitis B virus endemicity in donors and immunity in recipients that national control authorities may wish to modify this testing requirement to meet their special needs and conditions.

The label on the container or the direction circular should indicate the geographical source of the material as well as whether and how the material has been tested for hepatitis B surface antigen.

Liver function tests such as serum transaminases are used in some countries to detect liver damage that may be associated with hepatitis.

B.1.4 Red blood cell grouping

Each unit of blood collected shall be classified according to ABO blood group by testing the red blood cells with anti-A and anti-B sera and by testing the serum or plasma with known type A (or a single subtype A_1) cells and known type B cells. The unit shall not be labelled as to ABO group unless the results of the two tests (cell and serum grouping) are in agreement. Where discrepancies are found, in the testing or with the donor records, they shall be resolved prior to labelling the units.

Each unit of blood shall be classified according to Rh blood type based on the results of testing for the Rh_0 (D) red cell antigen. The Rh_0 (D) type shall be determined with anti-Rh_0 (anti-D) typing serum.

Before labelling blood as Rh_0(D)-negative it should have been tested using a technique designed to detect Rh_0(D) variants (D^u).

Some national authorities require further analysis of Rh phenotypes.

In many countries anti-(A + B) is included in order to detect weak subgroups of A.

B.2. SEPARATION OF RED CELL CONCENTRATES

The preparation of red cell concentrates shall be performed under aseptic conditions and wherever possible in a closed system. The sterility of all components shall be maintained during processing by using aseptic techniques and sterile pyrogen-free equipment. The methods shall be those approved by the national control authority, and a written description of the procedures shall be prepared for each product, describing each step in production and testing. Proposals for any procedural modifications shall be submitted to the national authorities for approval before they are implemented.

Whole blood for preparation of all components shall be collected as described in section A.6 and tested as described in section B.1.

The following red cell concentrates may be prepared for therapeutic purposes :

(1) whole blood, plasma-reduced
(2) red cell concentrate
(3) modified red cell concentrates
  (*a*) red cell concentrate, leukocyte-poor
  (*b*) red cell concentrate, washed
  (*c*) red cell concentrate, frozen and deglycerolized.

B.2.1 Methods of separation

Red cell concentrates shall be prepared from whole blood collected in plastic bags or in glass bottles.

> Multiple plastic bag systems are preferable because they minimize the risk of microbial contamination by providing completely closed systems. They are easy to handle and are disposable. They have two drawbacks : their high cost and the leaching into the blood and its components of substances such as plasticizers from poly(vinyl chloride) bags. The use of glass bottles is less costly but has the disadvantage of being an open or vented system ; thus separation steps must be carried out under strictly aseptic conditions in sterile rooms or laminar flow cabinets and with microbiological monitoring. The same conditions apply also to the separation procedure when plasma is transferred from disposable single plastic bags to separate containers.

All surfaces that come into contact with the blood cells shall be sterile and pyrogen-free. If an open plastic bag system is used—i.e., the transfer container is not integrally attached to the blood containers and the blood container is broached after blood collection—the plasma shall be separated from the cells with positive pressure on the original container and maintained until it has been sealed. If the separation procedure involves a vented system—i.e., an airway is inserted in the container for withdrawal of the plasma—the airway and vent shall be sterile and constructed so as to exclude microorganisms.

> In some countries the sterility of products prepared in open systems is monitored by testing a sample of at least 2% of the units. The national control authority should approve the system used.

The final containers for plasma reduced blood and red cell concentrates (but not modified red cell concentrates) shall be the container in which the blood was originally collected or a satellite container attached in an integral manner. If pilot samples are detached from the blood container during removal of any component, the pilot samples shall be reattached to the container of plasma-reduced blood or red cell concentrate. The removal and reattachment of the pilot samples shall be recorded conspicuously and signed on the label of the unit. The final containers for all other components shall meet the requirements for blood containers described in section A.6. At the time when the final container is filled and if a different container is used, it shall be given a number or other symbol to identify the donor(s) of the source blood. Whenever appropriate the secondary container shall be similarly labelled while attached to the primary container.

B.2.2 Time of separation

The timing and the method of separation (centrifugation, undisturbed sedimentation, or the combination of the two) depends on the components prepared from the given donation. When platelets and coagulation factors are being prepared from the same donation, separation of the components shall be performed as soon as possible after withdrawal of the blood from the donor.

> It is preferable to effect the separation within 4–6 hours of the blood donation.
>
> When platelet concentrates and coagulation factors are to be prepared, it is important that the venepuncture be performed with minimal tissue damage in order to prevent the initiation of coagulation. The blood should flow freely without interruption, as rapidly as possible, and be mixed thoroughly with the anticoagulant.

If platelet concentrate is to be prepared from a whole blood unit, the blood shall be kept at a temperature as close as possible to 20–24°C until the platelet-rich plasma has separated from the red blood cells.

Separation of blood cells by centrifugation shall be done in a manner that will not increase the temperature of the blood. Cells may also be separated by spontaneous sedimentation.

> Sedimentation is the least expensive method for red blood cell separation and there is no need for special equipment.
>
> Repeated washing with saline solutions and centrifugation and filtration are used to reduce the amount of leukocytes, platelets,

and trapped plasma in red cell concentrates. Repeated washings are also used for the removal of glycerol from frozen red cells after thawing.

B.2.3 Plasma-reduced blood and red cell concentrate (red blood cells)

Plasma-reduced whole blood is obtained when sufficient plasma has been withdrawn to yield a product with an erythrocyte volume fraction (" packed cell volume ") of not more than 0.6. Red cell concentrates are formed when more plasma is removed, yielding a product with an erythrocyte volume fraction of approximately 0.7–0.9.

Red cell concentrates may be prepared either by centrifugation or by undisturbed sedimentation prior to the expiry date of the original whole blood.

B.2.4 Expiry date

The expiry date of whole blood and red cell concentrates prepared in a closed system from acid citrate dextrose (ACD) blood as well as from citrate phosphate dextrose (CPD) blood is 21 days after collection in general. The time of removal of plasma is not relevant to the expiry date of the red cell concentrates.

When red cell concentrates are prepared with very high haematocrits, an expiry date of 17 days after collection is recommended in some countries because of glucose deficiency.

The shelf-life of stored blood has been extended to 35 days in some countries by collecting the blood in ACD supplemented with 0.5 mmol/l adenine or in a mixture of 0.5 mmol/l adenine and 0.25 mmol/l guanosine, with extra glucose. Recent studies indicate that it may be possible to achieve the same 35-day life of stored blood by collecting it in CPD supplemented with 0.25 mmol/l adenine and extra glucose.

The *in vitro* oxygen transport function of the red cell is substantially decreased after 5–7 days in ACD blood and after 10–14 days in CPD blood.

Provided that sterility is maintained, the expiry date of red cell concentrates is not influenced by the type of separation used. However, if an open system is used, which does not maintain sterility, the expiry date is 24 hours after separation, but the cells should be used as soon as possible. Red cell concentrates shall be stored at 4°C ±2°C and transported at 5°C ±4°C in ice in insulated boxes.

Refrigerated whole blood and red cell concentrates will warm up rapidly when placed at room temperature. Every effort

should be made to limit the periods during which the products are handled at ambient temperatures in order to prevent the temperature from rising above 10°C until the time when the products are used.

Sodium chloride solution (suitable for intravenous use) may be added immediately prior to use to facilitate both mixing and administration of red cell concentrates.

B.2.5 Modified red cell concentrates

B.2.5.1 *Red cell concentrate, leukocyte-poor*

Leukocyte-poor red cell concentrate is a red cell preparation containing at least 80% of the red cells and less than 25% of the leukocytes of the original whole blood. The number and type of residual leukocytes vary with the method of preparation.

Because of the possibility of reactions some countries require that the red cell concentrate contain less than 5% of the leukocytes of the original whole blood.

The preparation of leukocyte-poor blood can be performed by filtration, by freezing and washing, or by washing alone. It should be performed as soon as possible after the collection of the blood. Filtration is an efficient method for the elimination of the leukocytes if applied within 48 hours of blood collection, but the filtration of heparinized blood should take place within one hour. Freezing of the red cell concentrate should be performed as described below for frozen red cells.

B.2.5.2 *Red cell concentrate, washed*

Washing of red cells can be performed by interrupted or continuous flow centrifugation. By the former method the washing procedure shall be repeated three times.

The centrifugation should be carried out in refrigerated centrifuges. If such equipment is not available the washing solution should have a temperature of 4°C ±2°C.

The washing of red cells can also be performed by reversible agglomeration and sedimentation using sugar solutions.

Washed red cell concentrates should be transfused as soon as possible and not later than 24 hours after processing. They should be stored at all times at 4°C ±2°C.

Requirements for pilot samples, labels, and temperature of storage and transport are the same as those for red cell concentrates.

B.2.5.3 *Red cell concentrate, frozen and deglycerolized*

Frozen red cell concentrates are red cells that have been stored continuously at low temperatures in the presence of a cryoprotective agent. The red cells must be washed to remove the cryoprotective agent prior to use for transfusion. The methods of preparation, storage, thawing, and washing shall ensure a viability of at least 70% of the transfused cells 24 hours after transfusion.

The cryoprotective agent in most common use is glycerol. The temperature of storage should be between −70°C and −160°C depending on the glycerol concentration used.

The storage period of frozen cells is at least 3 years and may be much longer under certain circumstances, but the reconstituted (thawed and washed) red cells should be used as soon as possible and not later than 24 hours after thawing.

The usual method of shipment of frozen cells is in solid carbon dioxide or liquid nitrogen. Deglycerolized cells should be stored at 4°C ±2°C and shipped at 5°C ±4°C.

Requirements for pilot samples and labels are the same as those for red cell concentrate.

B.3. OTHER SINGLE-DONOR OR SMALL-POOL COMPONENTS

B.3.1 Single-donor plasma

B.3.1.1 *Plasma, fresh frozen*

Fresh frozen plasma shall be separated from the whole blood and frozen solid preferably within 6 hours of collection. It can be kept frozen or it can be freeze-dried.

Freezing may be accomplished in a mechanical freezer at or below −40°C or with a combination of solid carbon dioxide and an organic solvent such as alcohol. The latter procedure should have been shown not to allow penetration of the container by the solvent or leaching of substances from the container into the contents.

Prior to use for infusion, the frozen plasma should be thawep rapidly to 30–37°C.

When stored at or below −20°C (preferably below −30°C), fresh frozen plasma has an expiry date of 1 year from the date of collection. In freeze-dried form the expiry period is 5 years.

Before its expiry date, fresh frozen plasma may be used for preparing cryoprecipitated Factor VIII. It may be used for preparation of other pooled plasma fractions at any time, even after its expiry date.

B.3.1.2 *Plasma, frozen*

Frozen plasma shall be separated from whole blood 6 hours or more after collection of the blood, but the time should be as short as possible. Frozen plasma may be used directly for transfusion or fractionation, or it may be freeze-dried as single-donor units. In addition, it may be combined in small pools before freezing to prepare freeze-dried plasma.

The national control authority should determine specific requirements for frozen plasma.

If such plasma is intended to be used directly in patients without further processing, the blood shall be collected in such a manner and in such containers as to allow aseptic handling—e.g., by the use of closed systems.

Whenever the container is broached in an open procedure the method used for handling shall avoid microbial contamination, and, as an additional precaution, sterile rooms or laminar flow cabinets can be used. Delay in processing shall be avoided, and the ambient conditions shall be regulated to minimize the risk of contamination.

B.3.1.3 *Plasma, freeze-dried*

Freeze-dried plasma shall be made from fresh frozen plasma or frozen plasma using either single units or small pools.

Storage conditions and expiry dates of different forms of freeze-dried plasma shall be approved by the national control authorities.

The intended use for which freeze-dried plasma is suitable and the expiry date are related to the source material, storage conditions, and residual moisture in the product.

B.3.1.4 *Plasma, recovered*

Recovered plasma shall be separated from whole blood at any time up to 5 days after the expiry date of the blood. The method used for separation shall avoid microbial contamination. As an additional precaution, sterile rooms or laminar-flow cabinets can be used. It shall be stored and transported at temperatures not exceeding 10°C.

Recovered plasma is intended to be pooled for fractionation but should not be used directly for transfusion. It may be pooled at any time after collection. To avoid microbial growth in contaminated plasma, recovered plasma should be preferably stored and transported in the frozen state. A preservative should not be added.

B.3.1.4 *Plasma, platelet-rich*

Platelet-rich plasma is a preparation containing at least 70% of the platelets of the original whole blood.

The preparation shall be separated by centrifugation within 4–6 hours of the collection of whole blood, and the temperature and time of processing as well as of storage shall be consistent with the maintenance of platelet survival and function.

Platelet-rich plasma shall be transfused as soon as possible (but no later than 72 hours) after collection in order to achieve the desired haemostatic effect.

B.3.2 Platelet concentrates

Platelet concentrates can be processed by separation from whole blood, by separation from platelet-rich plasma, or by plateletpheresis.

The whole blood from which platelet concentrates or platelet-rich plasma is derived shall be maintained at 22°C $\pm$2°C until the platelets are separated. The separation shall be performed within 4–6 hours after the collection of whole blood or plasma. The bleeding of the donor shall be performed by a single venepuncture giving an uninterrupted flow of blood with minimum damage to the tissue of the donor. The time and speed of centrifugation must have been demonstrated to produce a suspension without visible aggregation or haemolysis. The suspension shall contain a minimum count of 12.5×10^{10} platelets from each litre of whole blood (i.e., 2.5 units of blood) in at least 75% of the concentrates tested at the maximum storage time.

A pH of 6.0 or higher shall be maintained throughout storage. The volume of original plasma to be used for resuspension of the platelets depends on the storage temperature. Platelets stored at room temperature shall be resuspended in approximately 50 ml of plasma. Platelets stored at 4°C $\pm$2°C shall be resuspended in 20–30 ml of plasma.

If platelet concentrates are stored at room temperature, continuous gentle agitation should be maintained throughout the storage period.

Recent studies indicate that platelet concentrates with high platelet counts and stored at 22°C ±2°C may require up to 70 ml of plasma to maintain the pH above 6.0 throughout the storage period, which may be as long as 72 hours.

The product should be ABO and $Rh_0(D)$ typed and it may be desirable also to know the HL-A type.

The material of the final container used for platelet concentrates shall not interact with the contents under normal conditions of storage in such a manner as to have an adverse effect on the product.

Requirements for the labelling of the final container are the same as those in section B.3.6. In addition to the customary data the label shall bear the following: (1) the recommended storage temperature; (2) if stored at 20–24°C, instructions to maintain a continuous gentle agitation of the concentrate during storage to obtain maximum haemostatic effectiveness; (3) instructions that the contents shall be used as soon as possible, preferably less than 4 hours after broaching the containers for pooling.

B.3.2.1 *Expiry date*

The expiry date of platelet concentrates processed in a closed system shall be 72 hours after the collection of the original whole blood.

Platelet concentrates prepared in an open system should be used within 4 hours of preparation if stored at 22°C ±2°C and within 24 hours if stored at 4°C ±2°C.

Single-donor platelet concentrates may be pooled under aseptic conditions prior to issue. Such small pools should be used as soon as possible, and no later than 4 hours after preparation if stored at room temperature and 24 hours if stored at 4°C ±2°C.

B.3.3 Leukocyte concentrate

Leukocyte concentrate is a concentrate of separated leukocytes, which may also contain a large number of platelets and red blood cells, depending on the method of preparation.

Methods of processing leukocyte concentrates shall comply with the requirements and recommendations described in sections B.2.1 and B.2.2.

The label of the final container shall bear, in addition to customary data, instructions to use the leukocyte concentrate as soon as possible but not more than 4 hours after the container has been broached for

pooling. Temperature of storage shall be 4°C ±2°C and of transport 5°C ±4°C.

Leukocytes are separated from the blood of healthy donors or, in some centres, from whole blood obtained from patients with chronic myeloid leukaemia.

Leukocytes can be separated from blood by centrifugation, by sedimentation, or by continuous-flow filtration or centrifugation (leukopheresis). The leukocytes from units obtained from several healthy donors may have to be pooled to obtain a sufficient amount of leukocyte concentrate. Leukopheresis by continuous-flow filtration or centrifugation is the most efficient way of obtaining leukocyte concentrates with large numbers of high-quality leukocytes from a single donor.

Leukocytes can also be extracted from blood by filtration of whole blood. Leukocytes adhere to, and can subsequently be recovered from, the surface of the filters. The chemical and physical properties of the filter should be such that the *in vivo* cell survival and cell function of the leukocytes are not impaired.

By centrifugation of whole blood, 30–60% of the leukocytes present in the original whole blood may be recovered.

Approximately 90% of the leukocytes of the original whole blood can be separated by sedimentation of the red cells accelerated by the addition of suitable substances with high relative molecular mass. Continuous-flow filtration (filtration leukopheresis) may give a final yield of 70% of the leukocytes of the donor blood.

The product should be ABO and $Rh_0(D)$ typed and it may be desirable also to know the HL-A type.

B.3.3.1 *Expiry date*

The expiry date of leukocyte concentrates shall be 24 hours after collection of the original whole blood.

B.3.4 Cryoprecipitated Factor VIII

Single-pool cryoprecipitated Factor VIII is a preparation of Factor VIII obtained from a single unit of plasma from whole blood or by plasmapheresis.

The product may also be prepared as a pool from a small number of donations, usually 4–6 and not exceeding 10. It may be freeze-dried.

The plasma shall be separated from red blood cells and frozen solid preferably within 6 hours of collection.

Freezing may be accomplished in a mechanical freezer at −40°C or below or with a combination of solid carbon dioxide and an organic solvent such as alcohol. The latter procedure should have been shown not to allow penetration of the container by the solvent or leaching of substances from the container into the contents.

The method of thawing and harvesting the cryoprecipitate shall have been shown to yield a product containing an adequate activity of Factor VIII (see section B.3.5.4).

B.3.4.1 *Expiry date*

The frozen product shall be stored at or below −20°C (if possible below −30°C) and shall have an expiry date of one year from the date of collection. The freeze-dried product shall be stored at 4°C ±2°C and shall have an expiry date of one year. It shall be used promptly after thawing or reconstitution. The thawed or reconstituted product should be kept at room temperature (20–24°C) prior to use. It shall be used as soon as possible and not more than 4 hours after its container has been broached for pooling or reconstitution.

B.3.5 Control of single-donor and small-pool products

B.3.5.1 *General*

Single-donor and small-pool products shall comply with any specifications established by the national control authority. Cellular blood components and some plasma components may deteriorate during their separation or storage. Therefore, whatever the method of separation (sedimentation, centrifugation, washing, or filtration) used for the preparation of cell components, it is important that a portion of plasma protein sufficient to assure optimum cell preservation be left with the cells except when a cryoprotective substance is added for prolonged storage in the frozen state.

Methods employed for component separation should be checked before their implementation and at regulated intervals for the quality of the final products. The characteristics assessed should include yield, purity, *in vivo* recovery, biological half-life, functional behaviour, and sterility.

The intervals at which such checks are carried out should be determined by the national control authorities.

Immediately before issue for transfusion the components shall be inspected visually. They shall not be issued for transfusion if abnor-

malities of colour are observed or if there is any other indication of microbial contamination or of defects in the container.

Components shall be stored and transported at the temperature most suitable for the given component. Refrigerator or freezer compartments in which components are stored shall contain only whole blood and blood components.

B.3.5.2 *Red cell concentrate, single-donor plasma, and leukocyte concentrates*

When red cell concentrates and leukocyte concentrates are obtained from units of whole blood, such units shall comply with the requirements in sections A.6 and B.1. Single-donor plasma shall be obtained from units of whole blood that comply with the requirements in sections A.6 and B.1 or by plasmapheresis.

B.3.5.3 *Platelet concentrates*

Platelet concentrates shall be obtained from units of whole blood that comply with the requirements in sections A.6 and B.1 or by plateletpheresis.

Randomly selected units at the end of their shelf-lives shall be tested on a regular basis. They shall be shown to have :

(1) platelet number concentrations (" counts ") of at least 125×10^9/l (i.e., per 2.5 units) of whole blood ;

(2) plasma volumes appropriate to the storage temperature (see section B.3) ; and

(3) a pH between 6.0 and 7.4.

The number of units to be tested shall be specified by the national control authorities.

B.3.5.4 *Cryoprecipitated Factor VIII*

Cryoprecipitated Factor VIII shall be obtained from units of whole blood that comply with the requirements in sections A.6 and B.1 or by plasmapheresis.

Randomly selected units shall be tested on a regular basis within 30 days of their preparation. The number of units to be tested shall be specified by the national control authorities. The freeze-dried Factor VIII preparation shall dissolve completely in the solvent recommended

by the manufacturers within 30 minutes when held at a temperature not exceeding 37°C. The solution kept at room temperature shall not show any signs of precipitation in the first 3 hours after it has been dissolved.

In many laboratories an average potency of 400 International Units of Factor VIII per litre of starting plasma is reached. The average potency of freeze-dried cryoprecipitate is then at least 300 International Units of Factor VIII per litre of starting plasma. Whether this potency can be reached depends on local technical possibilities. In several countries the yields will be much lower, and the national control authority in the country will have to decide on the level of acceptability.

B.3.6 Labelling of single-donor and small-pool products

When testing is completed and before issue for transfusion, units of single-donor and small-pool products shall be identified with container labels that clearly indicate at least the following information :

(1) the proper name of the product ;

(2) the unique number or symbol identifying the donor(s) ;

(3) the expiry date ;

(4) any special storage conditions or handling precautions that are necessary ;

(5) a reference to a leaflet containing instructions for use, warnings, and precautions ;

(6) the name and address of the blood donor centre and, where applicable, the manufacturer and distributor.

The results of red blood cell grouping shall be on the label of whole blood, red cell concentrates, plasma products, platelet concentrates, and leukocyte concentrates but not necessarily on cryoprecipitated Factor VIII.

B.3.7 Placental source material

Whole placenta, placental blood or serum, and retroplacental blood or serum may all serve as source material for certain plasma fractions.

This source material should be used only in methods of production and for products that have not been associated with the transmission of hepatitis, such as heat-treated albumin products and immunoglobulins prepared by the Cohn fraction-ation process. If another fractionation method is used, clinical evidence should prove that no transmission of hepatitis occurs.

Where it is impracticable to test individual source material for the presence of hepatitis B surface antigen the pooled material shall be assumed to be contaminated. Because hepatitis B surface antigen would be diluted in pooled material and may escape detection even with highly sensitive techniques, the label or package insert of the final product shall state whether a test for hepatitis B surface antigen was carried out, and if so, whether it was carried out on the individual source material or after pooling.

It is important that national control authorities assume the responsibility for the method of testing, the production method, and the use of the products obtained from this source material.

PART C:

REQUIREMENTS FOR THE MANUFACTURING OF HUMAN BLOOD PRODUCTS

C.1. BUILDINGS

The buildings used for the fractionation of plasma shall be of suitable size, construction, and location to facilitate their proper operation, cleaning, and maintenance in accordance with general rules of hygiene. They shall comply with the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) [1] and in addition provide adequate space, lighting, and ventilation for the activities listed below.

> Each of the activities listed below is an important integral part of the production procedure, and countries wishing to start manufacturing human blood products and related substances should not do so unless adequate provision can be made for all activities.

C.1.1 Storage of whole blood and its components

Whole human blood and its components shall be stored in separate refrigerated rooms in which they may be frozen or refrigerated and which are used only for this purpose. The products shall remain in the rooms until the results of testing show that they are suitable for introduction into the fractionation premises.

[1] WHO Technical Report Series, No. 323, 1966, p. 13.

C.1.2 Separation of cells and fractionation of components

The separation of cells and fractionation of components shall be done in a building isolated from the manufacture or processing of nonhuman proteins or microbiological materials, such as vaccines, and separate from the animal house.

In some countries the separation of cell constituents is carried out in a separate area from that in which components are fractionated.

C.1.3 Supply and recovery of ancillary materials

Adequate facilities shall be provided for the supply of ancillary materials, such as ethanol, water, salts, and poly(ethylene glycol).

Facilities for the recovery of organic solvents used in fractionation may also be provided.

C.1.4 Freeze-drying and filling

The sterile filling of final containers shall be done in a separate area.

A separate area and separate equipment should be used for the freeze-drying of bulk product and of final product.

C.1.5 Packaging, labelling, and storing

Separate facilities shall be used for the labelling and packaging of containers. A separate area shall be provided for the storage of labels, leaflets, and packages. Another separate area shall be used for the storage of final containers prior to despatch.

C.1.6 Keeping of records

Adequate provision shall be made for the keeping of records of all materials, fractionation steps, quality control procedures, results, the distribution of the final product, and the disposal of potentially infective materials.

C.1.7 Provision for control

Provision shall be made for quality control, including haematological, biochemical, physicochemical and microbiological testing as well as pyrogen and safety testing.

It is desirable that those parts of the quality control laboratories that are hazardous to production be separate from the production area.

C.1.8 Disposal of infective material

Provision shall be made for the suitable disposal of potentially infective materials by autoclaving or incineration.

Local laws should be complied with in the disposal of these materials.

C.2. EQUIPMENT

Equipment used for the collection, processing, storage, and distribution of source materials and plasma fractions shall comply with the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories). [1]

Particular attention shall be paid to the following points.

(1) The reliability, maintenance, monitoring, and recording of continuously operating equipment and the provision of reserve facilities.

(2) The suitability and compatibility of the surfaces of all materials (e.g., filter medium, glass, stainless steel, plastic, and rubber) that come into contact with the products.

Metal surfaces that come into contact with proteins should be resistant to scratching. The surface of certain materials can denature certain proteins or activate certain coagulation factors.

(3) The ease and efficiency of cleaning and, where necessary, sterilizing equipment. Any bactericidal agent used shall be capable of being completely eliminated before the equipment is used.

If possible all surfaces with which the plasma and the solvents come into contact should be amenable to visual inspection, and stainless steel tubing should be dismountable.

Washing fluids in common use are sodium hydroxide or soda solutions, which are bactericidal and virucidal. Caution should

[1] WHO Technical Report Series, No. 323, 1966, p. 14.

be exercised in the use of detergents because of a possible effect on the final product; tests should be made to ensure that they do not have any adverse effect.

(4) The provision of suitable facilities for autoclaving and for the disposal of potentially infective materials and equipment.

C.3. PROVISION OF SUPPORT SERVICES

The fractionation of source materials requires a number of services essential for the operations involved.

C.3.1 Water supply

An adequate supply of suitable pyrogen-free water shall be provided for use during the fractionation process and for the reconstitution and/or dilution of the plasma fractions before filling and freeze-drying.

The two most commonly used types of water are pyrogen-free distilled water [1] and pyrogen-free deionized water, each of which should be maintained at 80°C. Water preparation and delivery systems should be tested at regular intervals for pyrogenicity and conductance. The water system should be continuously circulating and should have no dead ends.

Water for injection is generally used for the preparation of final products.

C.3.2 Steam supply

An adequate supply of steam shall be provided for the cleaning of equipment and the operation of apparatus used to sterilize the equipment and containers. The steam shall be maintained to a standard of cleanliness such that it does not cause or leave a contaminating deposit on the equipment and containers so cleaned or sterilized.

C.3.3 Other support facilities

Other support facilities are listed below.

(1) A supply of electrical and thermal energy.

(2) A means of refrigeration for the following purposes :
   (*a*) storage of various source materials and fractions;

[1] *Specifications for the quality control of pharmaceutical preparations : second edition of the International Pharmacopoeia*. Geneva, World Health Organization, 1967, p. 50.

(*b*) temperature maintenance of various fractionation areas ;
(*c*) temperature maintenance of process equipment ;
(*d*) storage of final products under test ;
(*e*) storage of final products awaiting despatch.

(3) A system of ventilation providing two grades of filtered air :
(*a*) a supply through a filter of pore size 5.0 µm to the entire work area, and
(*b*) a supply through a filter of pore size 0.5 µm at a positive pressure to areas where aseptic dispensing is to take place.

Other support facilities may include the recovery of solvents and a sewage disposal service. Sewage disposal must be carried out in accordance with the sanitary standards of the health authority.[1]

It should be noted that proteinaceous sewage from a plasma processing plant is highly nitrogenous and, having a high biological oxygen demand rating, should not be discharged untreated.

The equipment providing such services shall be located separately from the main process areas and in a place where conditions (light, physical access, etc.) are conducive to the establishment of effective and routine preventive maintenance programmes. The equipment shall incorporate devices capable of monitoring and recording the functioning of the equipment so that the safety of material in process and the safety of the process operators are ensured. In this way a proper record of function can be maintained and, where necessary, entered into the process record of the product batches.

The equipment should be adequate to ensure that the fractionation process as well as the proteins are protected in the event of an interruption in the support services.

In order to ensure this, there should be adequate spare equipment and emergency reserve systems serviced by engineering staff skilled in the maintenance and repair of such equipment.

C.4. PERSONNEL

The plasma fractionation plant shall be under the direction of a designated qualified person who shall be responsible for ensuring that all operations are carried out properly and competently. This director

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[1] WHO Official Records, No. 226, 1975, p. 88 (Annex 12 : *Good practices in the manufacture and quality control of drugs*).

shall have a good working knowledge of the scientific principles involved. The director shall be responsible for ensuring that employees are adequately trained in the work involved and have adequate practical experience and that they are aware of the application of accepted good practices to their respective functions. The director shall have the authority to enforce discipline among employees.

Personnel involved in quality control functions shall be separate from the staff involved in production and shall be responsible only to the director.

Where appropriate, personnel shall wear gowns, masks, boots, gloves, and eye protectors.

Personnel known to be carriers of specific pathogenic organisms (e.g., *Salmonella*, tuberculosis, viral hepatitis) shall be excluded from the production areas.

> Personnel should be medically examined at regular intervals. In view of the occupational hazard of infection with hepatitis virus, employees engaged in plasma fractionation should take special precautions against infection.

C.5. FRACTIONATION OF SOURCE MATERIALS

The general conditions for carrying out the fractionation procedure for preparing prophylactic or therapeutic plasma protein fractions from source material shall comply with good manufacturing practices [1] and shall be approved by the national control authority.

Most physical and chemical techniques of protein separation may be used for the preparation of plasma fractions, provided that the procedures lead to protein preparations that have been shown to be safe and effective.

Only fractionation procedures shall be used that give a good yield of products meeting the quality requirements of the international or national authorities. They shall be carried out in such a manner that the risk of microbiological contamination is reduced to a minimum.

> The safety of fractionation steps may be increased by using protected or closed systems. The reproducibility may be increased by the use of automation.

The fractionation procedures used shall not significantly denature the proteins.

[1] WHO Official Records, No. 226, 1975, p. 88.

The biological characteristics (such as antibody activity, biological half-life, and *in vivo* recovery of the proteins) should not be affected to such an extent that the product is unacceptable for clinical use.

When possible, methods shall be used that exclude or inactivate disease-causing agents, particularly hepatitis virus, from the final products intended for clinical use.

Certain plasma fractions, such as Factor VIII and Factor IX concentrates and fibrinogen, cannot yet be manufactured in a manner that ensures their freedom from hepatitis viruses.

In certain geographical regions there may be other known microbial contaminants. Attempts should be made to determine that the fractionation procedure will either remove or inactivate such contaminants.

Placenta-derived source materials require further initial treatment. These materials must initially be handled in rooms separate from those in which other fractionation processes are carried out and using separate equipment.

The operating manual shall specify the times of sampling and the volumes to be taken at each stage of the process as well as the tests to be made on the samples.

Where appropriate, ancillary materials used for fractionation shall be controlled for microbiological contamination, identity, purity, pyrogenicity, and toxicity according to the international or national pharmacopoeia.

Equipment, procedures, and ancillary materials that may introduce allergenic components into the final product shall be avoided.

It is advisable to use air filtration to exclude airborne allergenic dust.

C.6. STORAGE, HEAT TREATMENT, AND INCUBATION OF BLOOD PRODUCTS

At all stages of the manufacturing process, the source materials and resulting fractions shall be stored at temperatures and under conditions shown to be adequate to prevent further contamination and growth of microorganisms, to protect the identity and the integrity of the proteins, and to preserve the biological activities and safety of the products.

There shall be clear demarcation in the storage of similar materials.

There shall be full identification at all times, including batch number of all in-process fractions and unlabelled final containers.

C.6.1 Heat treatment of albumin

Albumin in solution shall be heated in the final container to 60°C ±0.5°C and maintained at that temperature for 10 hours.

C.7. IN-PROCESS CONTROL

It must be recognized that the source materials are subject to biological variability and the products resulting from protein separation are variously contaminated with the other protein components of plasma. It is essential, therefore, to establish a monitoring system such that the safe operating limits of each process be maintained.

The main information collected comprises variations in physical conditions (temperature, pH, ionic strength, timing, etc.) and variations in the number and species of microbiological contaminants.

Owing to the number and interdependence of the factors involved, there are no universally accepted specifications for such in-process quality assurance systems. For this reason, continued information collection should be combined with data from previous experience, using the same process to ensure production control appropriate to the quality requirements of the final product.

C.8. RECORD KEEPING

Records shall be kept of the performance of all steps in the manufacture, quality control, and distribution of blood products and related substances.[1]

These records shall :

— be original (not a transcription), indelible, legible, and dated ;

— be made concurrently with the performance of each operation and test ;

— identify the person recording the data as well as the person checking the data or authorizing continuation of processing ;

[1] WHO Official Records, No. 226, 1975, p. 88.

— be detailed enough to allow a clear reconstruction and understanding of all relevant procedures performed ;
— allow tracing of all successive steps and identify the interrelationships of dependent procedures, products, and waste materials ;
— be maintained in an orderly fashion permitting the retrieval of data for a period consistent with dating periods and legal requirements ;
— indicate that processing and testing were carried out in accordance with procedures established and approved by the designated responsible authority ;
— if necessary, allow a prompt and complete recall of any particular lot ; and
— show the lot numbers of materials used for specified lots of products.

PART D:
REQUIREMENTS FOR THE CONTROL OF PLASMA FRACTIONS

D.1. INTRODUCTION

There are a number of requirements common to albumin, plasma protein fraction, immunoglobulins, and coagulation factor concentrates. It is therefore convenient to produce a single set of requirements, making specific recommendations for each product under the relevant sections.

D.2. TERMINOLOGY

Bulk purified material : powder or liquid material prepared by the fractionation of pooled source material.

Final bulk : a sterile solution or powder of material prepared from bulk purified material. It is used to fill the final containers. The final bulk must be provided with the respective batch number.

In some countries the final bulk is filled through a sterilizing filter.

Filling lot (*final lot*)*:* a collection of sealed final containers that are homogeneous with respect to the risk of contamination during filling

and (where appropriate) drying or other further processing. A filling lot must, therefore, have been filled and (where appropriate) dried in one working session. If the total final bulk is not filled in one session, it must bear a sub-batch number.

D.3. CONTROL OF BULK PURIFIED MATERIAL

D.3.1 Storage

The bulk material, either as liquid or as powder, shall be stored in sealed containers under conditions that minimize the multiplication of microbial agents as well as denaturation.

Powder free from the precipitating agent and containing less than 50 mg moisture per g of powder can be stored at up to 5°C.

D.3.2 Tests on bulk purified material

Tests on the purified bulk powder or solution shall be made if the manufacturer sends the material to another institution for further processing. Samples for these tests shall be taken under conditions that do not impair the quality of the bulk purified material. Tests shall be carried out on a specially dissolved sample processed to a stage equivalent to the final product, after sterile filtration. The tests shall be those listed in sections D.4.3 to D.4.9 inclusive, except that the tests for sodium and potassium (D.4.8 and D.4.9) are inappropriate for immunoglobulins. The sterility test on the dissolved sample of the bulk powder (section D.4.5) may be excluded.

For the control of immunoglobulins the specially dissolved sample shall be made at a concentration of 100–180 g/l.

D.4. CONTROL OF FINAL BULK SOLUTION

D.4.1 Preparation

The final bulk solution shall be prepared from bulk purified powder or by the dilution of concentrates by a method approved by the national control authority. The final bulk solution shall meet each of the requirements herein described (sections D.4.2 to D.4.10 inclusive) except that the tests for sodium and potassium (D.4.8 and D.4.9) are unnecessary

for immunoglobulins whereas the tests for aggregation and potency (D.4.11, D.4.12, and D.4.13) are applied only to the immunoglobulins.

The final bulk solution of normal immunoglobulins shall be made using material from a large number of donors (approximately 1000).

> In the case of normal immunoglobulin a large number of donors is necessary in order to obtain adequate amounts of the various desired antibodies in the final product.
>
> In the case of specific immunoglobulin the number of donors represented is less important because the particular antibody present will be controlled.

D.4.2 Preservatives and stabilizers

No preservative shall be added to the albumin, plasma protein fraction, or coagulation factor concentrates either during fractionation or at the stage of the final bulk solution.

To prevent protein denaturation, stabilizers shall be added. Such substances shall have been shown to the satisfaction of the national control authority to have no deleterious effect on the final product in the amounts present and to cause no untoward reactions in man.

> Protein denaturation may be prevented by using, for example, either 0.16 mmol of sodium acetyltryptophanate or 0.08 mmol of sodium caprylate and 0.08 mmol of sodium acetyltryptophanate per g of albumin or plasma protein fraction.

Any stabilizers or preservative added to immunoglobulins either during production or in the final bulk shall have been shown to the satisfaction of the national control authority to have no deleterious effect on the final products in the amount present and to cause no untoward reactions in man. Antibiotics shall not be used as preservatives or for any other purpose in the fractionation of plasma.

> Stable solutions of immunoglobulins may be prepared in approximately 0.3 mol/l glycine or 0.15 mol/l sodium chloride. Thiomersal or sodium timerfonate may be used as preservatives.

D.4.3 Concentration and purity

The albumin concentration in the final bulk albumin solutions shall be between 35 and 265 g/l. Not less than 96% of the proteins present shall be albumin as determined by a suitable electrophoretic method. The test shall be carried out on a sample both before and after heating (see section C.6.1).

The protein concentration in the final bulk plasma protein fraction solution shall be at least 35 g/l.

Plasma protein fraction contains at least 83% albumin and not more than 17% globulins. Not more than 1% of the protein in plasma protein fraction shall be gamma globulin.

The immunoglobulin concentration in the final bulk of normal and specific immunoglobulin preparations shall be 100–180 g/l. If in a specific immunoglobulin preparation the concentration is lower than 100 g/l, it shall require the approval of the national control authority.

The immunoglobulin shall be composed of not less than 90% of immunoglobulin G, as determined by a method approved by the national control authority.

The methods in most common use are radial immunodiffusion and electrophoresis.

D.4.4 Hydrogen ion concentration

The final bulk solution, diluted to 1% protein concentration with 0.15 mol/l sodium chloride, shall, when measured at a temperature of 20°C ±2°C, have a pH of 6.9 ±0.5 for albumin and immunoglobulin and 7.0 ±0.3 for plasma protein fraction.

D.4.5 Sterility

The final bulk material shall be tested for sterility. Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) [1] shall apply.

D.4.6 Freedom from undue toxicity and pyrogens

The final bulk material shall be tested for freedom from undue toxicity and absence of pyrogens by the methods described in sections D.6.4 and D.6.5.

A manufacturer introducing a change in an established fractionation procedure is advised to test the albumin products for undesirable hypotensive and hypertensive substances using the most suitable methods available.

[1] WHO Technical Report Series, No. 530, 1973, p. 48.

D.4.7 Stability

Sterile samples of the final bulk albumin and plasma protein fraction solutions, which have been heated for 10 hours at 60°C, shall remain visually unchanged after heating further at 57°C for 50 hours and when compared to a control sample that has been heated for only 10 hours at 60°C.

For the immunoglobulin solutions the test shall be made by heating an adequate sample at 57°C for 4 hours. No gelation or flocculation shall appear.

D.4.8 Sodium content

The final bulk albumin and plasma protein fraction solutions shall have a maximum sodium concentration of 160 mmol/l.

D.4.9 Potassium content

The final bulk albumin and plasma protein fraction solutions shall have a maximum potassium concentration of 2 mmol/l.

D.4.10 " Heme like " content

A sample taken from the final bulk and heated for 10 hours at 60°C shall, when diluted with water to 10 g protein per litre and placed in a cell with 1 cm light path, have an absorbance not exceeding 0.25 when measured in a spectrophotometer set at 403 nm.

In some countries the absorbance limit for this test may not be 0.25. In such countries the national control authority should specify the limit.

D.4.11 Determination of aggregated and fragmented molecules in immunoglobulins

Tests shall be made on the immunoglobulin solutions to determine the proportion of aggregated and fragmented immunoglobulin. The test and limits shall be approved by the national control authority.

Ultracentrifugation or gel filtration chromatography may be used, and other methods are being developed.

D.4.12 Potency test for normal immunoglobulin

Normal immunoglobulin shall be prepared from pooled material by a method that has been shown to be capable of concentrating tenfold from source material at least two different antibodies, one viral and one bacterial, for which an international standard or reference preparation is available [1] (e.g., antibodies against poliomyelitis virus, measles virus, streptolysin O, diphtheria toxin, tetanus toxin, staphylococcal α toxin). The immunoglobulin solution shall be tested at the protein concentration at which it will be present in the final ampoule.

Since preparations of normal immunoglobulin produced in different countries can be expected to differ in their content of various antibodies, depending on the antigenic stimulation to which the general population has been subjected (either by natural infection or by artificial immunization), at least two antibodies should be chosen for the potency test by the national control authority. The final bulk passes the test if it contains at least the minimum antibody levels required by the national control authority.

D.4.13 Potency tests of specific immunoglobulin

The potency of the final bulk shall be tested in respect of the particular antibody that the preparation has been specified to contain.

The final bulk of antitetanus human immunoglobulin passes the test if it contains at least 50 IU of tetanus antitoxin per millilitre, as determined by a neutralization protection test in animals.

The final bulk of antimeasles human immunoglobulin passes the test if it contains at least 50 IU of measles antibody per millilitre, as determined by a tissue culture neutralization test or a haemagglutination inhibition test.

The final bulk of antivaccinia human immunoglobulin passes the test if it contains at least 500 IU of vaccinia antibody per millilitre, in terms of the International Standard for Anti-Smallpox Serum, as determined by a neutralization test in eggs or in tissue culture.

The final bulk of antirabies human immunoglobulin passes the test if it contains at least 50 IU of rabies antibody per millilitre, in terms of the International Standard for Anti-Rabies Serum, as determined by a neutralization protection test in animals.

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[1] The list of appropriate international standards and reference preparations is included in Appendix 4.

The estimated potency of the final bulk of anti-Rh_0 immunoglobulin shall be expressed in international units and shall be not less than 90% and not more than 120% of the stated potency, and the fiducial limits of error shall be within 80% and 125% of the stated potency.

The national control authority should specify the limits for other antibody concentrations.

D.4.14 Globulins for intravenous administration

Preparations of immunoglobulins intended for intravenous administration are now being made in several countries. Such preparations shall comply with all the specifications for normal and specific immunoglobulins unless otherwise agreed by the national authority. Certain additional tests will be required, and these should be specified by the national control authority.

D.5. FILLING AND CONTAINERS

The requirements concerning 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) [1] shall apply.

Special attention shall be paid to the requirement that albumin and plasma protein fraction solutions in the closed final containers shall be heated within 24 hours after starting the filling to a temperature of 60°C ±0.5°C and shall be maintained at that temperature for 10 hours, in order to inactivate any hepatitis B virus that may be present. In order to prevent protein denaturation a stabilizer shall be added to the albumin solution prior to heating (see section D.4.2).

D.6. CONTROL TESTS ON FINAL PRODUCT

D.6.1 Identity test

An identity test shall be performed on at least one labelled container from each filling lot to verify that the preparation contains protein of human origin only. The test shall be one approved by the national control authority.

[1] WHO Technical Report Series, No. 323, 1966, p. 16.

For immunoglobulins, as well as albumins and plasma protein fractions, additional tests shall be made to determine that the protein is predominantly immunoglobulin G or albumin respectively. The tests in section D.4.3 shall be used.

In the case of specific immunoglobulin an additional test shall be made to identify the specific antibody.

D.6.2 Protein concentration and purity

The protein concentration and purity of each filling lot shall be determined as described in section D.4.3.

D.6.3 Sterility

Each filling lot shall be tested for sterility. Part A, section 5, of revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) [1] shall apply. For albumin and plasma protein fraction, samples for sterility testing shall be taken from final containers drawn at random prior to the heating procedure at 60°C for 10 hours.

> In some countries the sterility test is applied both before and after heating at 60°C for 10 hours.

D.6.4 Freedom from undue toxicity

Each filling lot shall be tested for freedom from undue toxicity by appropriate tests involving injection into mice and guineapigs. The tests shall be those approved by the national control authority.

> The tests generally used are the parenteral injection of 0.5 ml into each of at least two mice weighing approximately 20 g and the intraperitoneal injection of 5.0 ml into each of at least two guineapigs weighing approximately 350 g. In some countries, if one of the animals dies or shows signs of ill health during the time specified, the test is repeated. The substance passes the test if none of the animals of the second group dies or shows signs of ill health in the time interval specified. For coagulation factor concentrates except fibrinogen, the test dose should not exceed 500 IU of the coagulation factor per kilogram of body weight of the test animal.

[1] WHO Technical Report Series, No. 530, 1973, p. 48.

The injection shall cause neither significant untoward reactions nor death within an observation period of 7 days.

D.6.5 Freedom from pyrogenicity

Each filling lot shall be tested for pyrogenicity by the intravenous injection of the test dose into three or more rabbits that have not previously received blood products. In general the dose shall be at least equivalent proportionately, on a rabbit body-weight basis, to the maximum single human dose recommended, but not more than 10 ml/kg body weight. For albumin 200 g/l and 250 g/l, the test dose for each rabbit shall be at least 3 ml/kg of body weight, for albumin 50 g/l and for plasma protein fraction the test dose shall be 10 ml/kg of body weight, and for immunoglobulins the dose shall be 1.0 ml/kg of body weight.

The criteria for passing the test shall be those specified by the national control authority.

Guidance for these criteria may be found in the International Pharmacopoeia.[1]

D.6.6 Determination of moisture

The residual moisture shall where appropriate be determined by a method approved by the licensing authority.

The methods in most common use are drying over phosphorus pentoxide for 24 hours at a pressure not exceeding 2.7 Pa (0.02 mmHg) and the Karl Fischer method. The acceptable level of moisture shall be determined by the national control authority.

D.6.7 Inspection of filled containers

All final containers of albumin and plasma protein fraction shall be stored at 20–35°C for at least 14 days following heat treatment at 60°C for 10 hours. At the end of this incubation period each final container shall be examined. Those showing abnormalities such as abnormal colour, turbidity, microbial contamination, or presence of atypical particles shall not be distributed.

When turbidity raises the possibility of microbial contamination, testing should be done to isolate and identify the micro-organisms.

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[1] *Specifications for the quality control of pharmaceutical preparations : second edition of the International Pharmacopoeia*. Geneva, World Health Organization, 1967, p. 746, Appendix 43.

D.7. 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) [1] shall apply.

D.8. 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] shall apply.

D.9. LABELLING

The requirements for labelling given 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 exception that the recommended human dose need not be specified on the label on the container, and with the addition of the following information.

For albumin and plasma protein fraction the label on the container shall show :

— the type of source material ;
— the protein concentration ;
— the oncotic equivalent in terms of plasma ;
— the absence of preservative ;
— the warning " Do not use if turbid " ;
— a warning to use within 4 hours of broaching ;
— the sodium and potassium concentrations.

For immunoglobulins the label on the container shall show :

— the type of source material ;
— the protein concentration ;
— (in the case of specific immunoglobulin) the content of specific antibody expressed in international units or equivalent national units ;

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[1] WHO Technical Report Series, No. 323, 1966, p. 17.
[2] WHO Technical Report Series, No. 323, 1966, p. 18.

— the statement " For intramuscular use only " (if the immunoglobulins are not specially prepared for intravenous use).

The label on the package, or the leaflet in the package, shall in addition show :

— the recommended dose for each particular disease or condition ;
— the fact that the preparation fulfils the requirements of this document.

D.10. DISTRIBUTION AND TRANSPORT

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) [1] shall apply.

D.11. 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] shall apply.

D.11.1 Storage conditions and expiry date

(1) Final containers with albumin solution shall have a dating period of 3 years provided they are stored at a temperature not higher than 30°C.

(2) Final containers with albumin solution shall have a dating period of 5 years provided they are stored at 5°C ±3°C.

Other storage conditions and expiry dates may be approved by the national authority.

(3) Final containers with plasma protein fraction solution shall have a dating period of 3 years provided they are stored at a temperature not higher than 30°C.

(4) Final containers with plasma protein fraction solution shall have a dating period of 5 years provided they are stored at 5°C±3°C.

Other storage conditions and expiry dates may be approved by the national authority.

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[1] WHO Technical Report Series, No. 323, 1966, p. 18.
[2] WHO Technical Report Series, No. 323, 1966, p. 19.

(5) Storage of liquid immunoglobulin shall be at 5°C±3°C. Storage of freeze-dried preparations shall be below 25°C.

The expiry date of the liquid preparations shall be not more than 3 years from the date of the first satisfactory potency test on material in final containers.

The expiry date of the freeze-dried preparations shall be not more than 5 years from the date of the first satisfactory potency test on material in final containers.

D.12. CONTROL OF PREPARATIONS OF COAGULATION FACTOR CONCENTRATES

Factor VIII concentrates are prepared both as a frozen product and as a freeze-dried concentrate. The frozen products are usually derived from a single donation and consist of the cryoprecipitate from one donor prepared in a closed system of separation. The control of this product is covered in the section on whole blood and related substances (section B.3.5.4 for Factor VIII).

The freeze-dried Factor VIII concentrate may vary both in the number of donors contributing to a pool of cryoprecipitate and in the degree of purification of the product. Generally the small-pool product is subjected to little or no purification and is handled and subdivided in such a way that many control tests are inappropriate. Such preparations of frozen or freeze-dried cryoprecipitate from pools of fewer than 10 plasma donations should be controlled by the national control authority in a manner similar to that applied to the frozen product from single donations (section B.3.5.4).

Plasma source material for Factor VIII concentrates shall preferably be plasma frozen within 6 hours of collection from the donor or from frozen cryoprecipitate. Such material shall be kept frozen at such a temperature that the activity of the Factor VIII is maintained. Processing of the freshly thawed pooled cryoprecipitate material shall be completed within a few hours in order to preserve Factor VIII activity.

D.12.1 Tests on final containers common to other blood fractions

The following tests on the final containers of Factor VIII and Factor IX concentrates and fibrinogen shall apply.

(1) Sterility (see section D.6.3).
(2) Freedom from undue toxicity (see section D.6.4).

(3) Test for pyrogenicity (see section D.6.5). For Factor VIII each rabbit shall be injected with 10 IU per kg of body weight. For Factor IX each rabbit shall be injected with 50 IU per kg of body weight. For fibrinogen each rabbit shall be injected with 30 mg of protein per kg of body weight.

In addition the following requirements shall apply.

(1) Records : as given in section D.7.

(2) Samples : as given in section D.8.

(3) Labelling : the requirements given in section D.9 shall apply, with the exception that the recommended human dose need not be specified and with the addition of the following :

(*a*) content of the factor expressed in international units (IU) ;
(*b*) the amount of protein in the container ;
(*c*) the volume of diluent for reconstitution ;
(*d*) a warning concerning the possible transmission of hepatitis virus.

(4) Distribution and shipping : as given in section D.10.

(5) Storage and expiry date : final containers of the freeze-dried preparation of Factor VIII and Factor IX will have a dating period of not more than 2 years from the date of the first satisfactory potency test, provided they are stored at 5°C±3°C. Storage at ambient temperature during transport and on short trips will not result in loss of potency.

For fibrinogen the shelf-life is usually 5 years at 5°C ± 3°C. Other storage conditions and expiry dates may be approved by the national control authority provided they are consistent with the data on the stability of the products.

D.12.2 Special tests for coagulation concentrates

D.12.2.1 *Identity tests*

Tests for other animal protein. An identity test shall be performed on at least one labelled container from each filling lot to verify that the preparation contains protein of human origin only. The test shall be one approved by the national control authority.

Identity test for activity. An assay for Factor VIII or Factor IX activity or fibrinogen content, whichever is appropriate, shall be made to identify the product.

One of the potency tests described in sections D.12.3.1, D.12.4.1, and D.12.5.1 may serve as the identity test.

D.12.2.2 *Detection of hepatitis B virus*

Each filling lot shall be tested for hepatitis B surface antigen by the tests indicated in section B.1.3.2.

> The most sensitive test available should be used for the detection of hepatitis B virus.

D.12.2.3 *Solubility and clarity*

Factor VIII preparations and fibrinogen shall dissolve completely in the solvent recommended by the manufacturer within 30 minutes when held at a temperature not exceeding 37°C. Factor IX preparation shall dissolve completely in the solvent recommended by the manufacturer within 10 minutes when held at 20–25°C. The solutions kept at room temperature shall not show any sign of precipitation or gel formation within 3 hours of dissolution of the coagulation factors.

D.12.2.4 *Protein content*

The amount of protein in a final container shall be determined by a method approved by the national control authority.

D.12.2.5 *Test for additives*

Tests to determine the concentration of additives (such as aluminium, heparin, poly(ethylene glycol), citrate, sodium, and glycine) used during production shall be carried out, if the national control authority requires them.

D.12.2.6 *Determination of moisture*

The residual moisture shall be determined by a method approved by the national control authority.

> The methods in most common use are drying over phosphorus pentoxide for 24 hours at a pressure not exceeding 2.7 Pa (0.02 mmHg), and the Karl Fischer method. The products should not lose more than 2% in weight.

D.12.2.7 *Hydrogen ion concentration*

When the product is dissolved in a volume of water equal to the volume of water for injection stated on the label, the pH in the resulting solution shall be 7.2 ± 0.4.

D.12.3 Special tests applicable to Factor VIII concentrates

D.12.3.1 *Potency test*

Each filling lot shall be assayed for Factor VIII activity by a test approved by the national control authority.

> The national standard and the manufacturer's house standard should be a concentrate rather than a plasma because the former has better long-term stability and provides more homogeneous assay results, especially when the partial thromboplastin time test is used.

The specific activity shall be at least 100 IU per g of protein. The estimated potency is not less than 80% and not more than 125% of the stated potency. The confidence limits of error are not less than 64% and not more than 156% of the stated potency.

D.12.3.2 *Test for alloagglutinins*

A test shall be made for the presence of alloagglutinins by a method approved by the national control authority.

> Although it is not possible to be specific about the tests for alloagglutinins or to specify an upper limit of titre in the coagulation factor preparations, nevertheless a test for alloagglutinins should be made and their presence declared on the leaflet.

D.12.4 Special tests applicable to Factor IX concentrates

D.12.4.1 *Potency test*

Each lot of the final product shall be tested for potency for Factor IX. The method used shall be approved by the national control authority.

Other coagulation factors may also be present in the final product, depending on the method of production, and products shall be tested for the presence of all coagulation factors claimed to be present at a therapeutic level. These may be Factor II, Factor VII, and Factor X. The methods used for their assay shall be approved by the national control authority.

D.12.4.2 *Test for the presence of activated coagulation factors*

The presence of activated coagulation factors shall be determined by a method approved by the national control authority.

In some countries the nonactivated partial thromboplastin times of normal plasma are measured after the addition of an equal volume of a number of different dilutions of the product under test.

In some countries a test for the presence of thrombin is made by mixing an equal volume of the product under test and fibrinogen solution. The mixture is held at 37°C and should not coagulate within 6 hours. The usual range of concentration of fibrinogen solution is 3–10 g/l.

D.12.5 Special tests applicable to fibrinogen

D.12.5.1 *Test for clottable protein*

Each filling lot shall be assayed for clottable protein by a test approved by the national control authority.

Usually the test consists of the addition of thrombin—with or without calcium chloride—to an appropriately diluted solution of fibrinogen and the determination of the clotting time.

The clotting time should be shorter than a given limit—usually 60 seconds—or should occur in not more than twice the time taken for clotting to occur in fresh normal plasma for the addition of the same amount of thrombin.

Not less than 70% of the total protein shall be clottable by thrombin.

PART E:
NATIONAL CONTROL REQUIREMENTS

E.1. GENERAL

The general requirements for control laboratories in Part B of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) [1] shall apply.

The national control authority shall provide the standards and reference preparations necessary for the quality control of human blood and blood products.

The national control authority shall approve the production and control methods used and give indications on all points left for its decision or approval in Parts A, B, C and D.

[1] WHO Technical Report Series, No. 323, 1966, p. 19.

The national control authority shall especially approve the use of materials that may carry any potential risk and shall approve any new method of production or the preparation of a new product.

New products or products prepared by new production methods should be monitored to determine their efficacy and safety before they are released.

E.2. RELEASE AND CERTIFICATION

Human blood and blood products shall be released only if they fulfil Parts A, B, C, and D, wherever applicable.

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 product in question meets all national requirements as well as Parts A, B, C, and D—whichever is relevant—of the present requirements. The certificate shall further state the date of the last satisfactory test for potency, if applicable, the number under which the lot is released, and the number appearing on the labels of the containers. In addition, a copy of the official national release documents shall be attached.

The purpose of this certificate is to facilitate the exchange of human blood and blood products between countries.

Appendix 1

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Appendix 2

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Appendix 3

SUMMARY PROTOCOL OF COLLECTION OF SOURCE MATERIAL

1. Name and address of collecting centre .....

2. Source material .....

3. Details of single donations, where applicable

   (*a*) Donor identification .....

   (*b*) Date of collection .....

   (*c*) Volume in container .....

   (*d*) Result of tests for HBsAg .....

   (*e*) Nature of test used .....

4. Special information

   (*a*) Anticoagulant used .....

   (*b*) Whether collected for special purposes (e.g., antibody specific) .....

   (*c*) Precautions in use of material .....

5. Conditions of storage .....

6. Does the donation comply with existing agreements between the supplier and manufacturer ? .....

7. Does the donation comply with WHO Requirements ? .....

Name and signature of responsible person ..... Date .....

Appendix 4

INTERNATIONAL STANDARDS AND INTERNATIONAL REFERENCE PREPARATIONS USED IN THE CONTROL OF BLOOD PRODUCTS[a]

Substance	Standard or reference preparation (IU per ampoule)	Year of establishment	How distributed	Distributing laboratory
Anti-A blood-typing serum	Standard (256)	1950	Dried material derived from human serum	Copenhagen[b]
Anti-B blood-typing serum	Standard (256)	1950	Dried material derived from human serum	Copenhagen[b]
Anti-Rh_0 (anti-D) incomplete blood-typing serum	Standard (32)	1966	Dried material derived from human serum	Copenhagen[b]
Anti-C (anti-Rh) incomplete blood-typing serum	Standard (64)	1976	Dried material derived from human serum	London[c]
Anti-D (anti-Rh_0) immunoglobulin	Reference preparation (150)	1976	Dried human immunoglobulin	London[c]
Anti-hepatitis B immunoglobulin	Reference preparation (50)	1977	Dried human immunoglobulin	Amsterdam[e]
Blood coagulation Factor VIII	2nd Standard (1.10)	1976	Dried Factor VIII from human plasma	London[c]
Blood coagulation Factor IX	Standard (5.62)	1976	Dried Factor IX from human plasma	London[c]
Haemiglobincyanide	Reference preparation (—)	1967	Haemiglobincyanide solution	Bilthoven[d]
Thrombin	Standard (100)	1975	Dried human thrombin with sucrose	London[c]
Heparin	3rd Standard (1370)	1973	Dried heparin from porcine intestinal mucosa	London[c]
Diphtheria antitoxin	Standard (10)	1922	Dried hyperimmune horse serum	Copenhagen[b]
Tetanus antitoxin	2nd Standard (1400)	1969	Dried hyperimmune horse serum	Copenhagen[b]
Antistreptolysin O	Standard (10 IU/ml)	1959	Dried human serum	Copenhagen[b]
Antipoliomyelitis sera, types 1, 2, 3	Standards (10)	1962	Dried hyperimmune monkey sera	Copenhagen[b]
Antirabies serum	Standard (86.6)	1955	Dried hyperimmune horse serum	Copenhagen[b]
Antimeasles serum	Reference preparation (10)	1964	Dried human serum	Copenhagen[b]
Antismallpox serum	Standard (1000)	1965	Dried pooled post-variole serum	Copenhagen[b]
Antirubella serum	2nd Reference preparation (1000)	1970	Dried human normal immunoglobulin	Copenhagen[b]

See Notes p. 93

Appendix 5

COMPOSITION OF COMMONLY USED SOLUTIONS OF ACD AND CPD

Acid citrate dextrose solution (ACD)

Trisodium citrate (dihydrate)	22.0 g
Citric acid (monohydrate)	8.0 g
Dextrose (monohydrate)	24.5 g
Water for injection to	1000 ml

15 ml of this solution are used for 100 ml of blood.

Citrate phosphate dextrose (CPD)

Trisodium citrate (dihydrate)	26.3 g
Citric acid (monohydrate)	3.27 g
Sodium dihydrogen phosphate (monohydrate)	2.22 g
Dextrose (monohydrate)	25.5 g
Water for injection to	1000 ml

14 ml of this solution are used for 100 ml of blood.

For further details see : MOLLISON, P. L., *Blood transfusion in clinical medicine*, 4th ed., Oxford, Blackwell, 1967.

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[a] See : *Biological substances : international standards, reference preparations, and reference reagents*. Geneva, World Health Organization, 1977.

[b] International Laboratory for Biological Standards, State Serum Institute, 80 Amager Boulevard, Copenhagen, Denmark.

[c] International Laboratory for Biological Standards, National Institute for Biological Standards and Control, Hampstead, London NW3 6RB, England.

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Annex 2

REQUIREMENTS FOR MENINGOCOCCAL POLYSACCHARIDE VACCINE

(Requirements for Biological Substances No. 23)
Addendum 1977

The WHO Expert Committee on Biological Standardization at its twenty-seventh meeting adopted Requirements for Meningococcal Polysaccharide Vaccine, which were published in its report.[1] At that time it was recognized that the technology involved in the quality control of such vaccines was changing rapidly but the Requirements were written because meningococcal meningitis is a serious problem in many countries and national health authorities, particularly in the developing countries, had requested guidelines for the quality control of such vaccines.

During the year following the formulation of the Requirements, progress had been made in the preparation of polysaccharides of higher molecular weight (greater potency) for both Group A and Group C. Furthermore a greater purification by the removal of endotoxin was achieved so that it became possible to inoculate 10 times the quantity of the polysaccharides into rabbits without a significant rise in temperature. Accordingly an addendum to the Requirements to take these developments into consideration was published in the twenty-eighth report of the Expert Committee on Biological Standardization. [2]

Even with these improvements the thermolability of Group A polysaccharide caused problems, and storage at −20°C was essential. The difficulties were critical when the reconstituted vaccines were used in countries with high ambient temperatures. A major step forward has been taken recently by the discovery that the inclusion of lactose stabilizes the Group A polysaccharide against thermal depolymerization. As it is known that efficacy in man is related to the relative molecular mass of the polysaccharide at the time of injection, further amendments to the Requirements are needed to ensure that only stabilized vaccines are made available in future.

Another important finding is that the moisture content of the freeze-dried product also plays a role in the stability of the product. Products

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[1] WHO Technical Report Series, No. 594, 1976, pp. 50–75.
[2] WHO Technical Report Series, No. 610, 1977, pp. 52–53.

with a moisture content of 4% are less stable than those with a content of 2%. An amendment relating to moisture content is included.

Much research is in progress on the production of polysaccharides from Groups Y, 29E, and WI35, which represent infections that occur with a lower frequency than those from Groups A and C. Future Requirements may have to take account of the polysaccharides from these additional Groups.

In order to avoid reference to three documents, the amendments published in the twenty-eighth Report of the Expert Committee on Biological Standardization are included in these amendments, all of which relate to Part A of the Requirements.

Amendment 1

Replace section 3.4.6 by the following:

3.4.6 *Molecular size*

The molecular size of each lot of purified polysaccharide shall be estimated by gel filtration using Sepharose 4B. Chromatography shall be carried out in a solvent having an ionic strength of 0.2 mol/kg. The molecular size shall be determined by measuring the recovery of the polysaccharide eluted before a K_D of 0.50 is reached. At least 65% of the Group A polysaccharide and at least 75% of the Group C polysaccharide shall be recovered from the column before a K_D value of 0.50 is reached.

Delete footnote 2.

Amendment 2

Replace section 3.5 by the following:

3.5 Preparation of final bulk

The final bulk shall be prepared either from a single lot of purified polysaccharide or from several pooled lots. The polysaccharide shall be dissolved under aseptic conditions in a sterile solution suitable for freeze-drying and free of pyrogenic substances. A stabilizer shall be added, the substance used and its concentration being subject to approval by the national control authority.

The mixture shall be sterilized by membrane filtration.

Membranes with a pore size of 0.22 µm have been found satisfactory.

A suitable stabilizer is lactose at a concentration of 2.5-5.0 mg per human dose of polysaccharide. It is important to calculate the concentration of lactose on the basis of the anhydrous lactose molecule and not on the basis of the pentahydrate, which is the form most commonly available.

Sections 3.5.1 and 3.5.2 remain unaltered.

Amendment 3

In section 4, dealing with filling and drying, replace the last sentence by the following :

The stabilized dried vaccine shall be stored at a temperature of 2–8°C.

Amendment 4

To section 5.3, dealing with concentration of polysaccharide, add the following sentence :

As the presence of lactose interferes with this test, the reconstituted stabilized polysaccharide shall be dialysed against 50 volumes of water at a temperature of 2–8°C for 12–18 hours before carrying out these determinations.

Amendment 5

In section 5.5.1, delete the first paragraph and replace by the following :

Each filling lot shall be tested for pyrogenicity by intravenous injection into rabbits. Three or more healthy rabbits that have not previously received injections shall be used. The vaccine, reconstituted in the form in which it is to be used, shall be diluted further in pyrogen-free physiological saline so that each rabbit shall receive, by injection into the ear vein, the following doses of dry weight polysaccharide per kilogram of rabbit weight :

Group A vaccine, 0.025 µg
Group C vaccine, 0.025 µg
combined Groups A and C vaccine, 0.050 µg.

Amendment 6

Replace section 5.6 by the following :

5.6 Estimation of molecular size

The molecular size of the polysaccharide in at least one final container from each filling lot shall be determined by Sepharose 4B gel filtration as outlined in Part A, section 3.4.6. At least 65% of the Group A polysaccharide and at least 75% of the Group C polysaccharide shall be recovered in the column effluent before a K_D value of 0.50 is reached.

Delete footnote 1.

Amendment 7

Replace sections 5.7 and 5.8 by the following:

5.7 Test for residual moisture

The moisture content of the dried material shall be determined as indicated in Part A, section 3.4. The method used for the determination of the moisture content shall be approved by the national control authority.

The test shall be performed on one vial per thousand vials, taking them at random throughout the freeze-drying lot. The average residual moisture shall be not greater than 2.5% and no vial shall have a residual moisture content of 3% or greater.

5.8 Storage

The stabilized freeze-dried vaccines shall be stored at a temperature of 2–8°C.

Amendment 8

Section 8, on labelling, should reflect the fact that for stabilized vaccines it is no longer necessary to store Group A polysaccharide at −20°C or lower. Delete the last paragraph and replace by the following:

Furthermore, the label on the container, or the label on the carton enclosing several containers, or the leaflet accompanying the container shall contain the following additional information:

— a statement that the stabilized vaccines shall be stored at 2–8°C;
— a statement that the reconstituted stabilized vaccine shall be stored at 2–8°C and shall be discarded if not used during the day on which it is reconstituted.

Amendment 9

Replace the last sentence of section 10.1 by the following:

The Group A and Group C vaccine shall be stored at a temperature between 2°C and 8°C.

Amendment 10

Replace section 10.2 by the following:

10.2 Expiry date

The expiry date for dried bulk polysaccharide when stored at −20°C or below shall be not more than 5 years from the date of harvest. The expiry date of the stabilized vaccine in the final containers when stored at 2°C to 8°C shall be not more than 2 years from the date of issue.

A further test for molecular size may be made and if it is found to be satisfactory the national control authority may wish to allow a further storage period of one year.

Amendment 11

In the Appendix to the Requirements, section 3, subsection (4), delete the last sentence of paragraph (*d*), together with footnote 1.

Annex 3

REQUIREMENTS FOR INACTIVATED INFLUENZA VACCINE

(Requirements for Biological Substances No. 17) Addendum 1977

Developments in technology that have taken place since the formulation of the Requirements for Inactivated Influenza Vaccine (1967) [1] and the Addendum (1973) [2] have shown that the International Reference Preparation of Influenza Vaccine Haemagglutinin (Type A) is no longer appropriate for controlling the haemagglutinin content of inactivated influenza vaccines. The International Reference Preparation established in 1967 no longer represents the haemagglutinin of the strains of influenza virus causing infection. The WHO Expert Committee on Biological Standardization, at its twenty-ninth meeting, accordingly discontinued the International Reference Preparation of Influenza Virus Haemagglutinin (Type A).

The Expert Committee accepted the generous offer of the National Institute for Biological Standards and Control, London, to provide on an annual basis the reference materials (haemagglutinin and specific antisera) necessary for the control of influenza vaccines.

It was recognized that, as such preparations cannot await formal establishment by the Expert Committee as international reference materials, it will be more appropriate to refer to each of them as the WHO Influenza Virus Reference Haemagglutinin and to identify it by the year in which it is made.

The following amendments are made to the Requirements for Inactivated Influenza Vaccine; all of them relate to Part A.

Amendment 1

Replace section 1.3 by the following:

1.3 *Reference materials for haemagglutinin*

WHO Influenza Virus Reference Haemagglutinin (identified by the year of preparation) is prepared by the National Institute for Biological Standards and Control, London, and distributed annually.

[1] WHO Technical Report Series, No. 384, 1968, pp. 43–56.

[2] WHO Technical Report Series, No. 530, 1973, pp. 15–17.

It is a haemagglutinin representative of that present in the current infectious strains of virus.

The preparation is distributed with a known quantity, measured in milligrams of haemagglutinin per millilitre of the vaccine. The preparation is used to specify the haemagglutinin activity of national reference preparations for use in the manufacture and laboratory control of inactivated influenza vaccines by an *in vitro* immunodiffusion test.

A specific antihaemagglutinin serum is also distributed by the National Institute for Biological Standards and Control for use in such tests.

Amendment 2

In Part A, section 3.4.2, delete the words " by haemagglutination " from the first sentence. Replace the second sentence by the following :

The test shall be made in comparison with the WHO Influenza Virus Reference Haemagglutinin distributed annually by the National Institute for Biological Standards and Control, London, on behalf of the World Health Organization or by comparison with a national preparation calibrated against the WHO Influenza Virus Reference Haemagglutinin preparation.

Delete paragraph in small type.

Amendment 3

In Part A, section 3.5.3, delete the words " by haemagglutination ".

Amendment 4

In Part A, section 8, delete the words " the number of international units or comparable units of haemagglutinin per dose for each strain " and replace them by " the quantity of haemagglutinin expressed in milligrams per human dose ".

Amendment 5

The Addendum of 1973 is withdrawn.

Annex 4

GUIDELINES FOR THE PREPARATION AND ESTABLISHMENT OF REFERENCE MATERIALS AND REFERENCE REAGENTS FOR BIOLOGICAL SUBSTANCES [1]

[1] Prepared by a team of WHO consultants and staff members whose names are given in Appendix 1.

INTRODUCTION

Many national control authorities and individual laboratories seek guidance on the establishment of national or laboratory working standards, and such guidance, where it differs from that appropriate to the establishment of international biological reference materials and reagents, is the subject of these guidelines.

They have also been formulated so that international associations and groups of experts, which are to an increasing extent making proposals for and becoming involved in the preparation and testing of international reference materials, may become familiar with the procedure followed for the establishment of international biological reference materials and reagents.

Some of the guidelines are based on suggestions made during correspondence in 1965 between several experts whose names are listed in Appendix 2. WHO is also indebted to the many scientists who provided advice and data during the preparation of the present text, whose names are similarly given in Appendix 2.

PART A:

GUIDELINES FOR THE PREPARATION, CHARACTERIZATION, AND CALIBRATION OF INTERNATIONAL BIOLOGICAL STANDARDS, REFERENCE PREPARATIONS, AND REFERENCE REAGENTS

A.1. INTRODUCTION

WHO establishes international biological standards, reference preparations, and reference reagents for those biological materials used in the prophylaxis, therapy or diagnosis of human and some animal diseases that cannot be characterized adequately by chemical and/or physical means alone.[1] International biological standards and reference

[1] In general, international biological reference materials that are primarily of veterinary importance are the responsibility of the Food and Agriculture Organization of the United Nations. However, these guidelines may be applied to such materials also.

preparations are provided primarily to enable the activity of biological preparations to be designated in uniform terms throughout the world. The prime function of the reagents is to check on specificity.

A.1.1 Definition of biological reference materials and reference reagents

An *international biological standard* is a preparation of a biological substance to which an international unit has been assigned by the World Health Organization on the basis of data obtained in an international study. The prime function of an international biological standard is to enable the activity of a sample of unknown potency to be measured in a biological test system and expressed conveniently in international units. A collaborative study is carried out to confirm that the proposed preparation is suitable to serve as an international standard and to assign an international unit to it.

An *international biological reference preparation* is a preparation of a biological substance which may be used for a purpose similar to that of a standard but which has been established without a full collaborative study or after such a study has shown that it is not appropriate to establish it as an international standard. In some cases an international unit may be assigned to an international biological reference preparation, especially when it is considered necessary to define a unit for such a preparation as quickly as possible to prevent the emergence of a multiplicity of systems for designating activity.[1]

Some international reference preparations have been established to serve other purposes—e.g., live virus suspensions for determining the sensitivity of biological test systems, diphtheria antitoxin for use in the flocculation test, a long-acting penicillin preparation for the determination (by animal tests) of penicillin blood levels likely to be obtained in man, and a thromboplastin to define the " international calibrated ratio " used in the control of anticoagulant treatment with coumarin and indanedione drugs.

International biological reference reagents are biological diagnostic reagents, biological materials, or antisera of high specificity for the identification of microorganisms (or products derived from them) or for the diagnosis of disease.[2] Since these reference reagents are not used for the quantitative assay of the activity of biological products, inter-

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[1] WHO Technical Report Series, No. 259, 1963, p. 6.
[2] WHO Technical Report Series, No. 293, 1964, p. 6.

national units are not assigned to them. However, they serve for long-term reference purposes.

International biological standards and reference preparations are intended for the calibration of national or laboratory standards and reference preparations. The international reference reagents are intended to provide a means of ensuring qualitative specificity of the relevant corresponding working reagents used in WHO reference laboratories and other institutions.

These international biological reference materials and reagents are not necessarily of high purity, nor are they obliged to comply with pharmacopoeial requirements. They are not intended for administration to human beings or for general experimental purposes.

Both the material used to produce a standard and the considerable contribution of experts who collaborate in its characterization and quantitation are donated without charge to WHO, and ampoules of standards are distributed free to WHO Member States. However, the actual distribution of standards is much wider than this, and many laboratories and scientists outside the category of national control laboratories receive these preparations on request.[1]

No international reference materials and reagents are available in sufficient quantity for routine laboratory use. Four national institutes have been designated by WHO as international laboratories for biological standards and as custodians of international biological standards, reference preparations, and reference reagents.[2]

In the past the preparation of international biological substances has been carried out largely by the international laboratories or by other laboratories in direct collaboration with them. In recent years, however, there has been a considerable expansion of the work undertaken by WHO in biological standardization. This has led to a number of laboratories and institutions becoming involved in the making of preparations, intended perhaps in the first instance for field trials or laboratory studies but ultimately offered for consideration as international biological reference materials and reagents. It has been suggested that the experience of the international laboratories would be useful to laboratories making preparations that might eventually be considered for establishment as international biological reference materials and reagents.[3]

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[1] WHO Technical Report Series, No. 293, 1964, p. 53.

[2] *Biological substances: international standards, reference preparations, and reference reagents.* Geneva, World Health Organization, 1977.

[3] CAMPBELL, P. J. *Journal of biological standardization*, **2**: 249–258 (1974).

The WHO Expert Committee on Biological Standardization has recommended that in future all proposals by other international associations for the establishment of international biological standards, reference preparations, and reference reagents should be channelled through WHO.[1] The Committee considered that priority should be given to the standardization of substances used in prophylaxis, therapy, or diagnosis for which, by use of international standards and reference preparations, a designation of activity could be made in quantitative terms. There may be many instances when the Committee will have to consider biological reference reagents for use in qualitative procedures, and in such cases a careful selection will have to be made of those substances that could serve as international reference reagents on the basis of their need and value in diagnosis. On the other hand, the Committee will not concern itself with the establishment of standards, reference preparations, or reference reagents for biological substances used only for research purposes.

A.2. ASSESSMENT OF NEED AND PROCUREMENT OF MATERIALS

A.2.1 Assessment of need

International biological reference materials may be needed for :

(1) the biological assay of any product used in medical practice and distributed in more than one country ;

(2) the biological assay of a new product that has a clear potential for use and distribution in more than one country ;

(3) the calibration of national reference materials used widely in biological or diagnostic tests ; and

(4) the comparison of research data related to materials requiring biological assay, in the fields of therapy and clinical diagnosis.

The evaluation of the need for international reference materials in the fourth group is the most difficult. However, the establishment of a reference material with a unitage for the measurement of activity should be encouraged, and international scientific associations can be influential in arranging such a step before the need for an international standard is clearly apparent.

[1] WHO Technical Report Series, No. 594, 1976, p. 7.

When a need for an international standard is identified by an independent scientific body, WHO should be informed if it is intended to proceed with the establishment of a reference material, in order to avoid unnecessary duplication of effort.

Where a case for establishing an international reference material is made a technique must exist whereby the activity can be measured, and preferably this should have been published. In many cases a variety of assay methods will be available, and the material chosen should be suitable for use with as many of the techniques as possible.

A.2.2 Nature, source, and storage of bulk material

Ideally a reference material should be chosen in which the composition of biologically active components resembles as closely as possible that of the samples that will be assayed against it. Special considerations relating to the choice of materials for standards to be used in the assay of hormones have been described.[1]

The bulk material selected should have a high degree of stability and sufficient specific activity to serve the purposes of the assay or test for which it is intended. The purity of the material should be such that there are no substances present that would interfere with the procedures for which the material is to be used, but it should be noted that the purest material is not necessarily the most suitable. Less pure forms may be preferable if they are more stable or if the substances to be calibrated may not be assayed validly against the pure form. The bulk material should be obtained preferably from a single source and prepared by personnel experienced in handling the substance.

Bulk material may consist of part or all of a single batch, or if necessary it may be prepared by pooling a number of different batches. If it is necessary to blend material from several sources it should be recognized that the properties of the material and its stability may be altered. When different batches are pooled, homogeneity of the pooled material must be ensured by thorough mixing. Whatever studies might have been made on the individual batches before pooling, the homogeneous bulk material itself should be studied for its suitability to serve as an international standard, reference preparation, or reference reagent.

In some instances there is no difficulty in obtaining a sufficient quantity of bulk material whereas in others only very small amounts of a

[1] WHO Technical Report Series, No. 565, 1975, p. 25.

suitable preparation may be available. In these latter cases the amount needed should be estimated with care. In general, to be suitable as an international standard a minimum quantity of material would allow for 3000–4000 assays. Smaller quantities of materials would suffice only if issues of such material could be restricted to a small number of laboratories. These guidelines for quantity are based on the use of the material only as an international standard, restricted to the calibration of national standards. It is thus normal practice to put material into each ampoule sufficient for several assays.

Whether this amount of material is subdivided into 3000–4000 containers or into a smaller number will depend in the case of freeze-dried preparations on the stability of the reconstituted materials. If the material is in the form of a powder that will be weighed for use, a reasonable minimum amount for accurate weighing using a precise microbalance would be 5 mg per container.

For biological reference materials used in radioimmunoassay, techniques have been developed allowing 3–20 µl of the material in solution to be distributed into microampoules,[1] freeze-dried and sealed under dry nitrogen.

As international standards are intended for use only in well equipped national laboratories, the volume of scarce materials to be sealed in each ampoule should be based more on conservation of the material than ease in use. Measuring devices having the required precision for small volumes should be readily available in such laboratories.

If the bulk material has to be stored before being distributed into ampoules, it is essential that it be held under conditions known to be suitable for maintaining specific activity without changing physical or biological properties. It should, therefore, be kept under conditions in which it will not become contaminated and in which the activity will not be affected by moisture, oxygen, light, or heat. It has been found that powders such as antibiotics can be stored successfully for long periods in screw-capped bottles in sealed polythene bags containing silica gel in the dark and at low temperature or in evacuated desiccators in the dark at −10°C. Sera may be stored at 2–10°C providing the serum is sterile or contains an antimicrobial substance. With some sera, however, it is advisable to store them in the frozen state, in which case special precautions should be taken to achieve complete freezing (see Appendix 3). In all cases the containers should be such as to tolerate the recommended conditions of freezing, freeze-drying, storage, and opening.

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[1] CAMPBELL, P. J. *Journal of biological standardization*, **2** : 269–272 (1974).

Advice on optimum storage conditions should be sought from the producers of the materials prior to receipt of the batch so that the appropriate storage conditions can be prepared in advance.

Prior to storage of the bulk material sufficient samples should be removed from the bulk to provide for all necessary testing. Such samples should be representative of the homogenized bulk batch. Until they are tested these samples should be stored under the same conditions as the bulk batch.

A.3. DISTRIBUTION INTO FINAL CONTAINERS

A.3.1 General considerations

An important prerequisite for any standard is that the material in one ampoule should be identical to that in every other ampoule in terms of potency and stability.

The bulk material, which may be in the liquid or the solid form, is distributed into a number of suitable containers to enable identical samples to be supplied to user laboratories without affecting the integrity or stability of the preparation. In order to ensure that the samples form a homogeneous collection, all the samples from any one preparation must be derived from the same homogeneous bulk and all must be processed together in one working session. It is considered best to store standards in the dried form provided that the material can be dried without losing its specific biological activity and provided that, on being dissolved, it retains adequate activity and suitable physical properties.

If the material is a liquid or if it is water-soluble, distribution into ampoules in the liquid form is to be preferred because of the ease of dispensing a liquid with the desired degree of precision. The aim is to reduce the inter-ampoule variation to a degree that is negligible compared to the precision of the assay applicable to the preparation. Those liquids that can be dried are dried from the frozen state, followed by secondary drying to reduce further the solvent content. Such a process may be applied also to insoluble solids that can be suspended in a suitable liquid. Exposure to phosphorus pentoxide under vacuum is a suitable method of secondary drying.

Materials in liquid form that cannot be dried satisfactorily may, after dispensing, be stored as liquids provided that stability is retained under the available storage conditions. Examples are procaine benzylpenicillin in oil with aluminium monostearate, vitamin D3 in vegetable oil, old tuberculin, and inactivated poliomyelitis vaccine.

Certain materials may also be filled as powders. This is necessary for materials that are relatively insoluble in water or less stable in a freeze-dried form.

Suitable precautions should be taken to protect personnel if there is any possibility that the material contains organisms pathogenic to man or if it is a substance that could be harmful.

A.3.2 Treatment of liquid bulk materials

Some liquids may be distributed into containers without further processing. The choice and extent of processing to prepare the final bulk for filling will depend on the nature of the liquid—whether it is a true solution, a colloid, or a particulate suspension. In all cases the processing must ensure the homogeneity of the product during filling, and measures should be taken at all stages to avoid contamination of the material.

Some materials may have to be treated chemically or physically to control microbial contamination or to remove particles or aggregates of active material. Double glass-distilled water should be used for the preparation of diluents or stabilizers.

When an antimicrobial preservative is used, it should be one that will not affect the preparation during the drying process or its stability subsequently and will not itself volatilize during drying. Thiomersal at a concentration of 0.1 g/l has been used successfully in many sera, in killed bacterial vaccines, and in some killed viral vaccines. A cresol or phenol should not be used in a preparation that is to be freeze-dried.

In all cases, it is important to appreciate that any diluent or added substance should be chosen so that it will neither have a deleterious effect on the activity of the material nor interfere with the assay or test for which the preparation is intended.

A.3.3 Treatment of solid bulk materials

If the material is to be distributed in the solid (powder) form, special precautions should be taken to ensure the homogeneity of the bulk material as well as of the samples taken from it. This may necessitate the use of special mixing devices.

A.3.4 Quality of final containers

It has been the established practice to use heat-sealed glass ampoules in preference to rubber-stoppered vials for international standards.

This is because in a sealed glass ampoule there is virtually no exchange of gases and moisture with the atmosphere.

Containers should be made of high-quality neutral glass [1] that complies with WHO tests.[2] The glass should be free from stresses and should be able to withstand both sterilization by heat and the temperature stresses to which the ampoules may be subjected, such as rapid freezing to −80°C or below. The walls of the ampoules should have a thickness of at least 0.5 mm, especially it the ampoules are intended to be sealed under vacuum. They should be impervious to gases and should have little tendency to form bubbles when the ampoules are sealed by the fusion of the glass. The shape and size of the ampoules should be such that they can be filled easily, sealed by fusion of the glass without detrimental effect to the contents, and opened easily and their contents removed without difficulty. The capacity of the ampoules selected depends on the amount of material required in each; a capacity of about 5 ml is generally suitable. In the case of reference preparations intended to be reconstituted in the ampoule by the addition of liquid, the capacity of the ampoules should be sufficient to hold the total volume of liquid required for reconstitution. For some preparations, such as some blood coagulation factors, it may be necessary to coat the ampoules with silicone, and where ampoules are siliconized there should be a warning on the oven that the glassware should not be baked in an oven in which other glassware is heated. Tinted glass ampoules may be needed for photosensitive materials.

Batches of ampoules should be tested for conformity to specification, especially regarding dimensions. The ampoules should be cleaned by heating in distilled water in an autoclave [3] or by steaming in hydrochloric acid (20 g/l) [4] or by ultrasonic treatment, followed by several rinses with clean water and finally with distilled water. Detergents should not be used. The washed ampoules should then be sterilized by dry heat in a clean grease-free oven. If the ampoules are to be stored at any stage after cleaning and before filling, they should be stored in sealed dustproof containers.

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[1] Addresses of suppliers of suitable glass may be obtained from Chief, Biologicals, World Health Organization, 1211 Geneva 27, Switzerland.

[2] *Specifications for the quality control of pharmaceutical preparations: second edition of the International Pharmacopoeia.* Geneva, World Health Organization, 1967, pp. 869–870.

[3] Steam admitted to autoclaves for the cleaning or sterilization of glassware must be free of the volatile compounds that may arise from boiler water additives.

[4] Should steaming in hydrochloric acid be carried out in an autoclave, great care must be taken to remove traces of the acid from the vessel after the cleaning treatment.

A.3.5 Filling of ampoules

Ampoules should be filled from a single homogeneous bulk of material. To ensure that homogeneity is maintained throughout the filling process, the bulk material should be held at a constant temperature during filling and, if necessary, constantly stirred. It should also be shielded from direct sunlight. The quantity to be filled into each ampoule should be that convenient for use in the assay.

Before filling, each ampoule should be marked indelibly[1] for identification, and the filling must be carried out in such a way as to avoid any form of contamination (e.g., by microorganisms, chemicals, or dust).

Liquids intended to serve as biological standards or reference preparations should be filled with an accuracy within the range of ±1%. Filling with this accuracy usually permits the stated amount contained in each ampoule to be used for assay purposes without the need for weighing at the time of use. Reconstituting the entire contents of an ampoule is essential in the case of freeze-dried materials because the active principle may be distributed unevenly in the freeze-dried mass. In filling liquids intended to serve as reference reagents the same degree of accuracy of fill should be aimed at, but for some preparations greater variations such as ±5% may be permissible. During any filling run, a total of 1–2% of the filled ampoules should be selected and weighed to control the consistency of filling. Such samples should be taken at the beginning and end of the filling session, and at regular intervals throughout it.

In distributing solid materials, large variations in amounts per ampoule may be unavoidable. This is unimportant, however, since an exact quantity of the contents will be weighed at the time of use. Most powders can be put into ampoules by an automatic filler, but suitably sized spoons may also be used. In some cases, the powder may acquire an electrostatic charge and stick to the inside of the ampoule. To avoid the charring of such adherent powder when the ampoule is sealed, it may be necessary to use special devices for filling—for example, a funnel with the stem reaching to about 10 mm from the bottom of the ampoule. During filling with some substances—e.g., those that are hygroscopic or efflorescent—special precautions should be taken to control the humidity of the atmosphere.

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[1] Such marking should be achieved by procedures such as ceramic printing or etching but never by scratching with a diamond point.

A.4. PROCESSING OF FILLED AMPOULES

A.4.1 General considerations

Filled ampoules should be taken through the remaining stages of processing with the minimum of delay. It is essential that from the time of filling to the end of the operation all the ampoules in a batch should be processed together so that they are subjected to the same conditions at the same time.

If phosphorus pentoxide is used for drying, it is essential that adequate measures be taken to prevent contamination of the material with phosphorus pentoxide powder. If material in unsealed ampoules has been kept below room temperature, the ampoules must be allowed to reach room temperature before the enclosure used for desiccation is opened, otherwise there may be some condensation of moisture onto the product.

In all cases, the final sealing of the ampoules should be by fusion of the glass. Sealing by rubber or plastic closures is unsatisfactory for long-term preservation of international reference materials.

A.4.2 Processing of materials that are to be freeze-dried

A.4.2.1 *Freezing*

The liquid in the ampoules should be frozen to −60°C or lower to ensure complete freezing before the drying process begins. The rate at which freezing is carried out may be important for maintaining the specific activity and solubility of the material, and the most suitable rate may need to be determined experimentally. The temperature required may be determined from measurements of the electrical conductivity of the material or by differential thermal analysis.[1] Freeze-drying of complex biological materials can be achieved satisfactorily only if the materials have been cooled below the minimum temperature of incipient melting (lowest eutectic point). Care should be taken to avoid contamination of the material with any of the coolant used. If the amount of material in each ampoule is large, it may be necessary to increase the surface area of the contents by freezing as a shell or as a slant (see Appendix 3).

[1] REY, L. R. *Annals of the New York Academy of Sciences*, **85** : 510 (1960).

A.4.2.2 *Freeze-drying*

It is desirable that the whole batch of filled ampoules be freeze-dried in a single run. Where this is not possible, it is necessary to demonstrate that there is no significant difference in potency of the material between the different runs. It is necessary also to identify uniquely the ampoules from each run.

The preferred freeze-drying equipment is that of the " shelf " type, in which the temperature of the material in the ampoules is recorded continuously. If heat is applied to the shelves during the process, care should be taken to ensure that it is applied evenly and is not excessive for the particular material.

The precise conditions for successful drying of a particular substance may be determined only from previous experience with similar freeze-drying runs. Where such experience is not available experimental runs should be carried out, but in any event it is advisable to keep the temperature of the material as low as possible, even at the cost of prolonging the period of freeze-drying. It is desirable that the duration of drying be extended well beyond that which has been determined as the minimum, unless the completion of lyophilization is shown with the aid of a suitable device.

Secondary drying may be carried out in the freeze-drier if it is designed for this purpose. Otherwise, the ampoules should be transferred to desiccators containing active phosphorus pentoxide for this stage of the process.

Until the ampoules are sealed they should be protected from light and held at a suitable temperature (4–20°C), depending on the duration of drying and the stability of the material. The drying should be continued until the weight of the contents of selected ampoules is constant.

Unless the ampoules have been sealed within the equipment, the product in the freeze-drier is warmed to room temperature or a little higher before being exposed to atmospheric humidity in order to prevent the condensation of moisture on the product.

A.4.2.3 *Sealing*

The ampoules may be sealed on a manifold under vacuum (i.e., 1–4 Pa, or 0.01–0.03 mmHg) or after filling with a dry inert gas such as nitrogen or argon. The inert gas should be pure and completely free of oxygen. It should be dried by passing it through a desiccant such as a molecular sieve. A large batch of ampoules may have to be dealt with in portions, the size of each being dependent on the number of ampoules that can be accommodated on the manifold. It is essential

to ensure that each batch of ampoules is subjected to the same conditions and that the product is exposed to the atmosphere for the shortest possible time. For the latter reason it is usually necessary, before sealing the ampoules on the manifold, to transfer them direct from the freeze-drier to the desiccators, which are then evacuated and filled with the inert gas.

Alternatively, techniques may be used in which the ampoules are sealed with stoppers in the freeze-drier and finally by fusion of the glass after removal from the freeze-drier,[1] or in which the ampoules are closed by specially designed stoppers that allow only very slow diffusion of gases.[2]

After sealing, the ampoules should all be tested for pinholes and cracks and the defective ampoules discarded. Ampoules sealed under vacuum may be tested with a high-frequency vacuum testing coil, but the tester should never be applied to the part of the ampoule where it has been sealed. Sealing failures in ampoules filled with an inert gas may be detected by submerging them in methylene blue solution and exposing them to a partial vacuum for 15–20 minutes.[3] Several minutes after return to atmospheric pressure those ampoules having cracks or pinholes will be found to contain a quantity of the dye solution. Alternatively, a pressure test may be used by those laboratories having the necessary equipment.[4]

A.4.3 Procedure where freeze-drying is not used

When used for liquids or solids that cannot be dried, the ampoules are filled with an inert gas before sealing (see section A.4.2.3). This may be achieved by placing the ampoules in a chamber that is evacuated and filled with the gas. This process should be repeated several times to remove residual air. The inert gas should be pure, especially oxygen-free, and dried by passing it through a desiccant such as a molecular sieve.

When solids are to be dried in the solid state, the ampoules should be placed over active phosphorus pentoxide in a desiccator, which is then evacuated to a pressure of 1–4 Pa (0.01–0.03 mmHg). From this point onwards, the procedure is as described in section A.4.2.3.

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[1] SPAUN, J. & LYNG, J. *Dansk Tidsskrift for Farmaci*, **44** : 292 (1970).

[2] CAMPBELL, P. J. *Journal of biological standardization*, **2** : 259–267 (1974).

[3] Gross defects can be detected in this way, but minute cracks, to which it is alleged all heat-sealed ampoules are prone, cannot be detected by the conventional tests. See : MELTON, H. ET AL. Some problems in sealing gas-filled glass ampoules. In : *International Symposium on Freeze-Drying of Biological Products, Washington, DC, 1976*. Basle, Karger, 1977 (Developments in biological standardization, vol. 36), p. 145.

[4] BRIZELL, I. G. & SHATWELL, J. *The pharmaceutical journal*, **211** : 73 (1973).

A.4.4 Labelling

The indelible identification mark applied to the ampoules before they are filled is generally sufficient to identify them during storage. *Materials intended as proposed international biological standards or reference preparations should not be labelled as such until they have been established by WHO as definitive standards or reference materials.*

Before preparations are issued to users the ampoules should be labelled to show the following items of information.

(1) The name " World Health Organization ".

(2) The name of the preparation in the form " International Standard for ... ", " International Reference Preparation of ... ", or " International Reference Reagent of ... ". The name should be preceded by " 1st ", " 2nd ", " 3rd ", etc. as the case may be.

(3) The year in which established by WHO.

(4) The mass of solid containing one international unit; or the number of international units per milligram; or the number of international units contained in the ampoule.

(5) The storage conditions.

(6) The name and address of the distributing laboratory.

(7) A statement that the material is not for use in man.

A.4.5 Storage of sealed ampoules

The sealed ampoules should be stored in the dark and kept at a low temperature (generally −20°C). The actual temperature selected will depend upon the nature of the particular preparation.

A.4.6 Characterization of final product

To verify that a satisfactory final product has been obtained, the preparation should be tested for potency, stability of specific activity, moisture content, residual oxygen, and (where necessary) microbial contamination.

A.4.6.1 *Potency*

Potency tests are essential in order to confirm that the preparation has adequate specific activity for the assay or test for which it is to be used and to confirm that there has been no undue loss of potency during

processing. For this purpose it is desirable that samples be taken at appropriate times during processing and preserved over liquid nitrogen.[1]

A.4.6.2 *Stability*

Stability tests are necessary to estimate the period during which the preparation can reasonably be expected to retain an adequate level of potency under storage and to define suitable conditions for distribution to laboratories (e.g., by post). For these tests a number of ampoules are held, for various periods, at three or more different temperatures including the temperature at which the ampoules are normally stored. They are then assayed for specific activity, using a precise method. The elevated temperatures and the periods chosen must be such as to demonstrate losses of potency at two of the elevated temperatures at least. For example, ampoules of dried serum containing specific antibodies might be held at the temperature of storage, at 37°C, and at 56°C for two months. From the results of the tests the rate of loss of specific activity of the preparation at various temperatures can be estimated.[2]

A.4.6.3 *Moisture content*

The moisture content must be determined to verify that the drying process has been adequate. No completely satisfactory method of determining absolute moisture content, when the moisture is present in very low concentrations in small samples of biological materials, has yet been devised. However, the available methods give results that may be used for comparative purposes. The Karl Fischer method is frequently used.[3] A convenient method for international standards and reference substances, which is accurate to within ±10% of the result obtained, consists in drying the material over phosphorus pentoxide under vacuum (1–4 Pa, or 0.01–0.03 mmHg) at 56°C to constant weight or for a specified period. The contents of at least three ampoules should be tested separately, provided each ampoule contains not less than 10 mg of material. If each ampoule contains less than 10 mg a sufficient number of ampoules should be taken so that three portions each of not less than 10 mg can be tested.

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[1] CAMPBELL, P. J. *Journal of biological standardization*, **2**: 259–267 (1974).

[2] JERNE, N. K. & PERRY, W. L. M. *Bulletin of the World Health Organization*, **14**: 167 (1956).

[3] *Specifications for the quality control of pharmaceutical preparations: second edition of the International Pharmacopoeia*. Geneva, World Health Organization, 1967, Appendix 40.

Alternatively, the sealed ampoule may be heated in a special oven and broken open within the chamber. The water vapour evolved is carried in a stream of gas through an electrolytic detector cell.[1]

Where a low moisture content would result in loss of potency of the material (e.g., rabies vaccine and certain proteins) or where water of crystallization is essential for stability (e.g., certain antibiotics), a higher moisture content is required.

A.4.6.4 *Residual oxygen*

The oxygen content of the atmosphere within the ampoule should not exceed 1 ml/l, as measured with a mass spectrometer.

A.4.6.5 *Sterility*

Sterility is most desirable in preparations that are intended to have long-term stability, although it is not always mandatory. Standards and reference materials should be prepared in such a way that microbial contamination is prevented. Suitable tests should be made to show that the preparation is acceptable for the purpose intended. Where assay methods include incubation, sterility is essential.

A.4.7 Records

Full records should be kept of all procedures and tests to which the material has been subjected in preparing international standards, reference preparations, and reference reagents. A complete and accurate inventory should be kept of stocks and of all the ampoules issued, and an account of such an inventory should be sent to WHO annually.[2]

A.5. INTERNATIONAL STUDIES

International studies comprise collaborative studies and collaborative assays, although the borderline between them is not always sharply defined.

The term " collaborative study " is used to denote a study carried out in an agreed way by two or more laboratories in different countries,

[1] BÜCHLER, W. ET AL. *Zeitschrift für analytische Chemie*, **237** : 104 (1968).

[2] Addressed to Chief, Biologicals, World Health Organization, 1211 Geneva 27, Switzerland.

generally on a topic of broad interest such as safety testing or clinical effects and reactions in the field. In the context of this document, collaborative studies on methods of potency testing are especially relevant. Complex studies have been developed for the assessment of problems in the standardization of hormones and blood products.

A " collaborative assay " is a more narrowly defined type of international study, used when one standard is assayed in terms of another by previously established assay methods. Details of the design of such collaborative assays and on the reporting and analysis of data are given in section A.6.

A.5.1 Organization of international studies

The organization of international studies involves much work by a number of laboratories and should be undertaken by a scientist familiar with the appropriate biological field. Usually this will be someone in one of the WHO international laboratories for biological standards or WHO collaborating centres, although occasionally someone outside these groups may be requested to organize a study. Close cooperation with a biometrician who has experience in the relevant type of work is often essential, especially in the case of collaborative assays (see section A.7). In the organizing laboratory there should be facilities necessary for the preparation, handling, storage, and dispatch of materials. If the laboratory is also to analyse the results, access to computer facilities is essential. Sometimes the analysis can be performed by a collaborating biometric centre, locally or within WHO.

It is important that such an international study be authorized by the body sponsoring it, and it is desirable that WHO be kept informed of plans for such studies in order to avoid unnecessary duplication. Advice on their planning and execution is available from WHO.

A.6. COLLABORATIVE STUDIES

A.6.1 Examples of collaborative studies

Collaborative studies may be of varying size and complexity and may include research with the following aims.

(1) To determine which of two or more candidate materials is most suitable for use in medical work. The materials may be apparently similar or they may be of clearly different types — e.g., a highly purified substance and a crude preparation.

(2) To determine whether the candidate material is suitable to serve as a standard for the assay of preparations from different manufacturers.

(3) To determine whether different assay methods measure different properties of a proposed reference material—e.g., bioassays and immunoassays.

(4) To set up a reference material for a substance for which assay methods may not be of proven validity. This generally occurs in setting up a first standard for a new type of substance—e.g., coagulation Factor VIII and pyrogen.

(5) To confirm, when initially necessary, that the material has the biological activity expected of it and to determine those activities that are most suitable to form the bases of biological assays. Research may be directed to the correlation of activity as measured in the laboratory with the activity experienced in clinical work—e.g., the protective effect of a vaccine, the desired *in vivo* anticoagulant effect of a heparin preparation, or the antitumour effect of an antibiotic.

(6) To calibrate by bioassay, a first international reference material intended for use in immunoassays.[1]

(7) To assess the stability characteristics of a proposed international reference material by accelerated degradation tests. This is particularly desirable for those substances for which assays are imprecise, costly, or time-consuming and for which it is appropriate to share the work with more than one laboratory.

(8) To assess purity by chemical and physical tests, by assay of contaminating substances that have biological activity, by limit tests to demonstrate absence of specific contaminants, and by tests for specificity.

(9) To compare materials with samples from " normal populations " —e.g., the comparison of a standard for a coagulation factor with samples of fresh normal plasma from many individuals.

A.6.2 Planning

After wide consultation with appropriate experts, the aims of the study should be formulated. The size of the study depends on the nature and complexity of the problems to be investigated and the ease, cost, and accuracy of the assay procedures.

It is necessary to obtain and prepare samples of other possible materials, in addition to the reference materials, for inclusion in the

[1] WHO Technical Report Series, No. 413, 1969, p. 8.

study. A preliminary inquiry may be sent to possible participants in the study inviting them to take part. This inquiry should give an outline of the aims of the study and a brief description of the materials to be included.

Prospective participants in collaborative studies should be asked to state :

(1) the assay methods and the assay design of which they have experience ;

(2) the number of materials they could compare conveniently as a multiple assay ;

(3) the number of assays they could provide, with an indication of expected average accuracy ; and

(4) whether they could use a draft result sheet and any modifications they would like to see incorporated.

When it is desirable to ask participants to use a " common assay method " the procedure should be explained in detail. In such a case ample time should be allowed for laboratories to become familiar with the method before carrying out the assays for the collaborative study.

A.6.3 Participants

The participants in collaborative studies may include national control laboratories, manufacturers of the relevant biological products, and academic research laboratories. For studies on proposed international reference materials it is desirable to include laboratories in several countries, unless the appropriate expertise is limited to only a few countries.

Prospective participants should be asked if they would be prepared to accept and handle potentially harmful pathogens or material that may be contaminated with hepatitis antigen and to sign a form agreeing to handle and dispose of such materials on their own responsibility.

Participants should be required to agree not to publish information on a proposed standard without the prior agreement of WHO and certainly not until a decision has been taken on its establishment. The premature publication of papers, whether adversely critical or not, on a proposed standard that is not eventually accepted for establishment can cause scientific confusion and needless concern.

It should be explained to participants whether the report of the study will identify the results from each laboratory by name or whether anonymity will be preserved by the use of a code number for each.

The number of participants will depend on the nature of the study. Generally 4–10 laboratories will be sufficient but when very complex studies are undertaken (such as one involving a first evaluation of test methods), or when assays are inaccurate or costly, or when it is intended to obtain estimates of " normal values " in populations (such as those for hormones and blood products), it may be necessary to include more laboratories. In general it is preferable to plan for many laboratories to carry out a few assays, rather than a few laboratories to carry out many assays.

A.6.4 Materials included in collaborative studies

Biological materials included in collaborative studies must be prepared, subdivided, stored, transported, and handled in such a way that all samples of a preparation are identical and all are adequately stable for the duration of the study. To satisfy these criteria it is desirable to handle and freeze-dry the materials with the same careful procedures used in preparing ampoules of the standard.

Human blood, tissues and extracts must be tested for hepatitis B surface antigens.

Materials in ampoules should preferably be code-labelled so that participants cannot identify materials or duplicates. Sufficient ampoules of each material should be prepared to provide each participant with a generous supply for all the assays required. In deciding on the amount to be sent, due allowance must be made for the stability of the material.

In instances where the materials included in collaborative studies may be useful for further studies (e.g., assessment of new methods), it may be advisable to prepare a large number of ampoules and to store them under suitably stable conditions.

The type of materials on which tests are carried out are as follows.

(1) More than one candidate ampouled material where it is not known what type of material is to be preferred (e.g., coagulation Factor VIII [1]).

(2) A coded duplicate of the candidate standard. Results of assays of such duplicates provide evidence of accuracy of assays, since dissimi-

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[1] BANGHAM, D. R. ET AL. *Bulletin of the World Health Organization*, **45**: 337 (1971).

larity between standard and candidate is eliminated as a cause of variability (e.g., heparin [1]).

(3) Coded ampoules of a known dilution (e.g., 1 : 3 or 1 : 30) of the standard, the candidate material, or any other preparation included in the study. Results can provide evidence of accuracy of method of assay (e.g., renin [2]).

(4) Samples of a preparation shown to have clinical efficacy and preferably supplied at two concentrations that have been shown to give quantitatively different clinical responses (e.g., anti-Rh_0 immunoglobulin [3]).

(5) A second or third batch of ampoules of the bulk material from which the proposed standard was made in order to assess the reproducibility of successive batches intended for use as a working standard (e.g., corticotropin [4] and immunoglobulins [5]).

(6) A widely used national standard (or a proposed international standard).

(7) Samples of serum or plasma each with a different concentration of the substance or of its precursor or its metabolic forms. Such samples would be required in, say, the assessment of specificity, especially in immunoassay systems.

(8) A reagent (e.g., enzyme substrate preparation) to be used in parallel with local reagents in order to study whether the nature of various agents obtained locally may be a cause of variation in assay results, especially when the reagent is unstable or scarce (e.g., thrombin [6]).

(9) Fresh samples of body fluids obtained locally (e.g., comparison of coagulation Factor VIII with " fresh normal plasma "). Guidance should be given on the selection and handling of such samples.

(10) National or local laboratory standards (e.g., coagulation Factor IX [7]), when it is planned to demonstrate whether the expression of relative potencies in terms of a single standard leads to a reduction of

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[1] BANGHAM, D. R. & WOODWARD, P. M. *Bulletin of the World Health Organization*, **42** : 129 (1970).

[2] *Clinical science and molecular medicine*, **48**, Suppl. 2 : 135 (1975).

[3] Unpublished working document WHO/BLG/BLOOD/8.

[4] BANGHAM, D. R. ET AL. *Bulletin of the World Health Organization*, **27** : 395 (1962).

[5] ROWE, D. S. ET AL. *Bulletin of the World Health Organization*, **46** : 67 (1972).

[6] ROBERTSON, I. ET AL. *Thrombosis et diathesis haemorrhagica*, **34** : 3 (1975).

[7] BROZOVIĆ, M. ET AL. *Thrombosis and haemostasis*, **35** : 222 (1976).

variability of results among laboratories. Their inclusion should not otherwise be regarded as part of the collaborative study.

A.6.5 Transport of test materials

Test materials should be transported to participating laboratories under conditions that ensure the stability of the materials and that conform to official postal or other relevant transport requirements within and between countries.

Thus samples sent frozen must have an adequate supply of ice, solid carbon dioxide or liquid nitrogen, and the insulation of the containers must be sufficient to withstand the temperature conditions encountered during transport and the time of exposure to high temperatures.

Inquiries should be made of the appropriate authorities regarding special requirements for, or an embargo on, materials carrying special risks. Certain countries, for instance, forbid the introduction of samples of viruses, and certain authorities may not agree to transport samples contaminated with hepatitis B antigen.

A.6.6 Report on a collaborative study

The report on a collaborative study should include the following points.

(1) Historical introduction and description of a former standard if applicable.

(2) The aims of the study and the number of laboratories taking part.

(3) The proposed standard—its source, nature, handling and ampouling. Results of chemical and physical tests on the bulk and ampouled material. Descriptions of the nature of other materials included in the collaborative study.

(4) The planning and design of the assay, with approximate dates, and the form in which data were returned by participants.

(5) A brief description of the statistical methods used to treat the data, including any specialized problems of treatment.

(6) The total numbers of valid and invalid results, the reasons why some results were not included (e.g., criteria for nonparallelism or non-

linearity), and the combined estimates of relative potencies by different methods, calculated both with and without outlying results.[1]

(7) Comparisons of different materials and different methods, together with a biological interpretation of these observations. Comments on particular factors such as frequency distribution and causes of differences in potency estimates. Comments on dose-response curves.

(8) The final figure for the combined estimate of potency of the proposed standard, the confidence limits and the method of deriving them, and comments on the validity of the combined estimate.

(9) In studies on proposed first international standards, an assessment of the degree to which the use of potencies relative to the proposed standard reduced differences between laboratories and between methods.

(10) A recommendation on the suitability of the material studied to serve as a standard, together with any recommended limitations on its use (e.g., for certain assay methods only). A recommended potency in international units.

(11) An assessment of the stability of the material, based on accelerated degradation studies.

(12) A list of names and addresses of participants. Unless it has been agreed to the contrary, the participants are referred to in the body of the report only by anonymous code numbers, which do not correspond to the order of the list.

(13) Tables and histograms.

(14) Acknowledgements, summary, and references.

The draft report should be sent to participants together with their laboratory code numbers. They should be asked for their agreement that their data have been interpreted correctly in the analysis, that the material is (or is not) suitable to serve as a standard for the purpose defined, and that the proposed unitage to be assigned is correct.

The final report, amended in the light of any corrections and stating the agreement of the participants, is then submitted to WHO. The final report on an established standard should be made available to any user of the standard.

Reports intended for publication should include the date of establishment of the standard and the definition of the international unit. Any information that contributes to current research on the subject (e.g.,

[1] WHO Technical Report Series, No. 565, 1975, p. 5.

comparisons between assay methods) should be included. Sometimes this draft also is submitted to the participants.

A.7. COLLABORATIVE ASSAYS

A collaborative assay is necessary if, say, an existing international standard or international reference preparation has to be replaced and the second preparation has to be carefully calibrated in comparison with the first. It can also be used to calibrate a first international standard in terms of a national standard.

Some of the requirements described in sections A.6.2–A.6.6 may be applicable to such collaborative assays.

For a straightforward collaborative assay with methods giving high accuracy, about 4–10 laboratories are generally sufficient.

A.7.1 Design

Assays of standards and reference preparations should be designed to obtain from each laboratory :

(1) *either* an estimate of the relative potency of the candidate material in terms of the international material (or other primary standard) by direct comparison of the materials, *or*, if there is no primary standard, the potency of one or more test preparations relative to the candidate material.

(2) in some instances data from which a dose-response curve may be calculated that extends beyond the range of doses used in the assay.

Each assay should be designed to allow an assessment, from its own internal evidence, of statistical validity (in the case of parallel-line assays evidence of linearity and parallelism) and accuracy. For the purpose of these studies an *independent* assay is defined as one made using fresh dilutions starting with a newly-opened ampoule or a fresh weighing of each material. A *duplicate* assay is a repeat assay using the same solutions ; it does not include all the assay variables of weighing and dilution errors and is thus not truly independent. A *multiple* assay is a comparison of more than two materials in the same assay.

The participants may be asked to carry out a specified minimum number of independent assays, or (for very inaccurate assays) to do as many assays as might provide a specified accuracy defined by the statistical weighting determined from the combination of all valid assays. Duplicate or multiple assays may also be requested. Normally it is desirable to

request an assay design with at least three dilutions for each preparation so that linearity may be tested.

A.7.2 Reporting of data

Each participating laboratory should be asked to report details of the assay method used, including, for example, details of animals (e.g., species, strain, weight range, sex, pretreatment, and method of randomization) or other test organisms and details of substrates (e.g., nature, source, preparation, and characterization).

Accurate detailed recording of the nature of diluting solutions and the procedure of making dilutions of test and standard materials is of special relevance for the calculation of results and the detection of causes of variation, bias, or inaccuracy.

In many studies all the essential information for each assay can be entered on an assay result sheet. This sheet is designed in the light of the preliminary information received from the participants at the time of the initial invitation. Such a result sheet also greatly facilitates the processing of the data for the computer. It should be as simple as possible and its use explained by annotations.

Participants should be encouraged to supply their own statistical calculations for each assay because this helps to show whether their data are interpreted in the same way as that used by the biometrician in analysing all the data.

A.7.3 Analysis of data

It is advisable to have the data analysed by a biometrician or someone experienced in the statistical evaluation of various types of bioassay. The data are analysed using standard statistical procedures. The use of a full statistical procedure rather than short-cut methods is recommended, and access to adequate computer facilities is therefore desirable.

Each set of results should be checked on receipt, preferably by both the scientist and the biometrician organizing the collaborative study, so that uncertainties and deficiencies can be discussed promptly with the participating laboratory.

The extended dose-response curve for each method obtained by each laboratory should be plotted graphically. The results of assays should be plotted as a histogram because this helps to detect peculiar features that may be overlooked in the study of numerical data alone.

Each assay should be analysed separately, the validity tests carried out, and the relative potency and accuracy calculated (perhaps as confidence or

as fiducial limits). The results of all assays for each laboratory and for each method should be combined and the potencies and confidence limits calculated.

Analysis of variance can be carried out to assess the significance of differences between methods and laboratories and other factors, such as between candidate reference materials included in the study. An assessment is made of possible factors causing significant heterogeneity, non-linearity, and consistent differences in slope.

A final figure should be obtained for the combined estimate of potency of the preferred candidate material and the confidence limits given for that value.

A.7.4 Report on a collaborative assay

The report on a collaborative assay of a standard should assemble all the essential information about it, including data on the material and its characterization and the results of the collaborative assay. This body of information is the evidence that the preparation is suitable to serve the purpose for which the standard is set up. It should be written by the scientist and biometrician who organized the collaborative assay.

A.8. THE ESTABLISHMENT OF AN INTERNATIONAL BIOLOGICAL STANDARD, REFERENCE PREPARATION, OR REFERENCE REAGENT

When the report on a collaborative assay has been approved by all participants, and if it is agreed that the preparation should be proposed as an international standard, reference preparation, or reference reagent, the report together with any appropriate supporting documents should be submitted to WHO.[1] The report will then be sent to members of the Expert Advisory Panel on Biological Standardization and other experts for comments. Any queries raised by such experts must be answered satisfactorily before the preparation is offered formally for approval. If there are no further objections, the preparation can be established either by the Director-General or by the WHO Expert Committee on Biological Standardization. This procedure can be expedited by discussing with WHO the form that the report should take.

A list of biological substances comprising the international standards, international reference preparations, and international reference reagents

[1] Chief, Biologicals, World Health Organization, 1211 Geneva 27, Switzerland.

is published from time to time. Any substances that have been established or discontinued since the last publication of the list are included in an appendix to the next report of the WHO Expert Committee on Biological Standardization. Such appendices will be cumulative until such time as the list is revised and republished.

When a substance has been approved as an international preparation, a document is prepared by the coordinator of the study that describes the product, draws attention to relevant references, and gives instructions concerning its reconstitution and use.

A.9. USE OF INTERNATIONAL BIOLOGICAL REFERENCE MATERIALS

International standards and reference preparations are intended to be used for the calibration of national standards by comparative bioassay. These national standards can then be used to measure the potencies, in international units of activity, of the appropriate biological materials within the countries concerned. Measurements of the same activity in different countries, each in terms of a national standard, would then be comparable. In many countries national control laboratories do not exist or have not been able to prepare national standards. In such countries international reference materials may be made available directly to other laboratories. The supply or distribution of materials under such conditions is considered only after the receipt of a letter describing the purpose for which the particular reference material is needed. This letter should be addressed to the appropriate WHO international laboratory for biological standards.[1]

Many of the international reference materials are suitable for use only in a specially equipped laboratory. When hygroscopic materials have to be used on a weight basis, it may be necessary to use special weighing facilities—e.g., an enclosure of very low relative humidity or a continuously recording balance. Precautions have also to be taken in the opening of ampoules in order to avoid contamination of the contents by particles of glass.[2] Care should also be taken in opening ampoules sealed under vacuum.

In the case of freeze-dried international reference materials the entire contents of an ampoule must be dissolved in a suitable solvent. Care must be taken to wash out the whole of the parts of the opened ampoule

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[1] See : *Biological substances : international standards, reference preparations, and reference reagents*. Geneva, World Health Organization, 1977.

[2] See Appendix 4 as an example of instructions for opening an ampoule.

thoroughly since the small quantity of active component may be unevenly distributed throughout the freeze-dried residue. No attempt should be made to weigh out portions of the ampoule contents.

It is possible that an ampoule of international reference material may contain material sufficient for many assays, particularly where radioimmunoassays are concerned. In such cases a solution of the reference material may be redistributed by the user in suitable small volumes, which may then be stored frozen, preferably at −20°C or below. Such further processing of an international reference material should be carried out only by personnel with appropriate experience since the choice of a suitable diluent (e.g., containing carrier protein free of enzymatic activity) may be important in order to prevent adsorption of the active constituent on the surface of the containers. Each such international reference material must be considered as a unique problem.

The correct and efficient use of many international reference materials may well be outside the scope of some laboratories performing biological assays for routine purposes. They should be used by national laboratories to calibrate working standards, which may then be supplied in a more convenient form for routine use.

International biological reference materials should be used as soon as possible after reconstitution. If it is intended to use a preparation in several test systems it can be divided into aliquots, which may be held for a few days in the dark in a refrigerator (2–6°C). The period allowable depends on the nature of the preparation. Certain reference materials can be held for a few weeks at −20°C after reconstitution.

Suitable precautions should be taken to protect personnel (see section A.3.1).

A.10. THE "MASTER AMPOULE" CONCEPT FOR BIOLOGICAL STANDARDS AND THE PROVISION OF WORKING STANDARDS

In the past the principle that international biological reference materials should be provided to national authorities for the purpose of establishing national reference materials has been rigidly upheld. The provision of working standards [1] in quantity has been discussed on a number of occasions by the WHO Expert Committee on Biological

[1] Working standards are reference materials established by a laboratory for the purpose of inclusion in every test.

Standardization, but hitherto it has always been considered impracticable. Recent developments in technology, however, by which the activity of biological substances such as those used in endocrinology and haematology may be measured in minute quantities, demand a reconsideration of this question.

There are two principles involved. The first concerns the establishment of " master ampoules " of a scarce or particularly costly material for the preparation of international reference materials. In such a case the bulk material could be filled into a number of master ampoules and stored under suitable conditions—e.g., freeze-dried or frozen. The contents of two master ampoules would then be reconstituted and each distributed into identical large batches of ampoules and processed, one to become the international reference material and the other a working standard, after which an international study to characterize and assay the potency of both batches would be carried out. When the stock of working standard ampoules began to run low a third master ampoule would be reconstituted, distributed, and processed with the same equipment and in an identical manner to that used for the first working standard. It would be assumed that the third batch was identical to the first two, and it would then be necessary only to calibrate the third batch in terms of the international reference material in order to establish it as the second working standard. The process would continue until all but the last master ampoule had been reconstituted, by which time a replacement bulk for another set of master ampoules would be obtained.

Such a system is not new. It has been operated successfully for a number of the international standards and has been used in the USA for national standards since their inception. A similar principle, " the seed system " for vaccine production, is now well established. In order for this system to work as intended, the processing of each master ampoule must give demonstrably identical results, and storage conditions must be shown to cause no change between the first and the last master ampoule used in the system.

The advantages of the system are :

(1) that the burden on freeze-drying resources is much reduced at any one time ;

(2) that the demand for expensive refrigerated storage space is much reduced ; and

(3) that, if developments in technology subsequently show the reference material to be no longer satisfactory, little is lost in discarding the remaining undistributed master ampoules.

The second principle concerns the desirability of providing in quantity WHO working standards prepared from such master ampoules, and here also there are cogent arguments in favour of such a course, as enumerated below.

(1) Certain materials are very scarce and intrinsically valuable. This is particularly true of some human hormones, e.g., parathyroid hormone.

(2) Some materials are of necessity a variable mixture of several active components, so that it may be impossible to reproduce the reference material exactly except by the use of the master ampoule principle.

(3) Some of the finished reference material may have a limited stability after reconstitution so that the contents of each ampoule have to be used quickly, thus necessitating a larger supply of ampoules.

(4) The cost of a collaborative assay may be very high and beyond the reach of many national authorities.

(5) Modern techniques such as hormone radioimmunoassay, with their need for specificity, demand a direct comparison with the primary standard.[1]

For such materials the provision of WHO working standards in reasonably large quantities based on a master ampoule system would have many advantages. Much expense would be spared by the elimination of the need to establish successive preparations, with their necessary collaborative studies, since the relatively limited calibration assay used to allocate the potency of each successive batch of ampoules is less costly. Moreover, national authorities would be relieved of the burden of establishing a national reference material. It is even doubtful whether they could obtain supplies of some of the rarer materials.

The master ampoule procedure has been used for the supply of a working standard for corticotropin [2] for many years and the advantages described above have been amply demonstrated.

A.10.1 Operation of master ampoule system

Where the master ampoule concept is to be applied, bulk material of appropriate concentration would be filled into a number of large ampoules known as the master batch, each ampoule of which would contain, when

[1] WHO Technical Report Series, No. 565, 1975, pp. 21–28.

[2] BANGHAM, D. R. ET AL. *Bulletin of the World Health Organization*, **27**: 395 (1962).

dispensed, sufficient material to supply a working standard to appropriate testing laboratories throughout the world for a 1–2-year period. The master batch would comprise also enough master ampoules to prepare several batches of the international reference material, working standard, or national standard.

If liquid, the bulk should be of such concentration that the ampoule size and the volume it contains will be appropriate for freeze-drying. In some instances it may be necessary to fill with a quantity that will necessitate the use of two or more master ampoules in order to yield sufficient ampoules for the international reference material and a batch of the working standard.

Ideally the bulk material should be sufficiently concentrated to enable a 10–15-year supply of the international reference material to be contained in a single master ampoule. To provide a batch of working standard, one or more master ampoules may be needed depending on the expected use. In either case bulk material will be needed to provide sufficient batches of working standard for the 10–15-year period of use of the first international reference material.

After the contents of the master ampoules have been frozen or dried to constant weight and the ampoules sealed, the first international reference material and the first batch of working standard ampoules may be prepared. It may be necessary to carry out preliminary tests before distribution into ampoules in order to determine the appropriate dilution procedure for the contents of a master ampoule so that each ampoule of the international reference material and working standard has a convenient amount of activity. Using this predetermined procedure, a sufficient quantity of the master batch should be prepared by dissolving or suspending the contents of one or more master ampoules. Thereafter, all applicable steps described in sections A.3 and A.4 should be followed.

The number of substances that would justify the provision of WHO working standards from master ampoules would be strictly limited, but where demonstrably necessary their provision would be a great service to the world's scientific community. International associations contemplating the use of this system should contact WHO [1] so that plans can be made for the correct storage of all the master ampoules as well as for the reconstitution of two master ampoules and their processing into two batches of standard material. The batch of ampoules derived from one master ampoule would be designated as the proposed definitive international reference material while those from the other master

[1] Chief, Biologicals, World Health Organization, 1211 Geneva 27, Switzerland.

ampoule would be regarded as the proposed working standard. Both batches of ampoules would be included in the international collaborative study used for establishing the international reference material in order to demonstrate that the batches of ampoules resulting from the two master ampoules are identical and indistinguishable in the assay systems concerned. Subsequent batches of reference material prepared from further master ampoules by identical techniques would require simple calibration in terms of the definitive international reference material.

PART B:

GUIDELINES FOR THE PREPARATION, CHARACTERIZATION, AND CALIBRATION OF NATIONAL OR LABORATORY WORKING STANDARDS, REFERENCE PREPARATIONS, AND REFERENCE REAGENTS FOR BIOLOGICAL SUBSTANCES

B.1. INTRODUCTION

International biological reference materials are distributed free of charge to national control authorities for the purpose of calibrating national reference materials so that their activity may be expressed in international units. Frequently a manufacturer, involved in the assay of many batches of a biological substance for which excessive demands would be made on the supplies of the national standard, is obliged to establish a laboratory working reference material. The activity of such preparations should also be calibrated in international units by comparison with the national reference material or, where this does not exist, a direct comparison may be made with the international reference material.

The definitions of national standards, reference preparations, and reference reagents for biological substances as well as those of the laboratory reference materials are similar to those of the international counterparts (see section A.1).

Where an international reference material does not exist, a national control authority may need to establish independently a national reference material and, if appropriate, define a unit of activity.

B.2. ASSESSMENT OF NEED AND PROCUREMENT OF MATERIAL

A national standard may be needed for the following assessments.

(1) Any product or device used in medical practice that requires biological assessment in its quality control and is manufactured in or imported into the country concerned.

(2) A new product or device that requires biological assessment in its quality control and that is being considered for licensing in the country. A national standard for such material should be established as soon as possible.

(3) Clinical laboratory tests that are dependent on biological reagents with standardized biological activity.

(4) The comparison of research data related to material requiring biological assay in the fields of therapy and clinical diagnosis.

The same considerations that apply when choosing an international reference material also apply to a national standard (see section A.2.2). Material will usually be obtained from a manufacturer by special arrangement. Material packaged for therapeutic use is often not satisfactory since it may contain diluents, buffers, or preservatives. The purpose for which the material is required should be explained to the manufacturer or research scientist responsible for producing the material. Homogeneity is the most important criterion. Good long-term stability is desirable but less important than for an international reference material since the expected life of a national standard may be shorter if a corresponding international standard exists. Its composition should be typical of that of the material to be assayed against it.

In some instances bulk material will be obtained for distribution into containers by the national control laboratory; in other cases material may be obtained already distributed into vials by the manufacturer. These may be glass-sealed or rubber-capped, but in either case the details should be agreed with the manufacturer. When only a single manufacturer is concerned separate samples of the batch used by him for his house standard may be used.

The quantity required for a national standard may vary from an amount sufficient for less than 50 assays to one adequate for several thousand. In some instances only the national control laboratory, which holds the national reference preparations, will require a supply of the standard, and then only for perhaps one or two assays a year. On the other hand, a reference preparation may be used in clinical laboratories throughout the country on a routine basis.

If repeated calibration assays have to be carried out the amounts required will be greater, but this will depend on the number of laboratories involved (which could vary from one to perhaps 20) and the frequency of checking against an international standard. Supplies should be sufficient to allow limited stability studies. The amounts in each container and the form of presentation should allow ready use without the need for the specialized equipment mentioned in section A.9.

With regard to the storage of bulk material prior to distribution into separate containers, the same precautions as those applied to proposed international materials should be observed (see section A.2).

B.3. DISTRIBUTION INTO FINAL CONTAINERS

In preparing national and laboratory reference materials, the considerations set out in section A.3 should be taken into account, but the procedures may sometimes be modified and may be simplified in some respects. It may be possible to arrange for a reference material to be distributed into final containers and processed into final form by a manufacturer.

Homogeneity is essential, and all the precautions directed to this end in section A.3 should be taken. Long-term stability is necessary and should be investigated in the same way as for international standards. After the initial thorough testing in comparison with the international standard, the preparations should be compared regularly with the international standard. How often this needs to be done depends on the results of the stability already established. If there is any indication of a loss of activity then steps should be taken to replace the material. The same considerations apply to laboratory standards in comparison with the national standards.

Subject to the considerations above, stoppered vials may be acceptable for certain substances.

If the reference material is sufficiently stable over its intended life, storage in the liquid state may be considered.

B.4. PROCESSING OF FILLED CONTAINERS

The majority of the recommendations in section A.4 are pertinent to national or laboratory reference materials.

B.5. THE CALIBRATION OF NATIONAL REFERENCE MATERIALS

The calibration of national reference materials should be organized by the national control laboratory or other national body or by a laboratory authorized by the national authority.

The calibration assay of a national standard in terms of international units defined by an existing international standard or reference preparation generally comprises a straightforward comparison of the two materials. The assay might be expanded to include (coded) duplicates of the national standard and manufacturers' or other local working standards. The stability tests and the special tests listed in section A.6.1 could also be carried out in more than one laboratory.

The organization of such national calibration assays should be undertaken by a scientist and a biometrician, and the assay should be planned along the same lines as the calibration assay described in section A.7. In selecting participating laboratories, the manufacturers should be encouraged to take part, as well as any national academic and research laboratories that have appropriate expertise and resources. In some instances as few as 2–4 participants may be sufficient, although more may be needed to obtain the precision of the final estimate of potency considered necessary by the national authority.

The selection, handling, and transport of materials included in the study should be the same as for international reference materials (see sections A.6.4 and A.6.5). The design of the study would be generally that of a calibration assay, and the raw data could be reported on assay result sheets.

The analysis of the data should be as outlined in section A.7.3. The report of the assay need not be as full as the report outlined in section A.6.6, but the same principles should be followed in reporting the results of tests and the assay results, their statistical treatment, and the derivation of a combined estimate of potency. On the basis of this estimate a potency is assigned to the standard in international units where a relevant unit exists.

Copies of such records should be retained by the national control authority and a summary made available to users of the national standard.

B.6. USE OF NATIONAL REFERENCE MATERIALS

National reference materials are used either directly as working standards or for the calibration of working standards. They are often required by national legislation to be used in conjunction with national pharmacopoeial specifications or other similar forms of quality control. They are usually supplied by a national agency. In certain circumstances, where special problems exist in setting up national working standards (see section A.1), international working standards may be available. In some cases a number of different countries may collaborate in preparing a number of secondary standards calibrated against the international standards. An example is the provision by the Commission of the European Pharmacopoeia of such calibrated standards, which are the official national standards for those countries recognizing the specifications of the European Pharmacopoeia.

Appendix 1

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Appendix 2

ACKNOWLEDGEMENTS

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Appendix 3

NOTES ON THE STORAGE OF PROTEIN SOLUTIONS AT LOW TEMPERATURES [1, 2]

When a dilute solution of sodium chloride is cooled slowly, ice crystals consisting of pure water will form. As these crystals grow, they exclude sodium chloride molecules until the latter reach a critical concentration at which the whole mixture freezes solid. This concentration is called the eutectic concentration, and the temperature at which the solution freezes is characteristic for different substances. Even minor differences in the composition of solutions (especially of small molecules in, say, different buffers), significantly influence this temperature. When serum is cooled slowly below 0°C, crystals consisting initially of pure water form and grow. These crystals tend to exclude the other molecules (such as electrolytes, sugars, and proteins) which tend to become concentrated in pockets of solution between the ice crystals. Depending on the rate of cooling and the temperature reached, the greatly increased salt concentration and associated pH changes may damage proteins and result in loss of their biological activity. Certain proteins are more susceptible to this damage than others, while certain antibodies, clotting factors, and enzymes are notoriously liable to such denaturation. Although the freezing point of a eutectic solution of chloride (the main electrolyte present in serum) is around −22°C, serum does not appear to be " completely frozen " until around −50°C to −60°C,[3] owing to the other small molecular constituents present. Ideally the temperature at which any given solution is completely frozen should be determined, but the apparatus for doing so is not generally available. This can be achieved by differential thermal analysis [4] (which is suitable for simple solutions of electrolytes, but the endpoint is masked in complex solutions such as serum) or by measurement of change of electrical resistance. These techniques show that a solution sometimes does not begin to freeze until well below its normal " freezing temperature ", a phenomenon known as supercooling. It is thus generally desirable to cool a solution well below its " freezing point " to ensure that it is completely frozen.

Certain general recommendations for storing serum and other protein solutions can thus be made.

(1) For maximum safety of serum samples that are precious, known to be unstable, or for which testing of biological activity is costly or imprecise the following points should be borne in mind.

(*a*) The sample should be cooled rapidly (i) in or just above liquid nitrogen, (ii) by surrounding with solid CO_2, or (iii) by immersion in a CO_2-solvent mixture. The rate of cooling in a liquid of good thermal conductivity at a temperature at which it remains liquid has been shown to be considerably more rapid than in a coolant such as liquid nitrogen that is used at its boiling point.

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[1] These notes are based on instructions issued by the National Institute for Biological Standards and Control, London.

[2] Useful information concerning the denaturation of lipid–protein complexes by freezing can be found in : LOVELOCK, J. E. *Proceedings of the Royal Society. Series B.*, **147** : 427 (1957).

[3] GREAVES, R. I. N. *Cryobiology*, **5** : 76 (1968).

[4] REY, L. R. *Annals of the New York Academy of Sciences*, **85** : 510 (1960).

(*b*) The sample should be stored preferably in or over liquid nitrogen. Otherwise at −70°C or below.

(*c*) When required for use the sample should be thawed rapidly at temperatures not exceeding 40°C with gentle agitation.

(*d*) As containers for plasma, plastic tubes that inhibit contact activation are preferable, but the plastic selected should be one that does not fracture at very low temperatures.[1]

(*e*) Most thin plastic containers readily allow diffusion of gas or even water vapour. Where the gas (e.g., CO_2) may alter the contents (e.g., change the pH) the container should, for long-term storage, be kept in a thick glass vessel that is well sealed (e.g., screw-cap).

(2) For routine laboratory use of protein solutions, the following rules should be observed.

(*a*) The solution should be cooled rapidly as described above, and this is especially important for large volumes.

(*b*) The solution should be stored at −30°C or below.

(*c*) If it is impossible to store the solution at −30°C, storage at −10°C *may* be less harmful than storage at −20°C.

(*d*) If the material is sterile or has had a bacteriostat added, storage at +4°C *may* be less harmful than storage at −10°C.

(*e*) Protein solutions containing cresols or bacteriostatic agents of a similar nature should not be frozen. Although smallpox vaccine, in which phenol has been used to kill bacteria, has been successfully freeze-dried, maintaining the viability of the virus, it is a general rule that phenol should not be used in biologicals that are to be lyophilized.

[1] Many laboratories now use polypropylene tubes, with polyethylene screw caps and silicone rubber washers ; nominal capacity 1.5 ml.

Appendix 4

INSTRUCTIONS FOR OPENING AN AMPOULE

1. Tap the ampoule gently on the table top to collect the material at the bottom end.

2. Score the ampoule by sawing evenly and firmly with a *sharp ampoule file* about 3–4 cm from the top (empty) end.

3. Heat a solid glass rod to red heat and apply firmly to one end of the file mark.

4. If a crack does not result, deepen and extend the file mark, reheat the glass rod and reapply.

5. When a crack appears, hold the ampoule almost horizontal and gently remove the top (empty) end ; taking care that :

(*a*) no particles of glass fall into the material, and

(*b*) no material falls out.

6. An ampoule file of good quality should be used.

Annex 5

REQUIREMENTS FOR BIOLOGICAL SUBSTANCES AND OTHER SETS OF RECOMMENDATIONS

The specification of requirements to be fulfilled by preparations of biological substances is necessary in order to ensure that these products are safe, reliable, and potent prophylactic or therapeutic agents. International recommendations on requirements are intended to facilitate the exchange of biological substances between different countries and to provide guidance to workers responsible for the production of these substances as well as to others who may have to decide upon appropriate methods of assay and control.

Recommended requirements and sets of recommendations concerned with biological substances formulated by international groups of experts and published in the WHO Technical Report Series are listed hereunder :

No.	Year	
178	1959	Requirements for Biological Substances :
		1. General Requirements for Manufacturing Establishments and Control Laboratories [1]
		2. Requirements for Poliomyelitis Vaccine (Inactivated) [1]
179	1959	Requirements for Biological Substances :
		3. Requirements for Yellow Fever Vaccine [1]
		4. Requirements for Cholera Vaccine [1]
180	1959	Requirements for Biological Substances :
		5. Requirements for Smallpox Vaccine [1]
200	1960	Requirements for Biological Substances :
		6. General Requirements for the Sterility of Biological Substances [1]
237	1962	Requirements for Biological Substances :
		7. Requirements for Poliomyelitis Vaccine (Oral) [1]
274	1964	WHO Expert Committee on Biological Standardization :
		8. Requirements for Pertussis Vaccine
		9. Requirements for Procaine Benzylpenicillin in Oil with Aluminium Monostearate [1]

[1] Refer to subsequent revised requirements.

No.	Year	
293	1964	WHO Expert Committee on Biological Standardization : **10. Requirements for Diphteria Toxoid and Tetanus Toxoid**
323	1966	WHO Expert Group : Requirements for Biological Substances (Revised 1965) 1. General Requirements for Manufacturing Establishments and Control Laboratories 2. Requirements for Poliomyelitis Vaccine (Inactivated) 7. Requirements for Poliomyelitis Vaccine (Oral) [1] 5. Requirements for Smallpox Vaccine
329	1966	WHO Expert Committee on Biological Standardization : 11. Requirements for Dried BCG Vaccine 12. Requirements for Measles Vaccine (Live) and Measles Vaccine (Inactivated)
361	1967	WHO Expert Committee on Biological Standardization : 13. Requirements for Anthrax Spore Vaccine (Live—for Veterinary Use) 14. Requirements for Human Immunoglobulin 15. Requirements for Typhoid Vaccine 9. Requirements of Procaine Benzylpenicillin in Oil with Aluminium Monostearate (Revisions adopted 1966)
384	1968	WHO Expert Committee on Biological Standardization : 16. Requirements for Tuberculins 17. Requirements for Inactivated Influenza Vaccine [2]
413	1969	WHO Expert Committee on Biological Standardization : 4. Requirements for Cholera Vaccine (Revised 1968) [2] 18. Requirements for Immune Sera of Animal Origin
444	1970	WHO Expert Committee on Biological Standardization : 19. Requirements for Rinderpest Cell Culture Vaccine (Live) and Rinderpest Vaccine (Live) 20. Requirements for *Brucella abortus* Strain 19 Vaccine (Live—for Veterinary Use) [2]
444	1970	WHO Expert Committee on Biological Standardization : Development of a National Control Laboratory for Biological Substances (a guide to the provision of technical facilities)
463	1971	WHO Expert Committee on Biological Standardization : 21. Requirements for Snake Antivenins

[1] Refer to subsequent revised requirements.
[2] Refer also to subsequent addendum.

No.	Year	
486	1972	WHO Expert Committee on Biological Standardization : 7. Requirements for Poliomyelitis Vaccine (Oral) (Revised 1971)
530	1973	WHO Expert Committee on Biological Standardization : 4. Requirements for Cholera Vaccine (Revised 1968) (Addendum 1973) 6. General Requirements for the Sterility of Biological Substances (Revised 1973) 17. Requirements for Inactivated Influenza Vaccine (Addendum 1973) 22. Requirements for Rabies Vaccine for Human Use
565	1975	WHO Expert Committee on Biological Standardization : Recommendations for the Assessment of Binding Assay Systems (including Immunoassay and Receptor Assay Systems) for Human Hormones and their Binding Proteins (a guide to the formulation of requirements for reagents and assay kits for the above assays and notes on cytochemical bioassay systems) Development of national assay services for hormones and other substances in community health care
594	1976	WHO Expert Committee on Biological Standardization : 3. Requirements for Yellow Fever Vaccine (Revised 1975) 23. Requirements for Meningococcal Polysaccharide Vaccine [1] 20. Specifications of tests used in the Requirements for *Brucella abortus* Strain 19 Vaccine (Live—for Veterinary Use) (Addendum 1975)
610	1977	WHO Expert Committee on Biological Standardization : 23. Requirements for Meningococcal Polysaccharide Vaccine (Addendum 1976) [1] 24. Requirements for Rubella Vaccine (Live) 25. Requirements for *Brucella melitensis* Strain Rev. 1 Vaccine (Live—for Veterinary Use) 26. Requirements for Antibiotic Susceptibility Tests. I. Agar Diffusion Tests using Antibiotic Susceptibility Discs
626	1978	WHO Expert Committee on Biological Standardization : 27. Requirements for the Collection, Processing and Quality Control of Human Blood and Blood Products 23. Requirements for Meningococcal Polysaccharide Vaccine (Addendum 1977, incorporating Addendum 1976) 17. Requirements for Inactivated Influenza Vaccine (Addendum 1977) Guidelines for the Preparation and Establishment of Reference Materials for Biological Substances

[1] Refer also to subsequent addendum.

Annex 6

BIOLOGICAL SUBSTANCES: INTERNATIONAL STANDARDS, REFERENCE PREPARATIONS, AND REFERENCE REAGENTS

A list of international biological standards, international biological reference preparations, and international biological reference reagents previously included as annexes to the reports of the WHO Expert Committee on Biological Standardization is issued as a separate publication,[1] revised from time to time. The most recent list was published in 1977 and copies may be obtained direct (or through booksellers) from the agents shown on the back cover of this report or they may be ordered from: World Health Organization, Distribution and Sales Service, 1211 Geneva 27, Switzerland.

* * *

The Expert Committee, at its twenty-eighth meeting, made the following changes to the lists already published.

Blood Products and Related Substances

Established

Ancrod	International Reference Preparation 1976
Anti-c (anti-rh′) incomplete blood typing serum, human	International Standard 1976
Anti-D (anti-Rh_0) immunoglobulin, human	International Reference Preparation 1976
Blood coagulation factor VIII, human	Second International Standard 1976
Blood coagulation factor IX, human	International Standard 1976
Plasmin	International Reference Preparation 1976
Thromboplastin, human, combined	International Reference Preparation 1976

The above substances are held and distributed by the International Laboratory for Biological Standards, National Institute for Biological Standards and Control, London NW3 6RB, England.

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[1] *Biological substances: international standards, reference preparations, and reference reagents*, 1977. Geneva, World Health Organization, 1977.

Reagents

Established

Adenovirus antisera, equine, types 4, 19, 20, 22, 23 and 24	International Reference Reagents 1976

These antisera are held and distributed by the Center for Disease Control, Atlanta, GA, 30333, USA. Antisera prepared from the same batch of material as these reference reagents are available in the WHO Virus Reference Centres and are available also on application to the Chief Medical Officer, Virus Diseases, World Health Organization, 1211 Geneva 27, Switzerland.

* * *

The Expert Committee, at its twenty-ninth meeting, made the following changes to the list.

Antibodies

Discontinued

Antipneumococcus serum (type 1), equine	First International Standard 1934
Antipneumococcus serum (type 2), equine	First International Standard 1934

Antigens

Discontinued

Influenza virus haemagglutinin (type A)	First International Reference Preparation 1967

Blood Products and Related Substances

Established

Anti-hepatitis B immunoglobulin [1]	First International Reference Preparation 1977
Fluorescein-isothiocyanate-conjugated sheep anti-human IgM (anti-μ chain) [2]	First International Standard 1977

[1] Held by Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Plesmanlaan 125, Amsterdam, Netherlands.

[2] Held by Immunology, World Health Organization, 1211 Geneva 27, Switzerland.

Six human serum proteins : albumin, alpha-1-antitrypsin, alpha-2-macroglobulin, ceruloplasmin, complement C3 and transferrin, for immunoassay [1]	First International Reference Preparation 1977

Hormones

Established

Human placental lactogen for immunoassay	First International Reference Preparation 1977
Lysine vasopressin	First International Standard 1977

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[1] Held by Immunology, World Health Organization, 1211 Geneva 27, Switzerland.

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610	(1977) **WHO Expert Committee on Biological Standardization** Twenty-eighth report (133 pages)	11.—
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612	(1977) **Pesticide residues in food** Report of the 1976 Joint Meeting of the FAO Panel of Experts on Pesticide Residues and the Environment and the WHO Expert Group on Pesticide Residues (35 pages)	6.—
613	(1977) **Child mental health and psychosocial development** Report of a WHO Expert Committee (71 pages)	7.—
614	(1977) **WHO Expert Committee on Specifications for Pharmaceutical Preparations** Twenty-sixth report (53 pages)	7.—
615	(1977) **The selection of essential drugs** Report of a WHO Expert Committee (36 pages).	5.—
616	(1978) ***Neisseria gonorrhoeae* and gonococcal infections** Report of a WHO Scientific Group (142 pages)	12.—
617	(1978) **Evaluation of certain food additives** Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives (41 pages)	5.—
618	(1978) **WHO Expert Committee on Drug Dependence** Twenty-first report (49 pages)	6.—
619	(1978) **Steroid contraception and the risk of neoplasia** Report of a WHO Scientific Group (54 pages)	6.—
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624	(1978) **Surveillance for the prevention and control of health hazards due to antibiotic-resistant enterobacteria** Report of a WHO meeting (54 pages)	6.—
625	(1978) **Financing of health services** Report of a WHO Study Group (117 pages)	11.—
626	(1978) **WHO Expert Committee on Biological Standardization** Twenty-ninth report (147 pages)	14.—
627	(1978) **Research in human reproduction : Strengthening of resources in developing countries** Report of a WHO Study Group (16 pages)	4.—
628	(1978) **Arterial hypertension** Report of a WHO Expert Committee (58 pages)	6.—