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The Standardization of Factor VIII

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Introduction

The principles of biological standardization are applicable whether the drug requiring standardization is insulin, heparin or blood coagulation Factor VIII. Indeed, these principles apply to all biological drugs, which can be defined as those drugs that cannot be adequately characterized solely by chemical or physical means. To measure the potency of biological drugs a suitable standard is needed, and the World Health Organization (WHO) is responsible for establishing international standards in this field. WHO is advised by its Expert Committee on Biological Standardization which meets regularly in Geneva. The National Institute for Biological Standards and Control (NIBSC) in London has the responsibility, on behalf of WHO, for distributing world-wide the International Standards for Factor VIII.

General Principles

Certain general principles of biological standardization have been established over the years (Table 1). The first principle is that a standard must be established, its potency agreed, and a mechanism for distribution organized. Although biological standards have existed for at least 50 years, it was not until 1970 that WHO established the first International Standard for Factor VIII concentrate¹⁾. Secondly, it is an important concept in the field of biological standardization that “like must be compared with like”. In other words, apples cannot be compared with oranges! The third principle is that the relevant biological effect must be measured—obviously, a standard is not useful if it is measuring an irrelevant parameter. The fourth principle, that the potency ratio is independent of the assay system, is particularly relevant to Factor VIII standardization and raises the issue of whether the results obtained by a one-stage as compared to a two-stage Factor VIII assay is giving a truer picture of Factor VIII coagulant activity. The fifth principle is the need to employ statistically valid assays. This means using parallel line bioassays, comparing the test preparation with the standard at a minimum of three and preferably four dilutions, as outlined by Finney²⁾. Assays should also be tested for linearity and parallelism, to determine whether the bioassay is statistically valid. A potency estimate is always a mean figure, with 95% confidence limits. In other words, a potency of 500 units of Factor VIII concentrate with confidence limits of, say, 450 to 550 units, means that there are 19 chances out of 20 that the true potency of the drug is at least 450 units and not more than 550 units.

Table 1 Principles of Biological Standardization

1. A standard is required.
2. Like must be assayed against like.
3. The relevant biological activity must be measured.
4. The potency ratio is independent of the assay system.
5. Bioassays must be statistically valid.

Requirements of a Standard

The requirements of a standard are shown in Table 2. The appropriate biological activity is measured with a similar type of preparation, and a standard cannot be markedly different from the drug being measured (apples must be compared apples and not with oranges). A standard must be stable, and a 10-year life-

Table 2 Requirements of a Standard

1. The appropriate biological activity.
2. A similar type of preparation.
3. The standard must be stable.
4. A unit must be defined.

span is usual for most of our standards. Standards are usually freeze-dried and are kept permanently stored at -20°C . Finally, a unit of activity must be defined. In practice, this means that an international collaborative study is carried out by 20 to 30 leading laboratories located around the world, and the participants are asked to examine a proposed standard. Usually, the replacement standard will be compared with the existing standard, if there is one, as well as with other candidate preparations. The data are then collated and the results analysed, only statistically valid assays being accepted. A unit of activity is defined and, with the agreement of the participants in the collaborative study, the World Health Organization is asked to establish the standard or reference preparation with an assigned unitage. It is important to recognize that the assigned unit for a standard is a compromise figure and not every laboratory may agree with this unitage. However, it is essential that every user of the standard, be he physician, scientist or manufacturer, accepts that the defined unitage is the "true" value.

Standardization of Factor VIII

How do the general consideration discussed above bear on the problems of Factor VIII concentrates? In 1974, Bangham and Brozović³⁾ pointed out that an internationally recognized standard and unit for Factor VIII activity became desirable when it was understood how variable and unstable Factor VIII was in plasma. A standard became possible when stable, freeze-dried, preparations were available. A standard became essential when therapeutic concentrates were developed for sale. For example, Factor VIII concentrates manufactured in the United States and elsewhere are now sold around the world, usually on the basis of so many units of Factor VIII per unit of currency. There is therefore a strong financial incentive in ensuring the accuracy of Factor VIII measurements. There are various levels at which the standardization of Factor VIII can be approached, and the term the "hierarchy of quantitation" was used by Kirkwood and Barrowcliffe⁴⁾. The primary standard is the International Standard for Factor VIII, concentrate, first established in 1970. This international standard (currently the Third International Standard, established in 1982) is distributed world-wide on behalf of WHO by the National Institute for Biological Standards and Control, primarily to manufacturers of Factor VIII concentrate. The next level in the hierarchy of standardization is the national standard. For example, in the United Kingdom, there is the British Working Standard for Factor VIII concentrate, that is distributed to the National Health Service manufacturers of Factor VIII. There are only two such manufacturers and we are therefore able to provide them with sufficient working standard so that there is no need for them to have their own local standard. These centres use exactly the same standard that we do, as the regulatory authority, and as one might expect we usually have very good agreement between their potency estimates for Factor VIII concentrates and our own estimates. We cannot, however, provide sufficient material for all manufacturers throughout the world to use the International Standard itself as a working standard, and therefore a national standard has to be calibrated against the International Standard. From these national standards, obtained from the regulatory authority of the country concerned, local manufacturers can then establish their own house standards. This concept of working standards, calibrated against the relevant International Standard, has been shown to be a practical and effective scheme for several biological standards. A need also exists for a standard for Factor VIII measurements in plasma and cryoprecipitate. The most widely used standard for Factor VIII that we distribute in the United Kingdom is a British Standard for Factor VIII in plasma, that is also calibrated for Factor VIII:RAG, Factor IX and antithrombin III⁵⁾. The British system for Factor VIII Standardization has been in existence for 10 years or so, and works reasonably well in a small country, such as the United Kingdom. Clearly, it is more cost-effective to have a single centre with the responsibility for distributing standards on a national basis. Difficulties have existed in

the past—and to some extent still exist—in attempts to improve the standardization of Factor VIII. A major problem has been the existence of two types of assay, namely the partial thromboplastin time (the so-called “one-stage” assay) and the thromboplastin generation time (the so-called “two-stage” assay). Each assay method has its devotees, and there is currently no consensus about which is the better assay. While two-stage assays have tended to give more precise results, with less scatter, most laboratories world-wide now use the one-stage assay. There is some evidence that the one-stage assay gives a better prediction of *in vivo* recovery⁶⁾⁷⁾, and it is certainly true that most clinicians rely on one-stage assays, as these lend themselves better to automation in the laboratory. In any event, there is an urgent need for definitive studies correlating Factor VIII assays *in vitro* and the clinical response of patients to therapeutic infusions. Another problem that has been apparent since the first Factor VIII standard was established is that if a concentrate is compared with a concentrate, there is usually good agreement between the potency estimates obtained with either one-stage or two-stage assays. However, if a plasma is compared with a concentrate, there is on average a 20 per cent discrepancy between the results obtained with one-stage and two-stage assays⁴⁾⁸⁾. This problem is now well recognized, and its importance should diminish with the recent establishment of the International Standard for Factor VIII-related activities in plasma. This standard (discussed below) is a more appropriate standard for the calibration of plasma pools in haemophilia centres and hospitals generally. Manufacturers of Factor VIII concentrates have been very cooperative in attempting to place Factor VIII standardization on a scientific basis. It is also worth remembering how few doctors usually check the potency of the drugs they give their patients. How often do physicians check the number of units in a bottle before administering insulin to a diabetic? Yet this is often done by clinicians before they give Factor VIII to their patients. In fact, few therapeutic products are checked as carefully as Factor VIII concentrates. Not only does the manufacturer have to satisfy his own quality control people, the product has then to be cleared by the regulatory authorities, and finally the manufacturer has to satisfy the clinician treating the haemophiliac. He might well be forgiven for sometimes wishing that he had chosen to manufacture a simple drug like aspirin!

Recent Collaborative Studies

Two important collaborative studies were carried out in 1982, on behalf of the World Health Organization, that allowed the establishment of the Third International Standard for Factor VIII, concentrate, and the First International Standard for Factor VIII-related activities in plasma⁹⁾. These studies could not have been carried out without the help and support of numerous colleagues in many countries throughout the world, including Japan.

(a) Factor VIII concentrate

The material that was ampouled for the replacement Factor VIII concentrate standard was of so-called “intermediate purity”, and was very similar in characteristics to the existing standard. As part of the study, the participants were also asked to examine a freeze-dried normal plasma that was being evaluated in a separate study, as a reference preparation for Factor VIII-related activities in plasma (Factor VIII:C; Factor VIIIIR:Ag; Factor VIIIIR:RiCoF and Factor VIIIC:Ag). Participants were asked to send all their raw data back to London, where they were evaluated and analysed. The overall results of the study are given in Table 3, where the potency estimates are given for the results by one-stage and two-stage Factor VIII assays, respectively. When the proposed new standard was compared to the existing standard, there was excellent agreement between the one-stage and two-stage Factor VIII assays¹⁰⁾. Most laboratories performed one-stage assays, and the overall mean turned out to be the same as the result obtained solely by one-stage assays. However, if the concentrate was compared against the plasma reference standard, there was a marked one-stage/two-stage disparity. These results represent a good example of the hazard of comparing unlike materials, namely using a plasma standard to calibrate a concentrate, particularly when two-stage assays are employed. With one-stage assays, comparable results were obtained in this study whether a concentrate or a plasma standard was used. The collaborative study gave valuable information overall concerning the inter-assay precision within laboratories. The geometric coefficients of variation of the potency estimates provide an indication of the relative precision of the assay systems of the laboratories taking part in the study. There

Table 3 Potency Estimates by 1-Stage and 2-Stage Methods of the 3rd. International Standard Against the 2nd. International Standard for Factor VIII Concentrate, and Against the Standard for Factor VIII-Related Activities in Plasma (IU per ampoule)

	1-Stage	2-Stage	Overall
3rd. I.S. against 2nd. I.S.	3.89	3.91	3.89
3rd. I.S. against plasma standard	3.90	4.53	4.03

Table 4 Mean Geometric Coefficient of Variation (%) of Potency Estimates for Factor VIII Concentrates (26 Laboratories)

One-stage laboratories	Two-stage laboratories
9.9	8.9

was obviously little to choose between the precision of the one-stage and two-stage assays in this study (Table 4). A vital requirement of any standard is that it should be stable. Fortunately, this is not a significant problem with modern Factor VIII concentrates. The standard is in fact very stable indeed, and a negligible amount of activity is lost when the material is stored at +4°C. The predicted loss over a 5-year period at this temperature would still be less than 1% of the total activity. In fact, all our international standards are kept at -20°C. On the basis of the results obtained in this collaborative study, and with the agreement of the participants, the World Health Organization in 1982 assigned a value of 3.9 International Units per ampoule to the replacement standard for Factor VIII concentrate.

(b) Factor VIII-related activities in plasma

The measurement of Factor VIII in therapeutic concentrates is of course only one of the situations where we need to measure this clotting protein. Factor VIII frequently needs to be measured in plasma and cryoprecipitate, and there is therefore a need for an appropriate standard. Traditionally, Factor VIII has been measured by comparing the values found in a patient's plasma with that obtained from a fresh pool of plasma obtained from several normal donors. Indeed, this method is still used for measuring clotting factors where no standard currently exists. However, this approach has significant drawbacks, such as the need to define how "fresh" is "fresh blood" and how many donors constitute an adequate size pool—is it 10, 20 or 40? There is no general agreement on these points. Even more important, Factor VIII levels vary widely between populations, so that a so-called "normal" level in one area may be quite different from the "normal" level in another area. For example, we sent a freeze-dried plasma sample to 21 different laboratories in several parts of the world, and each laboratory was asked to measure the Factor VIII in the ampoule. The potency estimates that we received back varied from 0.56 to 0.92 units, with an overall geometric mean of 0.73 units per ampoule. Looking at the same question in a different way, and assuming that the value of Factor VIII in the ampoule is 0.73 units (the overall mean figure from all the results), what value did the same 21 laboratories now find for their local plasma standard? The values obtained ranged from a low of 0.63 to a high of 1.35 units per ml.—more than two-fold. It is also of interest that the number of donors that was considered adequate for providing a normal donor pool varied from 8 to 40 donors. This study demonstrated convincingly the hazards of using a local "normal plasma pool" for the measurement of Factor VIII in an unknown sample of blood. If more laboratories had been included in the study, and particularly if laboratories without special expertise had been included, there can be little doubt that the observed two-fold variation in Factor VIII levels would have been even higher. To overcome the limitations of the local plasma pool as a standard for Factor VIII, the freeze-dried plasma standard that we studied has now been adopted by WHO as an International Standard for Factor VIII:C in plasma, with an assigned unitage of 0.73 International Units per ampoule. In the same study, the participants had also been asked to measure other Factor VIII-related activities, in addition to Factor VIII:C. The results obtained for these values were, respectively, 0.87 IU for Factor VIIR:Ag, 0.80

Table 5 1st. International Standard for
Factor VIII-Related Activities in Plasma
(I.U. per ampoule)

Factor VIII:C	—	0.73
Factor VIII:Ag	—	0.87
Factor VIII:RiCoF	—	0.80
Factor VIII:Ag	—	0.95

Table 6 Predicted Potency Loss from Accelerated Degradation Data (%/Year)

Temp. °C	Factor VIII-Related Activities			
	C	R:Ag	R:RiCoF	C:Ag
+4°	0.64	0.06	0.46	0.53
-20°	<0.01	<0.01	<0.01	<0.01

IU for Factor VIII:RiCoF, and 0.95 IU for Factor VIII:Ag (Table 5). As with all standards, the long-term stability of the material must be established. Accelerated degradation testing demonstrated that all four Factor VIII-related activities are reasonably stable (Table 6). Although this standard is not as stable as Factor VIII concentrate, the material has acceptable stability when stored at -20°C. This reference material therefore now serves as the International Standard for all Factor VIII-related activities in plasma¹¹⁾. There is potentially a very large need for a Factor VIII plasma standard. Although it is not feasible for us to distribute this standard as a working standard to all clinical laboratories, the standard is available for calibrating local and regional plasma pools. In particular, it is hoped that commercial manufacturers of normal plasma standards will use the International Standard to calibrate their products. If this can be accomplished, then at least Factor VIII measurements in various parts of the world will bear a reasonable relationship to one another.

Conclusions

Measurement is central to science, and precise quantitation is impossible without appropriate standards. This concept has been accepted since time immemorial for the measurement of such things as weight, length and volume. However, in the field of biological sciences, we cannot as yet define our measurement solely in physical and chemical terms. Until we can, there is a continuing need for biological standards, and nowhere more so than in the field of blood coagulation. Appropriate standards are now available for the measurement of Factor VII, and it is hoped that physicians caring for haemophiliacs will make full use of them.

Summary

The principles of biological standardization have been discussed, particularly as they apply to the quantitation of Factor VIII. A World Health Organization (WHO) standard for Factor VIII concentrate has existed since 1970, and virtually all manufacturers now calibrate their house standards against the WHO standard, and label their products in International Units. In 1982, as the result of an international collaborative study, a replacement standard was established for Factor VIII concentrate (the 3rd. International Standard), with an assigned potency of 3.9 IU per ampoule. By the same token that concentrates should be calibrated against concentrate standards, a stable plasma standard is the appropriate standard for measuring Factor VIII in patients' plasmas. Accordingly, in 1982, WHO also established a standard for Factor VIII-related activities in plasma.

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