Review

International reference standards in coagulation

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ABSTRACT

Measurement of coagulation factor activity using absolute physico-chemical techniques is not possible and estimation therefore relies on comparative bioassay relative to a reference standard with a known or assigned potency. However the inherent variability of locally prepared and calibrated reference standards can give rise to poor agreement between laboratories and methods. Harmonisation of measurement between laboratories at the international level relies on the availability of a common source of calibration for local reference standards and this is provided by the World Health Organization (WHO) International Standards which define the International Unit for the analyte. This article describes the principles, practices and problems of biological standardisation and the development and use of reference standards for assays of coagulation factors, with particular emphasis on WHO International Standards for both concentrates and plasma.

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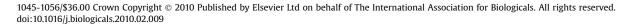
1. Introduction

The complex structure and function of coagulation factors, coupled with their low concentrations in blood plasma make their estimation by physico-chemical means virtually impossible. The estimation of coagulation factors therefore relies on the principle of comparative bioassay, relative to a reference standard containing a known amount of analyte. Reference standards prepared locally can provide consistency and continuity of testing within a single laboratory but do not address the issue of harmonisation in testing between multiple laboratories. International Standards (IS), established by the World Health Organization (WHO), fulfil this role by providing a common single route of calibration for all local and secondary working reference standards. Since the introduction of this concept in 1925, with the development of an IS for the biological activity of insulin [1], there have been WHO IS developed in many and diverse areas of biological science and medicine. The first IS for a coagulation factor, factor VIII (FVIII), was established in 1971 [2] in response to the need for harmonisation in the potency labelling of the "new" therapeutic concentrates and this approach has subsequently been applied to most plasma coagulation factors and coagulation inhibitors (Table 1). A description on the development and use of coagulation factor standards for plasma and therapeutic concentrates is provided.

2. The international unit

WHO IS for blood coagulation factors are labelled in International Units (IU) which are considered "methods-independent" being valid for all methods relating to a particular analyte, for example, a single assigned value applies for estimates of FVIII activity by both clotting and chromogenic methods. The derivation of the IU applied to coagulation factors is not arbitrary being traceable to the amount of analyte in 1 ml of fresh pooled normal plasma. A value of 1.0 IU per ml therefore relates to the average 100% normality in the population. This approach facilitates disease diagnosis by simplifying the definition of deficiency and when the same IU is used to label therapeutic concentrates it also assists in the calculation of adequate replacement therapy. In practice the assignment of a value to the 1st version of an IS relies on the assay of the candidate preparation relative to normal plasma pools collected locally by participating laboratories and the assignment of a consensus mean value for the IU. Local pools can vary considerably as found in the 1st international collaborative study on FVIII, where samples of pooled normal plasma in 20 laboratories differed by up to a factor of 2 [2]. For adequate representation of the "population mean" it is therefore important that collaborative studies include a sufficient number of local pools and constituent donors. Effective harmonisation of testing at the international level

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Table 1
International and working standards for coagulation factors and inhibitors.

Name	Code	Type of standard		Type of material		
		IS	WS	Pl	Co	Pu
Factor II	99/826	3rd		✔		
	98/590	3rd			✔	
	07/326		*6th		✔	
Factor V	03/116	1st		✔		
Factor VII	99/826	3rd		✔		
	97/592	1st			✔	
Factor VIIa	07/228	2nd				✔
Factor VIII	07/350	8th			✔	
	07/316	6th		✔	✔	
	02/122		*12th		✔	
Factor IX	99/826	3rd		✔		
	07/182	4th			✔	
	07/326		*6th		✔	
Factor IXa	97/562	1st			✔	
Factor X	99/826	3rd		✔		
	98/590	3rd			✔	
	07/326		*6th		✔	
Thrombin	01/580	2nd				✔
Fibrinogen	98/612	2nd		✔		
	98/614	1st			✔	
Antithrombin	93/768	2nd		✔		
	06/166	3rd			✔	
Factor XI	04/102	1st		✔		
Protein C	02/342	2nd		✔		
	04/252	1st			✔	
Protein S	03/228	2nd		✔		
VWF	00/514	1st			✔	
	07/316	6th		✔		
Factor XIII	02/206	1st		✔		
FEIBA (aPCC)	06/172		**1st		✔	

IS, International Standards WS, Working standards (* British Working Standard; ** NIBSC Working Standard); Pl, Plasma; Co, Concentrate; Pu, Purified.

can only be achieved if all users of the WHO IS apply the agreed assigned unitage when calibrating secondary working standards.

3. The "Like vs Like" principle

We know that variability in most comparative biological assays is reduced when the principle of "like vs like" is followed, i.e. when the standard and test samples are of a similar composition. This is based on the assumption that the test sample will mimic a dilution of the standard, if the standard and test are very similar to one another. If coagulation assays were very specific and were not affected by the matrix in which they were assayed then differences in composition would not matter. However, comparison of unlike materials, such as plasma and concentrates, tends to give higher variability and differences between methods. For example, when FVIII concentrates are assayed against plasma, or vice versa, variability between laboratories is always higher than for plasma vs plasma and concentrate vs concentrate assays [3,4]. For this reason there are both plasma, as well as concentrate, ISs for most coagulation factors. However the requirement for "like vs like" comparisons is not an absolute rule and should be examined on a case-by-case basis. For instance the measurement of FVII clotting activity in the collaborative study for the WHO 1st IS FVII Concentrate indicated similar inter-laboratory variability for

estimates of a FVII concentrate ($n = 13$) when calculated relative to the WHO IS Plasma (GCV 6.0%) and the WHO IS FVII Concentrate (GCV 7.2%). In contrast the inter-laboratory variability for estimates of an *activated* factor VII concentrate ($n = 14$) was greatly reduced when calculated relative to a "like" material, the WHO 1st IS FVIIa Concentrate, (GCV 7.0%) rather than relative to the WHO IS Plasma standard (GCV 17.0%).

4. Hierarchy of standardisation

WHO IS represent the primary definition of the IU for a given analyte. Limitations in the batch size of the WHO IS mean they cannot be used for routine testing and their primary function is in the calibration of secondary or working standards. The transference of the IU from the WHO IS to the secondary standards inevitably introduces some degree of bias or drift in the value of the unit. It is therefore important to minimise the layers of transference between the WHO IS and the actual laboratory test. Secondary working standards for product testing are available from various sources such as the Food and Drug Administration, Center for Biologics Evaluation and Research (FDA/CBER) in the United States of America and the European Pharmacopoeia (EP) in Europe. Working concentrate standards are also available for prothrombin (FII), FVIII, FIX, FX and FEIBA (Factor Eight Inhibitor Bypassing Activity) from NIBSC (Table 1). Secondary working standards for diagnostic use (calibrant plasmas) are generally available from commercial sources. The need for improved harmonisation in this latter area has led to the development of the SSC/ISTH Secondary Coagulation Standard Plasma which is available to manufacturers in larger amounts than the WHO IS.

5. Value assignment and replacement of International Standards

Establishment of ISs is the responsibility of the WHO, and the work on preparation, maintenance and distribution of these standards is carried out by the National Institute for Biological Standards and Control (NIBSC), which is a WHO Collaborating Centre for Biological Standards. WHO ISs have been established over the past 80 years, and although the procedure for establishment has advanced over this period, the basic concept has essentially remained the same. Detailed recommendations on the preparation, characterisation and establishment of IS can be obtained from WHO [5].

Value assignment for the 1st version of a WHO IS Plasma standard traditionally involves the assay of the candidate preparation relative to fresh normal plasma pools collected locally by participants in a multi-centre collaborative study. Detailed protocols are provided with instructions for the preparation of local pools and participants are encouraged to include as many donors as possible in order that the overall mean value for the IU can approximate to the normal population mean. This is important since the assigned value is determined by the overall consensus mean from all participating laboratories and cannot be defined in absolute terms. Consequently international collaborative studies frequently include over 20 different laboratories and a total number of donors exceeding 200 in the preparation of fresh pools [6–8]. Once the 1st International Plasma Standard has been assigned a value in IU it is accepted that the value of the IU for the particular analyte resides in and is defined by the lyophilised ampouled material. Subsequent versions of WHO IS Plasmas are assigned values in IU by assay against the previous WHO IS and against locally collected normal plasma pools. The former comparison provides continuity of the IU between successive standards and the latter comparison serves to