Report

WHO Technical Workshop on Stability of reference materials for biological medicines and in vitro diagnostics

Geneva, Switzerland

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Summary

In November 2005, the World Health Organization convened an informal technical workshop on the stability of reference materials for biological medicines and in vitro diagnostics. The meeting was attended by experts from WHO collaborating centres in the area of biological standardization, national control laboratories, industries and other relevant organizations. The consultation group discussed current practices and approaches to predicting and monitoring the stability of biological reference materials. The group agreed to the need for establishing a working group (i) to continue dialogue on potential issues encompassing the principles, strategies and practicality for assuring the stability of WHO international reference standards for biological medicines and in vitro diagnostics and (ii) to develop more detailed guidance for assessment of the stability of WHO international biological reference materials.

Keywords: Stability, Reference standards, Biological products, World Health Organization

1. Introduction

The World Health Organization (WHO) establishes international reference standards for biological substances that are used in the prevention, treatment or diagnosis of human diseases or conditions. These reference standards enable the activity of biological substances to be expressed in the same way throughout the world, in international units (IU) or other units, as appropriate and so provide a consistent basis for measurements.

Thus, physicians and scientists involved in patient care, regulatory authorities and manufacturers are able to communicate in a common language for designating the activity or potency of biological preparations used in prophylaxis or therapy and for ensuring the reliability of in vitro biological diagnostic procedures used for diagnosis of diseases and treatment monitoring.

The responsibility for establishment of WHO international biological reference standards lies with the WHO Expert Committee on Biological Standardization (ECBS). The published catalogue of WHO international biological measurement standards includes over 300 materials and is updated each time materials are added or removed from the list (http://www.who.int/bloodproducts/catalogue/en/index.html).

The process whereby such preparations are established and the technical specifications to which they comply are set out in the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards (adopted 2004) 1. In a previous WHO informal consultation held in June 2003 for the most recent revision of the document 1, a need for more detailed guidance on the stability testing of WHO international biological reference standards was identified. Another consultation on laboratory support for international biological standardization
held in 2004 identified a need for continued scientific and capacity building work in the area of biological standards.

The ECBS at its 55th meeting (15 to 18 November 2004) recommended that consideration should be taken for starting or continuing work specifically on predicting and monitoring the stability of biologicals; developing specific training modules for biological standardization, with the collaboration of the WHO global training network; and developing a manual to describe in detail calibration procedures for secondary standards.

In support of these recommendations, WHO convened an informal technical workshop on the stability of reference materials for biological medicines and in vitro diagnostics in November 2005. The meeting was opened by Dr D Wood, Coordinator of the Quality, Safety and Standards (QSS) Team of Immunization, Vaccines and Biologicals (IVB) Department of WHO. He highlighted the importance of international biological reference materials in control testing of vaccines and biologicals, laboratory diagnosis and biological standardization and elaborated on the procedure of the establishment of WHO international standards (IS).

Dr T Barrowcliffe was appointed Chairperson and Dr A Tahlan, Rapporteur.

2. WHO reference standards

Dr P Phillips, NIBSC, UK gave an overview of WHO international biological reference standards. Stability is an essential property of reference materials. Factors which may influence stability were noted. These include the effects of light, temperature, chemistry, moisture and oxygen. Control of these factors may help to increase stability. Stability needs to be considered for the reference standard: (i) while it remains in the storage of the custodian organization; (ii) during shipment; and (iii) within the user’s organization including post-reconstitution.

Many WHO international biological reference preparations are lyophilized and are themselves the ‘highest order’ standard, serving as the definition of the unit. Thus there is no reference method, or higher order standard relative to which stability of these materials can be determined.

Mr A Heath, NIBSC, UK described the Arrhenius model which is frequently used as the basis for predicting the rate of loss of potency of biological reference materials. The assumptions underlying this model, and some of the statistical issues which arise were also discussed. This model can be used to provide predictions of the rate of loss of potency for the intended storage temperature, and also for temperatures that may be encountered during transportation. Many biological products appear to exhibit Arrhenius type behaviour over a modest range of temperatures and a well designed study for such materials may give accurate and precise estimates of expected loss of potency. There are, however, classes of reference materials for which the Arrhenius model cannot be used. These include materials stored as liquid or frozen, live virus vaccine and nucleic acids
(DNA or RNA) standards. Some of the lyophilized materials may be sufficiently stable that detectable degradation has not occurred in a limited time and hence no prediction by the Arrhenius model can be made. The approaches to assessment of stability must thus be made on a case by case basis.

A number of questions were raised which may partially address some of these issues. Can information other than direct estimation of potency, such as properties of the material, freeze-drying process and residual moisture content, particularly if similar to those of a previous standard, be inferred as alternative correlates of stability? Can information on stability of one activity be useful in assessing another, e.g. mixed neutralizing antibodies in the same sample with different specificity for different microorganisms?

Statistical issues were also noted. The design of the stability study, e.g. the number of participating laboratories in a stability study, the assay methods to be used and design for ongoing monitoring are crucial for accurate assessment of stability. Methods used for combination of results from different laboratories, lack of independence in potency estimates from different temperatures, sensitivity of the model to potency estimates at higher temperatures and reliability of potency estimates for high temperature samples are also important in designing the stability studies.

3. Working reference standards

3.1 National control laboratory perspectives

Dr A Tahlan, Central Research Institute, India, Dr Y Horiuchi, NIID, Japan, Dr L Sirota, CBER/FDA, USA (by teleconference) and Dr S Phumiamorn, Ministry of Public Health Thailand presented some experiences from the national control laboratory (NCL) viewpoint.

Dr Tahlan detailed the products tested by his laboratory, and discussed the procedures for establishment and distribution of biological reference materials for vaccines and antisera in India. National reference standards are calibrated against the WHO reference material by a collaborative study and used by Indian manufacturers to calibrate their in-house standards for routine use. The NCL regularly evaluates the behaviour of the national reference standards using data from both the manufacturers and the NCL. If these data indicate loss of activity, replacement of the national reference standard would be undertaken.

Dr Y Horiuchi, described the problems arising in evaluating long-term stability: (i) Reference standards may degrade on long term storage and it is therefore difficult to have a stable scale; (ii) Consistency of absolute responsiveness of a biological test system (e.g. cells, animals) can not be ensured; and (iii) Discrimination between true degradation and variation in estimates when tests are carried out at different times is not always possible. Evaluating the reliability of accelerated degradation tests is also difficult because accumulation of sufficient data is generally not possible.
Dr S Phumamorn described the reference preparations tested in her laboratory and explained that working reference preparations in Thailand are calibrated in terms of WHO IS and standards from NIID, Japan. Stability of the working reference standards is evaluated annually by comparisons of the working reference preparation with the standard used for its calibration.

Dr L Sirota, CBER, FDA, USA participated by teleconference. Assay standardization studies has been carried out in the framework of the lot release program in CBER. Stable reference materials are essential for this.

### 3.2 Industry perspectives

Dr J Diment, representing the European Diagnostic Manufacturers Association (EDMA), discussed the in-vitro diagnostic industry’s perspective. He stressed that stability of in-process manufacturing, stability during long term storage, stability during shipping and stability of opened “in-use” reagents and working reagents all need to be considered. In the case of many diagnostic reagents, stability problems are detected at manufacturing stage and the users remain unaware of the problem. However, in some cases there is an inherent biological problem e.g. red cells which must be taken into account. The in-vitro diagnostic industry has established a definitive stability policy based on regulatory requirements (e.g. EN 13640, FDA requirements). This policy includes an element of real-time monitoring to validate the accelerated stability predictions. It was suggested that a similar policy might be adopted for assessing stability of biological reference standards. Dr Diment advocated the use of a documented stability procedure which must be followed for all materials and which would conform with recognised International Standards (e.g. ISO Guide 34).

Dr C Noël from Sanofi Pasteur discussed a mathematical model describing thermal viral inactivation $^2$. This model is used in the case of live attenuated vaccines to describe the inactivation of viruses as a function of time and temperature. Dr Noël concluded that this model can be used in the 5 to 50 °C temperature range and that parameter estimation was possible. The estimates and their precision are affected by many factors such as number of samples, sampling times and the choice of temperatures. Although this model has been studied only in two products, it was suggested that it may be generally applicable to live attenuated viral products.

Dr J Jorquera, representing the Plasma Protein Therapeutics Association (PPTA), discussed the design of stability studies for plasma derivatives and their related secondary standards. Studies carried out are International Conference on Harmonization (ICH) compliant, extend over a range of temperatures from about 5 to 50 °C, and apply to plasma derivatives stored refrigerated between 2 and 8 °C. Studies extended up to 42 months were illustrated and have shown product stability for some products in terms of primary or secondary reference standards. These results may impact sample handling $^3$. 
3.3 Perspectives from other organizations

Dr K Buchheit elaborated on the activity of the European Directorate for the Quality of Medicines (EDQM) regarding methodology adopted in preparation, types and uses of biological reference preparations (BRP). The official Ph. Eur. BRPs are established only after international collaborative studies. They are adopted by Ph. Eur. Commission and are available from EDQM. An accelerated degradation study (ADS) with candidate BRP materials is carried out at pilot phase for those cases where no stability data are available. ADS is performed to determine storage temperatures, shipment conditions and monitoring frequency. However, ADS does not replace monitoring. For monitoring, alert and action limits are set for the BRP and its biological activity which is evaluated against the relevant International Standard or against a part of the BRPs which are stored in plastic bags with water absorbing agent at –70 °C. The result of monitoring guides the decision whether repeat testing is required in another experienced laboratory or whether the BRP is required to be withdrawn and replaced.

Dr T Barrowcliffe, NIBSC, UK elaborated on the collaboration between the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH) and manufacturers of diagnostic reagents to develop a uniform plasma standard for use by the manufacturers. The SSC secondary plasma standard is stored and shipped by NIBSC on behalf of ISTH. The collaborative study for value assignments on the SSC plasma standard lot # 2 was organised by NIBSC and the Royal Hallamshire Hospital, UK. For the stability study Factor VII:C and Factor VIII:C were evaluated as representative of all components of the plasma standard. In the accelerated degradation study the predicted mean loss at –20 °C did not exceed 0.011% per year and at 37 °C predicted mean loss did not exceed 15% per year. In a longer term study, comparison of samples stored continuously at –20 °C with samples stored continuously at –70 °C showed no detectable difference between them.

Dr A Pauwels-Lamberty, Institute for Reference Materials and Measurements (IRMM), Geel, Belgium, discussed stability studies being undertaken in IRMM. A range of materials is produced by IRMM in accordance with internationally accepted guidelines, in particular ISO Guide 34. At certification stage, a short term study of typically four weeks using relatively high temperatures is carried out to determine dispatch conditions and a longer term study of typically one to two years and using lower temperatures is carried out to determine storage conditions and to estimate shelf-life. All stability studies are planned at the beginning, before processing and labelling is executed. Isochronous measurement schemes are used, in which samples of the material stored at elevated temperatures are removed to a ‘reference’ temperature of -70 °C at defined intervals and at the end of a pre-determined study period all samples are simultaneously analyzed. Results are used to derive a regression of the measured activity on time and an uncertainty of measurement based on the ISO Guide to the expression of uncertainty in measurement (GUM) is determined. Post-certification monitoring is performed after 4, 6 and/or 8 years of storage using samples stored at -70, -20, +4, +18 °C, and may provide the basis for re-assessment of the shelf-life. Approximately 150 CRMs including microbiological materials are controlled annually.
4. Case studies

Dr R Gaines Das, NIBSC, UK presented as examples of accelerated degradation studies for assessment of stability of reference materials, collaborative and in house studies carried out for Factors VII and VIII in freeze dried plasma, the WHO international standard of interferon alfa 2b, WHO international standards for thyroid stimulating hormone and the WHO international standard for epidermal growth factor. She also noted studies which have shown that storage of freeze-dried material in sealed glass ampoules may give better long-term stability than storage of the same material in vials. Interpretation of data from thermally accelerated degradation studies requires an understanding of the assay system(s) used, the precision of estimated potencies and the assay design. Independent studies carried out over a range of times may provide valuable insights into the degradation process.

Dr Y Horiuchi presented accelerated degradation studies carried out for prediction of the stability of Schick test toxin, Japanese encephalitis vaccine (inactivated, liquid form), and freeze-dried BCG vaccine. Infectivity titration for a reference preparation for smallpox vaccine has been performed for more than 20 years in Japan. These data were graphically presented by plotting log titre against time (year). Although a smooth regression could be fitted, the large variation in individual determinations was noted.

Dr J Shin introduced a stochastic approach to predicting the stability of reference standards. Maximum likelihood (ML) analysis has been applied to estimate the stability of many WHO reference standards for more than 20 yrs. The stochastic approach was initiated to provide complementary information regarding the uncertainty of the prediction and to overcome limitations of the available ML analysis software. Estimates by this approach were compared with those of ML analysis using a data set described previously by Kirkwood and Tydeman. There was a notable discrepancy between the two approaches which will be a matter for future research.

5. Report of WHO guidelines for vaccine stability

Dr I Knezevic, WHO, Switzerland outlined the development of WHO guidelines on stability evaluation of vaccines. A consultation was planned in June 2006 and the aim was to submit guidelines for adoption by ECBS in 2006. She explained that the intention of stability guidance is to assist manufacturers and national regulatory authorities in carrying out stability studies for vaccines and to complement current WHO recommendations for particular vaccines. These guidelines will have broader scope but will be compatible with ICH guidelines and should allow maximum product specific flexibility. She further informed the group that the current draft guidelines include some issues related to thermal stability, and the relevance of accelerated thermal degradation studies in assessing products for lot release. Maintaining the balance between general principles and specific recommendations for a vaccine or type of vaccine is an important issue for these guidelines. Combined vaccines raise important general issues including
assessment of stability after reconstitution, cumulative stability of intermediates in the selection of components for formulation of final vaccine, and whether there should be expiry dates for individual components.

6. Discussion

The above presentations formed the basis for wide ranging discussion by the Working Group. The need for guidelines was recognized, and it was noted that these could be helpful to industry as well as to those concerned with development of standards and to regulators. A number of issues need to be addressed. The degradative process and the mathematical models used to describe it require consideration for the variety of materials for which international biological reference preparations are produced. These include a number of special cases in addition to ampouled lyophilized preparations. The design, analysis and interpretation of stability studies require substantial statistical expertise. The sensitivity of the prediction of stability to the mathematical model in addition to the precision of assays and to the design of the study will be of particular concern. Other issues include the number and types of assays to be used, the range of temperatures to be used and the need for ongoing studies and the intervals at which these should occur. Guidance is needed on the appropriate action when a material appears to be stable, so that no measurable degradation occurs and hence no rate of degradation can be predicted. Other issues were noted without a clear indication of their role in the proposed guidelines. The role of quality assurance schemes in the standardization process, of which stability determination is one element, was noted. The relevance of monitoring other parameters, such as moisture, pH, oxygen, matrix properties, in addition to the biological activity of the preparation was also discussed.

7. Conclusions and recommendations

The international biological reference preparations provided by WHO appear to have shown stability. However, there is no clear guidance provided by WHO on how stability should be predicted and how it should be assessed in an on-going manner. It would be helpful for WHO to address these issues. In particular, many questions about the design of stability studies were raised.

At present, studies for the characterization and establishment of the international biological reference materials typically address stability of the material under storage at the custodian site. Stability of the materials after reconstitution have received less attention, and these should be more specifically addressed.

The consultation group recommended that:

- The instructions for use supplied with an international biological reference material should include the available information regarding its stability
• Additional data pertaining to stability of an international biological reference material should be provided on WHO websites as it becomes available.

• If possible, feedback from users about the behavior of WHO international standards should be obtained and documented.

• Guidance should be developed on:
  – a procedure for monitoring international biological reference materials so that stability can be confirmed and so that predicted stability based on short term studies can be related to that determined on the basis of longer term studies.
  – typical designs for thermally accelerated degradation studies including the range of times and temperatures at which assays should be carried out and the number of assays which might be required.

References


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