Standardization as a Private Enterprise

Standardization of analytical methods in clinical chemistry is important because it facilitates interpretation of laboratory results. Many chemical determinations are now well standardized, but this is less common with immunoassays. Standardization requires availability of a primary standard and a reference method. Although standards are available for most clinically important analytes, reference methods for immunoassays are available only for haptens (1). The study of Haese et al. (2) in this issue aims at standardization of assays for human kallikrein 2 (hK2); it highlights many of the problems associated with standardization of sensitive immunoassays and with the use of recombinant proteins as standards.

hK2 is a potentially important marker for prostate cancer, the most promising application being evaluation of tumor aggressiveness. Most prostate cancers grow slowly, with average tumor-doubling time being 2–4 years, but some tumors grow aggressively. Moreover, it is important to know whether a slightly increased prostate-specific antigen (PSA) value is caused by benign prostatic disease or by a cancer and, if so, whether this cancer needs to be cured. On the tissue level, PSA expression decreases with increasing tumor grade (and aggressiveness), whereas hK2 expression is constant or less affected with increasing tumor grade (and aggressiveness). The study of Haese et al. (2) in this issue also provides a pragmatic approach to the selection of a standard, i.e., they selected the one giving the best agreement when used to measure hK2 in serum by two different assays. This standard is not wild-type hK2, but a variant engineered to improve stability. Thus, it violates the rule that the standard should be identical to the analyte measured. However, this requirement is not always realistic. A more relevant requirement is that the standard and the analyte in the sample behave identically in the immunoassay used. The authors actually suspect that this is not the case because considerable differences between the two standardized assays were observed when they were used for analysis of serum samples. The authors attribute this to differences in recognition of putative variants of hK2 in plasma by the various antibodies used. Although this probably contributes to the variation, another likely factor is nonspecific interference by plasma components. The hK2 concentrations measured are ~1 billion-fold lower than those of serum albumin and more than 1 million-fold lower than those of complement factors and immunoglobulins, which are known to cause nonspecific interference in immunoassays (3). Thus, although the establishment of hK2 immunoassays with very high sensitivity is an admirable achievement, it is necessary to judge the results with caution.

The study also demonstrates possibilities and limitations of the use of recombinant proteins as standards. The various hK2 preparations differ slightly in structure, which may explain some differences in immunoreactivity. This shows that recombinant proteins need to be carefully characterized before being adopted as immunoassay standards. It would also be interesting to compare the immunoreactivity of recombinant and natural hK2 isolated from seminal fluid (4).

Protein standards are usually assigned values in mass units (g/L), but many currently used standards have been assigned international units (IU), which for hormones often are based on biological activity but may also be arbitrary. Some of the hK2 standards have been calibrated in mass units on the basis of amino acid composition and molecular weight, but the standard finally selected was calibrated by immunoassay using a PSA assay assumed to measure PSA and hK2 equally. The authors point out that this standard needs to be characterized by amino acid analysis and mass spectrometry (2). Because amino acid analysis reflects the molar content of protein, it would be advisable to start using substance concentrations (mol/L) for hK2 as has been done with the recently issued WHO standards for chorionic gonadotropin (7). Substance concentrations will also correctly reflect the concentrations of hK2-inhibitor complexes provided that these and free hK2 are recognized equally.

The recombinant hK2 preparation selected will be a putative international standard once it has been appropriately characterized. Standardization also requires, however, the availability of a reference method. For proteins, reference methods typically will require well-characterized antibodies. The establishment of an epitope map for PSA has facilitated development of assays that measure free and complexed PSA equally (8). The epitopes recognized by many hK2 antibodies have already been characterized (9), and it is to be hoped that the authors extend these studies by mapping all available antibodies. This would form the basis for development of a reference method for hK2.

Although more work is needed before hK2 assays can be considered finally standardized, the study of Haese et al. (2) is an important step in this direction. It is also an encouraging example of how voluntary international collaboration can lead to very useful results. It is unusual in
being initiated by a consortium of research groups rather than by an organization responsible for standardization. Because the capacity of these organizations is limited (1), the approach of Haese et al. (2) deserves to be used as a model for standardization of other new analytes.

References

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