Meeting Report

WHO Working Group on Stability of Reference Materials for Biological Medicines and In Vitro Diagnostics

Geneva, Switzerland

27-28 November 2006
Summary

The World Health Organization (WHO) convened a working group meeting on the stability of reference materials for biological medicines and in vitro diagnostics in November 2006. Participants were from WHO collaborating centres for biological standardization, national control laboratories, industry, and other international organizations. On reviewing (i) current principles and practices on the stability of WHO biological reference materials, (ii) problems in science and compliance with the current WHO recommendations, and (iii) design and analysis of thermally accelerated stability studies, the working group agreed that guidance on stability prediction and monitoring for biological reference materials should be prepared as an addendum to the existing WHO recommendations on the development of international and other standards.

Keywords


Introduction

The WHO Expert Committee on Biological Standardization (ECBS) sets written procedures for the preparation, characterization and establishment of international and other biological reference materials. The latest set of principles for predicting and monitoring the stability of international and other biological reference preparations in a final container was adopted in 2004 [1].

There are many quality attributes to be pre-defined before the adoption of WHO international biological reference materials by WHO ECBS. One of them is stability which is evaluated in many cases by thermally accelerated degradation studies before establishing reference materials. Routine stability testing is carried out on a case-by-case basis after establishment. ECBS has recently emphasized post-establishment stability monitoring, especially for unconventional preparations. These preparations include the materials filled in containers other than ampoules (such as vials), the materials showing wide variability of potency assays (such as live viral vaccines), or a new class of materials (such as nucleic acids standards).

In November 2006, WHO convened a working group meeting subsequent to the first technical workshop on the stability of reference materials for biological medicines and in vitro diagnostics held in 2005 [2]. Dr David Wood, Coordinator of the WHO Team of the Quality, Safety and Standards (QSS) opened the meeting. Dr Trevor Barrowcliffe (formerly NIBSC) and Dr Sigrid Nick (Paul-Erhlich Institute, PEI) were elected as chairperson and rapporteur, respectively.
Session 1: Update and review

Dr D Wood gave an overview on WHO international biological standardization.

It is a constitutional responsibility of WHO to develop, establish and promote international standards for biologicals. The three main standard products developed by WHO include:

- Global written standards, i.e. recommendations and guidelines, for biological products such as vaccines, blood products, biological therapeutics and selected IVD, which serve as a tool for harmonization of specifications worldwide;
- Global measurement standards, i.e. international standards and reference reagents, for qualifying secondary standards for regulatory bodies, manufacturers and end users, which serve a tool for comparison of and standardizing analytical tests worldwide; and
- Evidence-based standards relevant for risk management purposes or for global public health which serve a tool to promote regulatory preparedness.

The implementation of the organizational strategic objective for the quality of biologicals at WHO Headquarters is currently carried out by two units which follow a functionally integrated biological standardization programme. QSS deals with the quality of vaccines, cytokines, growth factors and endocrines while Quality Assurance and Safety: Blood Products and related Biologicals (QSD) for blood products and IVDs.

The strategic directions for implementing the strategic objective for biologicals include:

- Holistic approaches to regulatory support (i.e. integrating standards development with regulatory capacity building with safety monitoring);
- The promotion of regulatory research;
- The development of regulatory networks; and
- Improved prioritization by linking with other WHO Advisory Groups (e.g. Strategic Advisory Group of Experts, Global Advisory Committee on Vaccine Safety) and by engagement with broader community e.g. the International Conference of Drug Regulatory Authorities.

At the 57th meeting in 2006, ECBS established 15 new reference preparations as global measurement standards which guide manufacturers and regulatory authorities on the activity of biological medicines. The development of WHO reference preparations largely depends on support from the WHO International Laboratory for Biological Standardization at NIBSC. The sustainability of the WHO reference preparation programme is essential. WHO and NIBSC plan to develop, in consulting other stakeholders, a business plan to assure the long-term security of this global public health resource.
In the discussion, there were questions and answers on general issues of reference preparations. Dr J. Diment (Ortho-Clinical Diagnostics, a representative of the European Diagnostic Manufacturers Association, EDMA) questioned that to what extent ISO has been involved in setting WHO standards. Dr Wood explained that WHO strives to avoid unnecessary duplications but to ensure that the various activities are complementary. WHO also participates in relevant activities offered by ISO. Another issue was raised by Dr Y. Horiuchi (National Institute of Infectious Diseases, NIID) about the calibration of working standards against WHO standards serving as calibrator. Because in many Asian countries, usually 1 or 2 institutes are involved in calibration, it is difficult to obtain unbiased data for calibration. Network system for collaboration would be a good approach. Dr Wood added that WHO has been actively promoting the activities of regulatory networks relating to establishing working standards.

Dr J. Shin (WHO) reminded the participants of the background to the formation of the working group and also provided feedback on activities of the working group from the Expert Committee at the 57th meeting in 2006.

Upon reviewing the revised WHO recommendations on the establishment of biological reference standards [1], ECBS at the 55th meeting in 2004 recommended that WHO should start or continue work specifically on predicting and monitoring the stability of biologicals. Hence, an informal technical workshop was convened in Geneva, Switzerland in 2005. The Expert Committee's feedback on the conclusions of the 2005 workshop was the need for prioritization of standards required for special stability studies, which would entail the following specific needs:

- Guiding principles for prioritizing stability monitoring studies.
- Formal procedures for prescribing and monitoring stability monitoring studies.

The latest WHO recommendations [1] specify (i) that in some circumstances further stability monitoring studies during post-establishment period should be performed on a case-by-case basis taking into account data obtained from thermally accelerated degradation study and (ii) that the studies may be recommended by the Expert Committee. Several reference standards already recommended by the Expert Committee or noted by the study coordinator for stability monitoring studies include prostate-specific antigen (PSA), hepatitis A vaccine (HepA), somatropin, smallpox vaccine, IL-17/18, and nystatin preparations (Table 1). Some practical issues related with extended stability testing program or monitoring were noticed. These include appropriate timing for requesting report of stability monitoring studies and making a common approach for every standard.

The following expected outcomes of this meeting were summarized:

- Expert views and suggestions for current best practices for ensuring the stability of WHO biological reference preparations are collected;
• Agreement on developing guidance on predicting and monitoring the stability of reference materials to supplement the existing WHO guidance on reference standards are developed for consideration by ECBS; and
• Priority scientific research items in this area.

In the discussion, an issue was raised on limitations in performing regular stability monitoring studies for reference standards. Dr P. Phillips (NIBSC) pointed out that we should consider the best way of managing shortage of human resources involved in establishing and maintaining WHO biological reference standards at the WHO International Laboratory for Biological Standardization at NIBSC. Dr K.-H. Buchheit (European Directorate for the Quality of Medicines, EDQM) added that planning such studies also should consider difficulties where animal experiments are necessary.

Session 2: Principle and practice

Mr A. Heath (NIBSC) summarized the current practice in ensuring the stability of WHO international reference standards.

WHO ECBS is responsible for the establishment of international standards or reference reagents. The revised WHO recommendations positioned that, where international biological reference standards are to be assigned a value in arbitrary international units (IUs), an uncertainty value is not given. In order to serve as an international reference standard, it is important to obtain sufficient amount of filled containers of a reference preparation to meet the estimated demand, preferably for at least ten years. A crucial part for ensuring stability is an appropriate manufacturing process which needs to be controlled vigorously. Any processes associated with degradation needs to be reduced to a minimum. Many international standards are lyophilized. Since the freeze-drying method is especially critical, preliminary freeze-drying studies need to be conducted. Residual moisture, oxygen content, formulation nature of the ampoules and storage temperature greatly influence stability of a preparation. In addition, strong efforts are required to minimize heterogeneity.

Stability studies provide information on the length of time of stability (suitability for the intended purpose) of a preparation under the recommended storage conditions, after transportation, and after reconstitution. In general, there is no direct method of estimating the rate of loss of potency of a reference standard under its defined storage conditions. Therefore, indirect and approximate methods need to be used for estimating the rate of loss of potency or degradation. The selection of the methods for monitoring the stability depends on the nature of the reference material and on its application.

New materials are investigated in a collaborative study by a small number of laboratories to obtain information of the behaviour in different assay system. Collaborative studies carried out when a replacement is necessary could serve as a further source of information on stability, e.g. give an overview on the period the previous preparation was in use.
Wrapping up his overview, Mr Heath pointed out that the following issues were not included in the current WHO recommendations:

- Technical guidance on size or design of stability studies;
- Detailed specification of minimum acceptable stability; and
- Formal procedures for reporting continued stability monitoring of an International Reference Standard to WHO.

He also suggested that the instructions for use (IFU) of a reference preparation should contain the citation of the report that supported the establishment, the citation of any scientific publications describing the characterization of the reference material and information on recommended storage temperature and time. Where appropriate, the IFU should indicate the method of reconstitution and also storage conditions after reconstitution.

At the end of the presentation, Mr Heath questioned what degree of degradation should be acceptable. In the discussion it was suggested that an acceptance limit in the degree of degradation should depend on the intended lifetime of a reference material if the limit would need to be set out.

Dr R. Gaines Das (NIBSC) presented NIBSC's draft stability policy guidance notes. Stability has been a recognized requirement for international standards since the inception of the international standardization program in 1921. NIBSC procedures provide stability assessment for standards following the current WHO guidance [1]. However, more detailed guidance is necessary taking into account new external developments (e.g. ISO standards, external quality assessment and accreditation programs).

The current NIBSC draft guidance notes include requirements for assessment of stability and examples of possible study designs. In addition, there is an explicit requirement to provide more detailed information to users.

WHO international biological reference preparations are defined in units and no higher order standard is available. In general, a reference method is not available. In the absence of a reference method or a higher order standard, ‘real time’ studies are not possible. For this reason, accelerated studies under stress conditions provide the only approach for assessment of stability. The range of stress conditions used for these studies should, where possible, include conditions of less stress than the ‘normal conditions’ since this may provide further assurance regarding stability, especially for long term studies. For example, if the normal storage temperature is -20°C, then samples may be stored at -70 and -150 °C. Although sometimes referred to as ‘real time’ monitoring, this is a misnomer as all samples are ‘stressed’ with reference to the condition of least stress, and comparisons are of the same material after storage under different conditions.

The Arrhenius equation relating the rate of reaction to the temperature is a widely accepted theoretical approach for prediction of stability. It is based on the assumption of
first order kinetics and a linear relation between the degradation rate and the temperature. Moreover, it assumes that degradation follows the same kinetics at all temperatures. The approach may require extrapolation over a wide range of extreme temperatures as well as the recommended storage conditions and testing after different storage periods. In addition the Arrhenius equation may be a satisfactory model for freeze-dried materials, but may not be generally applicable for liquid stored preparations or for live vaccines.

In summary, the NIBSC procedure applies predictive stability testing and takes into account experience established with already existing lyophilized preparations and the expected lifetime of a standard. NIBSC experience with lyophilized materials stored in sealed glass ampoules indicates that the predictive approach based on relatively short term storage at elevated temperatures in general leads to an underestimation of real stability and longer-term storage will lead to different predictions. From the practical point of view, a study must be properly designed when the material is prepared in its final form considering the temperature range, the duration and the testing regime, as sufficient ampoules must be stored at the required temperatures. A pre-requisite for applying accelerated procedures is the occurrence of detectable degradation within a short period of time, which is likely to take place only at relatively extreme temperatures. For a reliable extrapolation temperatures close to the recommended storage temperature need to be covered, too.

The number of assays to be performed largely depends on the assays' precision and the ease with which an assay may be performed. At NIBSC, temperatures of -150, -70, -20 (the customary storage temperature), +4, +20, +37, +45, and +56 °C are routinely considered. Experience shows that the latter may however result in difficulties in reconstitution. Shipping simulation is covered by the storage at the extremely elevated temperatures. When designing a study, one needs to take into account the time constraints. In general, it takes 1-2 years before a new standard may be used.

It is important to consider the provision of necessary information to the user. This should comprise stability during storage at NIBSC, during transport, unopened at the user laboratory and stability after opening and reconstitution. Expiry dates are not attached to reference preparations. It is present practice that as long as reference materials are cited in the current on-line catalogue the declared potency or content remains valid. The cited materials are subject to further continuous stability monitoring.

Dr B. Toussaint (Institute of Reference Materials and Measurements, IRMM) explained the current stability testing regime applied at IRMM, Belgium. In contrast to NIBSC and WHO, an uncertainty value is assigned to all reference materials and real-time isochronous studies are the method of choice to evaluate the stability of the material at different storage temperature during different periods. In addition, the stability of the materials is still monitored over 10-12 years after characterization. The same material stored at a temperature where degradation is very unlikely to occur (e.g. -70 and -150 °C) is taken as a reference. Details of the method have been outlined earlier [2].
Dr J. Diment emphasized that there are no particular issues with the stability of WHO reference materials except that the WHO procedures do not comply with harmonized ISO guidelines.

He presented a written technical manual and pointed out the main differences of the IVD industry’s perspective compared to the NIBSC document:

- There is a need for all international standards for ongoing real-time studies and monitoring;
- Testing intervals could be a result of data and risk assessment;
- Besides the Arrhenius model, alternative prediction methods may be used if they are justified;
- A design dossier to be reviewed by an external expert is proposed;
- Stability trials should include patient samples to overcome variation of assays; and
- A standard operating procedure (SOP) on how to conduct stability trials is suggested.

He also questioned whether the experience of the users of a standard could be obtained and used to provide information about stability.

Dr Barrowcliffe suggested collecting information from users calibrating and recalibrating material on WHO reference standards. Dr Buchheit affirmed that the suggestion made by Dr Barrowcliffe is the only possible way for certain critical preparations, e.g. vaccines which would need to be tested in an animal model with rather high variability and that this is already current practice for several vaccines.

Dr Barrowcliffe proposed that the overall strategic plan should take input from the NIBSC guidance notes and a technical manual on product stability strategy proposed by Dr Diment. Harmonization of methods should be possible for similar materials between the collaborating centres PEI and NIBSC, but NIBSC cannot give as much detail as standard operating procedures (SOPs). Each laboratory should establish own working procedures.

Session 3: Scientific issue: What to do for those preparations that do not fit the Arrhenius model

Mr A. Heath (NIBSC) presented examples of stability studies on preparations where the Arrhenius model may not be applicable. These included international standards for nucleic acid amplification technology (NAT) assays, e.g. hepatitis B virus (HBV) DNA [3] and hepatitis A virus (HAV) RNA [4]. Problems during stability investigations arise from the insolubility of nucleic acid materials stored at higher temperature as well as from the heterogeneity of NAT assays and kit lots. In addition, studies suffer from poor precision of individual NAT assays which typically is in the range of ± 0.3 log10. For this reason, a need for continuing real-time stability studies for these types of preparations is generally recognized. It is not completely clear whether the Arrhenius model would be applicable, because the observed drop in RNA or DNA content does not follow the expected exponential decay over time (linear drop in log concentration) assumed by the
Arrhenius model. For the 2nd HBV DNA preparation, detectable degradation has still been absent when stored at +20 °C for 4 years.

Dr Y. Horiuchi (NIID) presented examples from the Japanese experience with stability prediction on the basis of accelerated trials. While Schick (diphtheria) toxin perfectly fits into the Arrhenius model, the degradation of Bacille Calmette-Guérin (BCG) vaccine does not follow Arrhenius behaviour. It is likely to exhibit the second order degradation reaction. Degradation curves of smallpox vaccine also do not fit in the Arrhenius model. Other examples are human immunodeficiency virus (HIV) and hepatitis C virus (HCV) RNA and HBV DNA preparations that become insoluble at higher temperatures. He suggested applying lower temperatures and longer periods for degradation studies for such materials.

In the discussion, Dr M. Chudy (WHO) explained that at higher temperatures nucleases may cause RNA degradation in a plasma environment in addition to the problem of insolubility.

**Session 4: Compliance issue (I): Design and analysis of stability studies**

Dr P. Phillips (NIBSC) led the discussion. Participants raised the following points:

- Consideration for what minimum data ECBS would need for acceptance of a reference material.
- The degree of degradation to be acceptable should depend on the nature of the reference preparation (blood product, antibiotic etc) and on anticipated time period over which the standard would be used.
- At least two laboratories are required for stability studies including the laboratory that did the filling.
- The importance to distribute blinded samples was stressed.
- A template should be created to optimize reporting of stability data.
- Statistical evaluation and support would be needed to track the data.
- Constraints should be considered for implementing ongoing stability monitoring studies, especially:
  - if only few laboratories worldwide are able to perform the stability tests;
  - if ethical issues arise, e.g. animal experiments need to be performed; and
  - if human resources are a limiting factor.

Dr Buchheit stressed again difficulties if in vivo stability assays are needed. Research on alternative test methods (e.g. biochemical, physicochemical methods or others) is needed.

**Session 5: Compliance issue (II): Stability monitoring**

Dr P. Phillips (NIBSC) reminded participants that data to support the establishment of a reference material should be sufficient to predict stability with estimated percent loss per year. For dispatch, confidence limits should be given including data at different storage
temperatures and at 37 °C. Data on post-establishment stability should be available for at least 1 year as a minimum, preferably for 2 years.

Participants raised the following points as to what is needed to improve implementing the current practice in stability monitoring:

- An initial programme for monitoring of long-term assurance of stability for the material should be available;
- A standardized template for summarizing stability data would aid in reporting and reviewing the data; and
- Once reference standards are established, their stability data should be published and made accessible to the public (e.g. through WHO webpage)

Dr Diment added his perspectives on behalf of EDMA's position:

- A formal renewal process is proposed for all WHO reference preparations in regular timeframes (every 3 years) using accumulated stability data including real-time long-term stability data as well as monitoring data from sponsoring laboratories and users; and
- Existing WHO reference preparations should be dealt with according to a risk-based programme of long-term stability assessment.

Dr J. Diancai (NICPBP) presented the Chinese position on stability prediction and monitoring for national reference materials. Accelerated studies are required as early as the research and development process is initiated. As a general rule, different temperatures (-20, +4, +25, and +37 °C) are applied. Dr Diancai explained about calibration, stability prediction and monitoring with different testing regimes and schedules for blood products, cytokines, live vaccines, and diagnostic kits, respectively. Some reference substances including blood products and inactivated vaccines will be given an estimated shelf-life for a specific temperature storage based on accelerated studies. Stability monitoring will be preferably carried out by direct comparison to WHO international standards. If an international reference preparation is not available, monitoring shall be performed using the first batch as a reference.

Session 6. Design and analysis for thermally accelerated degradation studies

Dr R. Gaines Das (NIBSC) described general principles in designing and analysing thermally accelerated degradation studies. Information is available in articles published by Kirkwood and his colleagues [5-8]. Design of study for the measurement and testing (formerly, Bureau Communautaire de Références, BCR) programme of the EU Member States was published by Moss and other colleagues [9]. Briefly, samples are coded in random order to avoid unintentional bias. Duplicate samples from baseline storage temperature permit measurement of reproducibility of assay system. Frequent testing at early stage permits refinement of data, assessment of consistency of prediction, and assessment of compliance with Arrhenius equation.
As shown in Table 2, an actual testing protocol is presented in a case study on freeze-dried pooled normal plasma, assessed for activity of two clotting factors, Factor VIII and Factor VII which are expected to be stable at -20 °C. The container was screw-capped vials and the assay methods were chromogenic (Factor VIII) or clotting (Factor VII) activity (Unpublished data provided by Dr A Hubbard, Division of Haematology, NIBSC). It should be noted that samples stored at higher temperatures were tested at shorter times. Testing of samples stored at lower temperatures before degradation can be detected is not productive.

In general, different assay systems may differentially detect degradation. Assay systems used must reflect assay systems for which reference material is intended although data from other assay systems may provide scientific information of interest.

In principle, the accelerated degradation study must be designed when the material is prepared. The design should consider reproducibility (duplicates) and a higher testing frequency at early stage is required. Assay systems to be used may detect degradation to a different extent, especially in vivo and in vitro assays. Independent studies using samples stored at different temperatures and for different times would be preferable.

Interpreting data from thermally accelerated degradation studies should consider assay systems used, assay designs, the nature of data, relationships among the various estimates, and the variability of estimates. Where possible, independent studies carried out over a range of time may provide valuable insights into the degradation process. Simple rules are unlikely to apply to many examples of real data.

Dr J. Shin briefly demonstrated a computer software program for use in personal computers [10]. The software program requires formatted data that can easily be created in Microsoft Excel. The program can be delivered by e-mail free of charge once requested.

Session 7: Conclusion

Dr S. Nick (PEI) presented main points captured during presentations and discussions in the 2-day meeting.

The working group reached the following conclusions:

- Written guidance on the evaluation of the stability of reference materials should be prepared as an addendum to the existing WHO recommendations on the development of international and other standards [1]. Such guidance should consider (i) resource issues, especially if few laboratories worldwide may perform stability tests; (ii) ethical issues of animal experiments, and (iii) the balance between what is achievable and what is ideal; and

- The plan for developing guidance should be based on the NIBSC draft guidance notes. The group agreed that further work on NIBSC draft guidance notes would be necessary.
Dr K.-H. Buchheit (EDQM) urged caution in considering a change in the assay(s) used for stability monitoring (e.g. replacing a microbiological assay for antibiotics by an HPLC assay) because the more sensitive assay methods may reveal signs of degradation which have not been seen with the original assay. T. Schofield (the International Federation of Pharmaceutical Manufacturers and Associations representative) added that accelerated studies could only represent a first step. Real information from permanent monitoring at real storage temperature is needed additionally. The guidelines should offer multiple options on the scientific design of studies.

References


**SUGGESTED AUTHORS**

Sigrid Nick  
Paul Ehrlich Institut, Langen, Germany

Trevor Barrowcliffe  
35 Oakhurst Avenue, East Barnet, Hertfordshire, United Kingdom

Rose Gaines Das, Peter Phillips  
National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, United Kingdom

Jinho Shin  
Department of Immunization, Vaccines and Biologicals, World Health Organization, Geneva, Switzerland

On behalf of the WHO Working Group on the Stability of Reference Materials:  
Mrs Florence Baudoux, GSK Biologicals, Rixensart, Belgium; Dr Karl-Heinz Buchheit, European Directorate for the Quality of Medicines, Council of Europe, Strasbourg, France; Dr Derek Calam, Pewsey, Wiltshire, United Kingdom; Dr Michael Chudy, Department of Immunization, Vaccines and Biologicals, World Health Organization, Geneva, Switzerland; Dr John Diment, Ortho-Clinical Diagnostics, High Wycombe, United Kingdom; Mr Alan Heath, National Institute for Biological Standards and Control, Potters Bar, United Kingdom; Dr Yoshinobu Horiuchi, National Institute of Infectious Diseases, Tokyo, Japan; Dr Diancai Jiang, National Institute for the Control of Pharmaceutical and Biological Products, Tiantan Xili, Beijing, People's Republic of China; Dr Mary Kimberly, Division of Laboratory Sciences, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; Dr Catherine Noël, Sanofi Pasteur, Marcy L’Étoile, France; Dr Supaporn Phumiamorn, Ministry of Public Health, Nonthaburi, Thailand; Dr Timothy Schofield, Merck Research Laboratories, West Point, Pennsylvania, USA; Dr Lev Sirotas, Center for Biologics Evaluation and Research, Food and Drug Administration, Rockville, Maryland, USA; Dr Brigitte Toussaint, Institute for Reference Materials and Measurements, European Commission, Geel, Belgium; Dr Ilka von Hoegen, Brussels, Belgium; and Ms Libby Wunsch, The Biovac Institute, Cape Town, South Africa.
<table>
<thead>
<tr>
<th>ECBS meeting</th>
<th>Year established</th>
<th>TRS no.</th>
<th>Name of standards</th>
<th>Study report</th>
<th>Records in TRS</th>
</tr>
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<tbody>
<tr>
<td>50th</td>
<td>1999</td>
<td>904</td>
<td>1st IS Prostate-specific antigen</td>
<td>BS/99.1902</td>
<td>Prostate-specific antigen standards were recommended for ongoing stability monitoring programme as they are filled in vials</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1st IS hepatitis A vaccine</td>
<td>BS/99.1914 &amp; rev.1</td>
<td>Ongoing stability monitoring programme as it was a liquid preparation and as predictions of long-term stability were not available because of the inherent variability of the assays</td>
</tr>
<tr>
<td>51st</td>
<td>2000</td>
<td>910</td>
<td>2nd IS Somatropin</td>
<td>BS/00.1929</td>
<td>Study coordinator reported that, although it appeared stable, stability monitoring will be continued</td>
</tr>
<tr>
<td>57th</td>
<td>2006</td>
<td>TBD</td>
<td>2nd IS Smallpox vaccine</td>
<td>BS/06.2037</td>
<td>Stability to be monitored and reported back to ECBS</td>
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<tr>
<td></td>
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<td></td>
<td>1st RR Interleukin-17</td>
<td>BS/06.2039</td>
<td>Need to resolve the stability of the preparation after reconstitution before it can be recommended for use in immunoassays</td>
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<td>1st RR Interleukin-18</td>
<td>BS/06.2040</td>
<td>Need to resolve the stability of the preparation after reconstitution before it can be recommended for use in immunoassays; Need for real-time stability studies</td>
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<tr>
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<td>Nystatin</td>
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<td>Preparation 06/04-01 is established as the 3rd IS for nystatin with a potency of 5,710 IU per mg pending suitable stability data, which need to be provided to WHO as soon as possible; no ampoules of the 3rd standard to be issued until this data is reviewed by WHO</td>
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TRS: technical report series; IS: international standard; TBD: to be determined; RR: reference reagent;
Table 2. Testing protocol based on previous in-house experience and familiarity with pooled plasma and these clotting factors

<table>
<thead>
<tr>
<th>Storage period (months)</th>
<th>Storage temperature (°C)</th>
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<tr>
<td></td>
<td>+4</td>
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<td>5</td>
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<td>7.3</td>
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<tr>
<td>19</td>
<td>X</td>
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<tr>
<td>31</td>
<td>X</td>
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<td>45</td>
<td>X</td>
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<tr>
<td>56</td>
<td>X</td>
</tr>
<tr>
<td>72</td>
<td>X</td>
</tr>
</tbody>
</table>

X: a set of samples. Usually duplicate or triplicate samples are used for intra-assay precision and statistical weights.