

Annex 3

Guidelines on stability evaluation of vaccines

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WHO recommendations and guidelines for production and control of vaccines and other biologicals are scientific and advisory in nature and provide guidance for national regulatory authorities and for vaccine manufacturers (www.who.int/biologicals). They form the basis for the acceptability of products globally. These recommendations feature stability as an important element and provide guidance for stability testing for individual vaccines. The following text is written in the form of guidelines instead of recommendations because vaccines represent a heterogeneous class of agents, and the stability testing will need to be adapted for the product in question. Guidelines allow greater flexibility than Recommendations with respect to specific issues related to particular vaccines.

1. Introduction

The stability of vaccines has a major impact on the success of immunization programmes worldwide. As part of its efforts to assure vaccine quality, WHO has acknowledged the importance of clearly defining the stability characteristics of a vaccine and emphasizes the role of national regulatory authorities in overall vaccine evaluation.

The aim of this document is to provide the scientific basis and guiding principles for evaluation of vaccine stability for the purpose of clinical trial approval, licensing, and post-licensure monitoring.

The temperature sensitivity of vaccine characteristics, particularly potency, led to the development of storage and cold chain requirements for all vaccines. In the 1980s and at the beginning of the 1990s, a major WHO focus was on thermostability testing, as measured by potency assays, as part of lot release. More recently, guidance has addressed the importance of studies performed under real storage conditions, in real time, and with other relevant environmental factors. In addition, the WHO guidelines for nonclinical and clinical evaluation of vaccines, stress a need for stability data to support approval of a clinical trial (1, 2). However, until now there has been no comprehensive guidance document available which deals with the evaluation of the stability of vaccines at different stages of their development, production, licensing, lot release and post-licensing.

At its fifty-first meeting, the Expert Committee on Biological Standardization recommended that WHO set up a working group on stability evaluation of vaccines to examine this issue. The first meeting of the working group was held at the Paul Ehrlich Institute, in Langen, Germany, in February 2002, when key issues to be included in guidelines were identified. At its second meeting, held at WHO, Geneva, in 2004, the working group suggested further additions and improvements to the proposed guidelines including guidance on the design of stability studies. Reviews of stability studies undertaken on different types of vaccines were carried out in 2004 and 2005. These revealed problems in the conduct, analysis and the interpretation of data. In particular, difficulties were identified with the application of the pharmaceutical accelerated stability testing programme to vaccines and with the mathematical models used in data analysis. Additionally, differences in current practice with regard to the selection of parameters measured and the frequency of testing were identified. Two extremes were noted. In some cases numerous parameters were evaluated while in others only potency was examined. Similarly, the frequency and the rationale for defining appropriate intervals of testing varied considerably.

Furthermore, the assignment of shelf-life to intermediates, as well as their cumulative age, was identified as a problem for both vaccine manufacturers and national regulatory authorities. The stability assessment of combined vaccines is an additional issue. A survey of current approaches to the stability testing of

vaccines targeting both manufacturers and regulatory practices was conducted in 2006. The outcomes of all these activities were used to define the scope and to provide the guiding principles set out in this document.

The intention of this document is to complement current WHO recommendations for stability testing of individual vaccines, as described in the WHO Technical Report Series, by providing a set of general principles and a description of their application. The first part of the document is devoted to general considerations on the stability evaluation of vaccines. This is followed by a discussion of the stability of vaccines during the manufacturing process and in subsequent use, focusing on intermediates and final products. Regulatory expectations for stability studies to be conducted at different stages of development (i.e. clinical trial approval, licensing, lot release and post-licensure monitoring) are indicated in a separate section. The selection of samples and assays employed in the studies performed for different purposes, as well as the expression of results, are discussed in the section on the design of stability studies and statistical considerations. Key issues in the analysis of data are also considered and approaches to the analysis of the results of stability testing are described. The document effectively gives manufacturers two options for stability testing with respect to the design and data analysis:

- the “traditional” method based on the compliance with the acceptance criterion and determination of shelf-life as the time associated with the last measurement within the specification; and
- the “new” method, where statistical evaluation is used to define an expiry date through extrapolation of the data.

Early in the development process the manufacturer is encouraged to discuss with the national regulatory authority these approaches for the study design and data analysis and their suitability for the product in question.

In developing this document, guidelines for stability evaluation of medicines, including biologicals, issued by WHO and other bodies (3–9) were considered. The present guidelines are not intended to conflict with any of these existing documents but rather to complement them with vaccine-specific considerations.

2. Scope

These guidelines apply to all vaccines against infectious diseases.

It is important to note that the focus of these guidelines is on how to evaluate vaccine stability, not to provide guidance on how to stabilize a vaccine. Genetic stability is not considered in these guidelines.

Thermal stability testing as part of lot release is only mentioned in the context of the overall stability assessment, whereas recommendations for specific vaccines are provided in the documents on each individual vaccine.

3. Glossary

The definitions given below apply to the terms used in these guidelines. They may have different meanings in other contexts.

Accelerated stability studies: studies designed to determine the rate of change of vaccine properties over time as a consequence of the exposure to temperatures higher than those recommended for storage. These studies may provide useful support data for establishing the shelf-life or release specifications but should not be used to forecast real-time, real-condition stability of a vaccine. They could also provide preliminary information on the vaccine stability at early developmental stages and assist in assessing the stability profile of a vaccine after manufacturing changes.

Adjuvants: substances that are intended to enhance relevant immune response and subsequent clinical efficacy of the vaccine.

Combined vaccine: a vaccine that consists of two or more antigens, combined by the manufacturer at the final formulation stage or mixed immediately before administration. Such vaccines are intended to protect against either more than one disease, or against one disease caused by different strains or serotypes of the same organism.

Conjugated vaccine: a vaccine produced by covalently binding an antigen to a carrier protein with the intention of improving the immunogenicity of the attached antigen. This technique is most often applied to bacterial polysaccharides for the prevention of invasive bacterial disease.

Expiry date: the date given on the individual container (usually on the label) of a final vaccine up to and including which, the product is expected to remain within specifications, if stored as recommended. It is established for each batch by adding the shelf-life period to the date of manufacture or the starting date of the last potency test.

Intermediates: material produced during the manufacturing process, which is not yet in the final product, but whose manufacture is critical for the successful production of the actual vaccine. As part of quality assessment, both quantifiable and qualitative parameters of an intermediate should be defined and specifications established to determine the successful completion of the manufacturing step prior to continuation of the manufacturing process. This includes material that may undergo further molecular modification or be held for an extended period of time prior to further processing.

Release specification: a specification that a lot of a product should meet at the time of release in order to assure that the lot will maintain adequate quality throughout its shelf-life.

Real-time, real-condition stability studies: studies on the physical, chemical, biological, biopharmaceutical and microbiological characteristics of a vaccine, during and up to the expected shelf-life and storage periods of samples under the expected handling and storage conditions. The results are used to recommend storage conditions, and to establish the shelf-life and/or the release specifications.

Shelf-life: the period of time during which a vaccine, if stored correctly, is expected to comply with the specification¹ as determined by stability studies on a number of batches of the product. The shelf-life is used to establish the expiry date of each batch. Shelf-life is used for the final product; storage period is used for the intermediates.

Stability-indicating parameters: parameters that are direct or indirect indicators of vaccine efficacy or safety demonstrated in clinical trials. They are used to assess product suitability throughout the shelf-life. Determination of these parameters should result in quantitative values with a detectable rate of change. Qualitative parameters such as sterility could also be considered but cannot be included in the statistical analysis.

Stability of vaccines: stability is the ability of a vaccine to retain its chemical, physical, microbiological and biological properties within specified limits throughout its shelf-life.

Stability tests: a series of tests designed to obtain information on the stability of a vaccine in order to define its shelf-life and utilization period under specified packaging and storage conditions.

Storage period: time period during which an intermediate may be held under appropriate storage conditions.

Supporting stability data: supplementary data, such as stability data on small-scale batches, related formulations, and products presented in containers other than those proposed for marketing, and scientific rationales that support the analytical procedures, the proposed retest period or the shelf-life and storage conditions.

Stress testing: studies performed to determine the impact of extreme environmental factors such as light and extreme temperature. These studies are not usually performed as part of a stability programme, but are used instead to establish protective packaging and container conditions, and to support exclusionary labelling.

¹ "Shelf-life specifications" are those specifications that should be met throughout the shelf-life of the vaccine (should not be confused with "release specification").

Thermal stability as lot release test: stability of a vaccine after exposure to a temperature higher than that recommended for storage, for a specified period of time, often expressed in terms of change in potency.

Utilization period: period during which a liquid or a reconstituted preparation of the final vaccine in an opened container can be used.

Vaccines: a heterogeneous class of medicinal products containing immunogenic substances capable of inducing specific, active and protective host immunity against infectious disease.

4. General considerations

The main principles of stability testing of pharmaceuticals, described in WHO guidelines (3) apply, in general, to biologicals. However, special considerations are needed in the application of these principles to vaccines.

Most vaccines are large and complex molecular assemblies that are highly susceptible to environmental factors that may significantly affect their activity. Vaccines consist of complex mixtures of proteins, carbohydrates, lipids, inactivated microorganisms or, in some cases, live attenuated microorganisms as active components, as well as stabilizers, adjuvants, preservatives and other substances which together contribute to the overall efficacy and safety of the vaccine. The intention of these WHO guidelines for stability evaluation of vaccines is to discuss vaccine-specific issues and to facilitate the development of “vaccine-tailored” stability assessment procedures. Such issues include the inherent sensitivity of biological substances to changes in environmental conditions, the importance of tests reflecting potency and their degree of uncertainty, and the fact that, in general, a single parameter is insufficient to document stability and that a stability profile has to be established. In addition, considerations of microbiological aspects such as bioburden or sterility of intermediates or final products and effectiveness of antimicrobial agents may have to be addressed.

Among the environmental factors considered to influence pharmaceuticals, the only one that affects characteristics of all vaccines over time is temperature. The impact of humidity is not relevant for the vast majority of vaccines due to their liquid formulation, and the protective nature of the packaging, providing that the closure system of the vial or ampoule is appropriate. In addition to temperature, other environmental factors (e.g. light) might be considered in the development of new vaccines. However, photostability is not considered as a mandatory test in vaccine stability studies. For those vaccines that are susceptible to the light, such as *Bacillus Calmette-Guérin* (BCG) vaccine, the use of amber glass is part of the usual practice in packaging and shipment. This is, however, a measure to protect the vaccine from light and does

not require the exposure of vaccines already known as being susceptible, to light in routine stability studies.

Stress testing of extreme environmental conditions such as light or extreme temperatures is not mandatory in vaccine stability studies. However, it should be considered when a vaccine is intended for a market where exposure to extreme temperature or other environmental factors is a real possibility. Stress testing also helps to determine the intrinsic stability of a vaccine by establishing degradation pathways in order to identify the likely degradation products and to validate the stability-indicating power of the analytical procedures used. These studies may include exposure of a vaccine to temperatures higher than those recommended for storage, to light, oxidizing agents and freeze-thaw, as well as susceptibility to hydrolysis across a range of pH values.

A major problem in assessing vaccine stability is that many vaccines possess a specific biological activity that cannot be fully characterized by physicochemical methods alone. Biological assays play an important role in the quality control of vaccines and are essential parameters of vaccine quality. Potency assays based on an *in vivo* challenge test (e.g. Kendrick test for whole cell pertussis and National Institutes of Health (NIH) test for rabies vaccine) are typical examples of the parameters used for testing vaccine stability. Vaccine stability testing is based on the determination of the change in a vaccine property which may be a direct or an indirect indicator of vaccine immunogenicity or efficacy. Safety should also be considered in vaccine stability studies and specifications defined at the licensing stage.

The potential reversion to toxicity and changes in vaccine component complexes are issues specific to vaccines. Examples of parameters that could be tested as part of stability studies include histamine sensitizing test (pertussis vaccines), level of free polysaccharide, desorption from adjuvant and aggregation of adjuvant. Sensitivity to detect change is an important attribute of assays that measure stability-indicating parameters that are likely to be clinically relevant.

Tests incorporated into vaccine stability studies used to determine vaccine characteristics, including biological activity (e.g. potency, antigen content and specific toxicity), are performed before or after vaccine exposure to:

- recommended storage temperature (real-time real storage conditions studies); or
- temperatures higher than those recommended for storage (accelerated stability studies).

Given the inherent variability of biological assays, the use of reference materials is of critical importance in the interpretation of the data generated in stability studies. Reference materials should be calibrated against the International Biological Standard when available. The purpose of International

Standards is to ensure comparability of vaccine potency worldwide. In response to the global need for reference materials, WHO's biologicals programme, together with its collaborative centres, has focused on the establishment of International Standards and Reference Preparations for vaccines and other biologicals (<http://www.who.int/biologicals/IBRP/index.htm>). The stability evaluation of International Standards and reference materials is an essential element in the establishment of such standards and is discussed in the WHO recommendations for international reference materials (10). It is also the subject of a separate WHO initiative and is not discussed further in this document. However, where an International Standard or Reference Reagent is a vaccine lot, some of the considerations described in this document may apply. The importance of stable reference preparations in the stability assessment of vaccines is critical and is discussed later in the section on the design of studies and statistical considerations (section 7.3).

5. Stability evaluation at different stages of production and use

The current concept of the quality assurance of vaccines is based on the overall consistency of production, involving several in-process controls, rather than being based simply on a single lot release assay. The adherence to good manufacturing practices is therefore of critical importance in establishing confidence in the production process. Stability testing should be seen as a continuous process from the development of the vaccine through licensing to post-licensure monitoring. Although the studies at different stages differ, for example, in terms of their design, parameters tested and the environmental conditions to which vaccines are exposed, in essence, this is an ongoing process for monitoring vaccine stability throughout the vaccine life-cycle.

5.1 Choice of stability-indicating parameters and frequency of testing

Depending on the nature of the antigen and other components as well as on the manufacturing process, stability-indicating parameters should be selected on a case-by-case basis.

In the selection of stability-indicating parameters, the potential clinical implications of the observed changes must always be considered. Ideally, stability-indicating parameters should reflect the link between vaccine quality and efficacy or safety as demonstrated in clinical trials. For most vaccines, potency is considered as a stability-indicating parameter that reflects potential impact of environmental conditions on the immunogenicity and subsequent protective efficacy of a vaccine. For example, upper and lower potency specifications for

live viral vaccines reflect the link of vaccine potency both with the minimum dose used to demonstrate the efficacy in clinical trials and the maximum dose shown to be safe.

Every effort should be made to identify stability-indicating parameters during the development of a vaccine, taking into account a potential link between biological activity (e.g. toxicity or potency) and safety and efficacy demonstrated in clinical trials.

Parameters that might change over time but have no correlation with efficacy and safety in clinical terms may in some cases be used to help to demonstrate consistency of production. Manufacturers should define the stability profile and propose stability-indicating parameters for the vaccine in question. This provides assurance that changes in product characteristics, including potency, will be detected by appropriate physicochemical and biological assays.

For live attenuated vaccines, the titre is an obvious stability-indicating parameter that can be directly studied on the intermediate and/or final lot. Parameters other than potency-indicating ones should also be considered since they indicate changes in vaccine quality with unknown effects on efficacy and safety. Such parameters may include, in addition to *in vivo* and *in vitro* potency, antigen content, appearance, pH, general safety, specific toxicity, antimicrobial agent content, completeness of adsorption, sterility, adjuvant (adsorbent) content and changes in physicochemical properties.

For non-live vaccines, it may not be possible or relevant to test the potency directly on an intermediate and this will have to be studied on formulated (e.g. adsorbed) vaccines. The current approach for testing frequency (at 3, 6, 9, 12 and 18 months and every 6 months thereafter) described for pharmaceuticals, does not apply to all vaccines. Therefore, appropriate time points for testing should be chosen to take into account the characteristics of the vaccine in question, the rate of change of the parameter measured, the purpose of testing, study design and subsequent data analysis. Time points as well as stability-indicating parameters should be discussed with the national regulatory authority in the context of study design and data analysis.

5.2 Intermediates

Vaccine production processes involve production of intermediates such as harvests, bulk purified antigens, bulk adsorbed/adjuvanted antigens and final bulks. Unless unstable or immediately needed for logistic reasons, such intermediates are usually not processed immediately and storage periods of up to several years are possible. Stability testing should be performed at different stages of production, namely single harvests, monovalent bulks, multivalent bulks and final bulks. Stability should be adequately tested and documentation

provided for each of the stages mentioned as appropriate for the product under consideration.

In view of these lengthy proposed storage times or shelf-lives, a full dataset demonstrating real-time stability may not be available at the time of authorization of a new product or of a change in the production process. National regulatory authorities may consider granting a licence under the condition that real-time/real-condition stability data would be provided on a continuous basis as they become available.

In such cases, accelerated stability testing may provide useful data to support licensing. It should be stressed that, irrespective of the design of accelerated stability studies, the conclusions will by definition be based on extrapolations from the data collected and therefore will have significant limitations as to their value in predicting real-time stability data. Final acceptance, pre-or post-licensing, of a storage period for an intermediate or a shelf-life for a final product should always be based on real-time/real-condition stability data.

Proposed storage periods should be validated by suitable stability studies and data submitted as part of the licensing dossier. The choice of stability-indicating parameters as well as frequency of testing should be justified. The cumulative nature of the actual age of an antigen by the end of the shelf-life of the final product should be taken into consideration.

5.3 Cumulative age of an antigen in the final product

The stability of the characteristics of a final product should be guaranteed during the whole shelf-life, irrespective of the age of the intermediates at the time they are used in the production process. Total age of all components at the end of shelf-life is considered as cumulative age of the product. In practice, stability data on the final product should include the data generated on the intermediates of different ages used in the final formulation.

Complete stability data covering the total cumulative age of all the antigens in a vaccine may not be available before approval of storage periods and the shelf-life or approval of their extension. Nevertheless, manufacturers are encouraged to collect such data on a continuous basis and to report them to the national regulatory authorities.

The storage conditions and periods for the intermediates should be specified until sufficient evidence has become available to demonstrate that the age of intermediates has no impact on the quality, safety and efficacy of the final product. Accelerated stability studies may also be performed to demonstrate that the stability of the final product is not affected by an aged intermediate.

National regulatory authorities are encouraged to request and assess the data.

5.4 Final lot

5.4.1 Vaccine formulation

The stability of a final lot of vaccine depends on the stability of all intermediates as well as that of the final formulation. Therefore, data on stability of the intermediates as well as on the final formulation should be submitted to the national regulatory authority. In the case of combined vaccines, the stability of each component should be assessed and these data included in the manufacturer's dossier. Cumulative stability and its potential impact on the stability and overall quality of the final vaccine should be carefully considered. Stability testing of the final lot could be performed for different purposes and the details on the design and subsequent data analysis are provided in sections 7 and 8 of these guidelines.

5.4.2 Vaccine presentation, container and closure system

In addition to the data on stability of the final formulation, other factors that may affect the stability of a vaccine during its use should also be tested in the stability study. Potential interactions between the vaccine and container and closure system are particularly important for vaccines in liquid form. The impact of the closure system on vaccine stability and quality in general should be tested by exposing samples to and maintaining them in different positions during a certain period of time. These positions should mimic possible situations that may occur during the transport and storage of the vaccine and that provide contact between vaccine and the closure system (in the upright, horizontal or inverted position).

5.4.3 Stability of freeze-dried vaccines

Data to support proposed use of vaccine after reconstitution, maximum storage period and storage conditions should be generated as part of the stability study performed on the final lot.

In the assessment of freeze-dried vaccines, residual moisture should be specified. Reconstitution period (time needed for reconstitution) and appearance of reconstituted vaccine should be defined.

The stability of a diluent should be tested as a stand-alone component as well as in the context of reconstituted vaccine.

5.4.4 Stability of a vaccine in the case of known "short-time excursions" outside the labelled storage conditions

In general, during production, storage, handling, transportation and use, a vaccine has to be kept under the recommended storage conditions, in particular, temperature, which guarantee the maintenance of its quality and hence its safety and efficacy. All possible measures should be taken to avoid exposure of the

product to inappropriate temperatures either too high or too low (e.g. freezing adversely affects adsorbed antigens). The use of temperature loggers or vaccine vial monitors (VVM) is intended to detect exposure of vaccine to temperatures outside or beyond the recommended limits (10, 11).

For logistic reasons “short-time excursions” outside the validated cold-chain may occasionally be inevitable, in particular during handling and transportation, and use of the vaccine in climatic zones with high temperatures. When such a need is identified for a given vaccine, studies under conditions that mimic, as far as possible, those of the foreseeable exposures should be performed. Such studies should involve exposure to suitable temperatures, higher than those recommended for storage, for a defined period. The studies usually involve parameters reflecting vaccine potency (e.g. immunogenicity, antigen content and molecular size distribution) but, in some cases, may also include other stability-indicating parameters (e.g. free saccharides for conjugated polysaccharide vaccines, tests for molecular integrity and degradation products, abnormal and specific toxicity, reversibility of detoxification and residual moisture). For freeze-dried vaccines, exposure studies on the reconstituted product may also provide useful results.

6. Stability evaluation of vaccines: regulatory considerations

Stability evaluation is a vital part of the assessment of the vaccine quality and safety subject to detailed regulatory oversight. The purpose of stability studies is to help assure that vaccines are of acceptable quality and hence have suitable safety and efficacy profiles at the end of their shelf-lives or storage periods, under the recommended environmental conditions. Stability studies on vaccines are conducted to determine the storage period of intermediates, to determine or modify a maximum shelf-life or minimum release specification for final product, and to monitor vaccine stability post-licensure. Another goal of stability studies is to provide information for subsequent comparability studies following changes in manufacturing or formulation. Stability data helps to ensure that the marketed product remains within specifications for its entire shelf-life.

A stability protocol is an important element of the manufacturer’s dossier and should include all tests performed to support the shelf-life of the vaccine in question. Given that the assurance of stability is a continuous process, the dossier submitted for licensing needs to be supplemented with the data from stability studies completed afterwards. Data provided for licensure should be generated on the lots representative of the intended manufacturing scale production as well as of the final formulation.

National regulatory authorities should ensure that the appropriate stability studies have been performed at all stages of production and that they adequately support the proposed conditions for storage. Changes in manufacturing will necessitate additional stability studies and regulatory approval.

6.1 Stability studies for approval of clinical trials

Vaccines under development should be fully characterized before initiation of phase III clinical trials (1, 2). Sufficient stability data should be generated to characterize stability of the lots during clinical trials. Since the correlates of protection are often not known at this stage, it is usually difficult to define an appropriate potency assay or other stability-indicating parameters. Data generated in previous phases of clinical evaluation (phases I and II) could be used to model doses and other parameters for phase III clinical trials. Potential degradation products that could develop over time should be identified.

In addition to real-time data, accelerated stability data may play a role in providing this information. Mathematical models may be used to estimate the potency of vaccines given in clinical trials.

All relevant documentation and data should be available to the regulatory authorities.

6.2 Stability evaluation for licensing

The stability of a vaccine, and therefore the proposed shelf-life, expiry date and storage conditions should be determined on the basis of the results of real-time stability studies. Stability studies should be performed on material representative of the final manufacturing process and final formulation. Data generated in accelerated stability studies may be used, in addition to real-time stability data, to support a proposed minimum release specification when the final product is subject to temperature excursions during handling and shipping.

Extensive testing during the development of a vaccine should provide the information on stability-indicating parameters. Moreover, it could also help to establish some predictive values of the parameters that could potentially serve for the extrapolation of the data at the later stage. The most accurate predictions are based on biologically relevant mathematical modelling of stability-indicating parameters. The prerequisites for the extrapolation of the data are consistency of manufacturing, quantitative results of the assays performed on clinically relevant parameters, the use of appropriate design of the study and analysis of the data. Further considerations on the design of the studies to support licensing and the analysis of the data are discussed in sections 7 and 8 of these Guidelines.

Studies that support the stability of a vaccine for the purpose of licensing have to be performed as appropriate for a particular vaccine and the

documentation submitted to the national regulatory authorities. Sufficient stability data should be generated to support the proposed shelf-life for the vaccine in its final container. This should be assessed on a case-by-case basis taking into account vaccine characteristics and their potential relevance to clinical efficacy and safety. Stability of final lots as well as stability of intermediates should be demonstrated and supporting data submitted in the manufacturer's dossier.

When a shelf-life of more than 6 months is proposed, and change in a stability parameter is linear, 6 months real-time, real-storage-condition data should be submitted as a minimum. Modelling of the minimum release specification with less than 12 months of data is highly unreliable. The calculated minimum release specification will be artificially high with fewer data. Pilot-scale data may be acceptable providing that manufacturing-scale batches are tested following approval and demonstration of comparability. Real-time, real-storage-condition data should be required for all vaccines. Stability of final bulk or final lot should be determined, the parameters to be measured defined, and specifications set. Accelerated degradation testing should be seen as a support to real-time, real-conditions studies, and not as a replacement for them.

However, some production processes may have very tight timelines (e.g. seasonal influenza vaccines) and in such cases extrapolation of the data generated in previous years may be considered acceptable.

6.3 Post-licensure stability monitoring

Following licensure, continuous monitoring of vaccine stability is recommended. For this purpose, different designs of studies may be employed. The aim of post-licensure stability studies is to support the shelf-life specifications and to refine the stability profile of the vaccine in question. Some details on the design and data analysis are provided in sections 7 and 8 of this document.

Data should be provided annually to the national regulatory authorities.

6.4 Thermal stability testing for lot release

Thermal stability should be considered as a vaccine characteristic that provides an indicator of consistency of production in the context of lot release. The thermal stability test is not designed to provide a predictive value of real-time stability, but to test for conformity with a defined specification for a tested vaccine.

Thermal stability testing is part of lot release specifications for live attenuated vaccines such as oral polio vaccine (OPV), measles, mumps and rubella vaccine (MMR) and yellow fever.

In the current WHO recommendations for individual vaccines, thermal stability is considered as shelf-life specification.

However, the appropriateness of such a test for lot release of inactivated vaccines should be carefully considered and the need for it justified. In principle, if the rate of change has no relevance for the safety and efficacy of a particular vaccine, it would be difficult to justify a thermal stability test at lot release other than as an indication of lot-to-lot consistency. For example, decrease of the antigen content could be detected after exposure of the vaccine to elevated temperatures, but may or may not be directly linked with immunogenicity and subsequent efficacy of the vaccine. Therefore, the appropriateness of such an assay should be carefully considered on a case-by-case basis.

For vaccines under development, the appropriateness of thermal stability testing as part of lot release should be explored. Scientific rationale should be based on the assessment of the actual value of the test in the overall understanding of vaccine quality and the effect of production variables. If there is no added value, then thermal stability test should not be required as a lot release assay.

7. Design of studies and statistical considerations

The objectives of stability studies differ throughout the lifecycle of a vaccine. Stability studies are conducted to:

- determine shelf-life and storage conditions, and to support licensing;
- monitor vaccine stability in the post-licensure period; and
- support manufacturing changes by demonstrating comparability of product manufactured by different processes.

Design of vaccine stability studies should clearly indicate the purpose of the study, the analysis of the results, and subsequent interpretation of the data. In addition, the variability of biological assays should be carefully considered and an appropriate design for the study and for data analysis should be selected.

The vaccine stability study should be supported by a protocol. The study protocol should include the stability assay format (i.e. the number of runs of the assay), as well as the number of and intervals between stability study time-points. The stability of the reference materials is also important. The results of a vaccine stability study may either be subject to acceptance criteria, or may undergo statistical analysis to estimate key vaccine stability characteristics.

7.1 Statistical considerations in design of the vaccine stability study

There are several statistical considerations associated with the statistical design and interpretation of the results of a vaccine stability study. These relate to estimating the risk to the vaccinees associated with receiving unsafe vaccine

(e.g. a vaccine with a potency higher than that demonstrated to be safe in preclinical or clinical studies) or ineffective (e.g. a vaccine with a potency lower than that demonstrated to be effective in clinical studies), as well as of the risk to the manufacturer that a truly acceptable product will not be supported by the analysis.

Stability studies supporting real-time, real conditions should be designed to minimize the uncertainty associated with characterizing the change in the product over time. This can be accomplished in several ways. Increased testing of larger numbers of lots at increased numbers of time-points reduces the statistical uncertainty in the loss rate of the product, and thus provides better information to assure adequate potency and safety throughout the shelf-life of the product. Due to the increased burden placed upon the analytical laboratories, this could be combined with bracketing and matrixing (section 7.2), which may be used to decrease the amount of testing required to determine vaccine shelf-life or the minimum release acceptance criterion. Uncertainty in the loss rate during accelerated stability studies is minimized by performing tests at the beginning and end of the study.

7.2 Selection and testing of samples

When the same final formulation is presented in different volume, unitage or mass, a bracketing design may be considered in the selection of samples. Bracketing is a design of stability study where the same strength and container closure system is used for three or more fill contents so the smallest and the largest container size are considered as representative of all. This design is based on the assumption that the stability of the intermediate condition samples is represented by the data generated at the extremes. This approach may require some data to demonstrate that this assumption is valid.

For each sample, a minimum of three lots of vaccine should be included in the study. An effort should be made to manufacture lots from independent components. If fewer than three lots are used in a study, this should be justified in the stability study protocol. This may result from constraints on availability of stability study material. More than three lots may be used in order to obtain a more reliable estimate of stability loss.

The use of a statistical design intended to ensure that tested samples are representative of all samples is known as matrixing. For this purpose different fractions of samples are tested at different sampling points.

7.3 Assays employed in stability studies and the expression of results

Every effort should be made to use quantitative assays that result in a defined value (e.g. potency in international units or antigen content in micrograms).

Descriptive results reported only as pass or fail should not be used if the assay can provide a defined value. The particular importance of quantitative results is in the determination of the rate of change where the actual measurements are needed for the proper analysis of the data. The use of pass or fail criteria is an exception and has limited value in stability studies. For example, in the case of a sterility test performed at the beginning and at the end of the stability studies, the result is usually presented as “pass”. The interpretation of such an outcome reflects that whatever change might occur over time, this did not affect the sterility of the product. However, such a result cannot be part of any analysis using mathematical models.

Validation of the assays is another issue of critical importance for the stability assessment of a vaccine. Suitable study designs may be used to mitigate the effect of stability assay variability. Calibration to a standard and the use of stable reference preparations in the stability study are crucial and should be carefully considered in the analysis of the data. Comparison with an unincubated sample from the stability lot can reduce the effect of long-term variability of methods such as bioassays. For this purpose, testing samples can be returned to a storage condition under which the vaccine is known to be stable (e.g. -70°C), then tested together with unincubated samples from the stability lot. This strategy is appropriate if the goal of the study is to estimate loss rate. Batch testing can be employed when a reliable estimate of the loss rate is required.

Due to assay variability, larger numbers of lots and shorter intervals, resulting in more frequent testing, increase the risk that individual measurements will appear not to demonstrate truly acceptable product, if each individual result is required to conform to an acceptance criterion. Moreover, this approach also increases the likelihood that inappropriately long expiration dates may be set, increasing the risk that the studies may support the release of product that is not effective throughout the expiration dating period. This risk may be mitigated through the use of a carefully documented protocol, describing the study objectives, the proposed data analysis and the interpretation of the results of the stability study.

7.4 Design of studies in support of product licensure

Vaccine shelf-life and/or release criteria should be supported by real-time studies. Such studies are conducted during the development of vaccines to examine the kinetics of vaccine potency or other attributes. These studies should be conducted on materials that are representative of final process intermediates and commercially packaged product, but can include studies on early development material when a scientifically sound justification can be made. Comparability of full-scale manufacturing and development lots should be demonstrated. The

goal of these studies is to support minimum release potency or maximum shelf-life that will ensure that the product maintains a minimally effective potency throughout its shelf-life, and in some cases, to ensure that degradation products do not exceed levels shown to be safe in clinical or preclinical studies.

Stability studies on a commercially packaged product should support planned exposures of a vaccine to temperatures associated with expected temperature excursions, as well as the storage temperature indicated on the label. This includes conditions for labelling, packaging and inspection, as well as shipping of vaccine to commercial distributors. Accelerated and long-term stability studies can be conducted in parallel rather than consecutively, when the vaccine is stable at a particular storage condition, or when it has been demonstrated that storage at one temperature does not affect stability of the vaccine under a subsequent storage condition.

Long-term stability studies on commercially packaged product should yield sufficient information to reveal the product kinetics as well as to establish shelf-life. Thus, if preliminary studies of packaged vaccine indicate nonlinear kinetics, with early rapid change in the product characteristics, more readings at early time points should be taken to better characterize the kinetics, while later measurements may be taken at wider intervals. More regular intervals may be selected when vaccine kinetics are linear. In this case, studies may also be designed to provide reliable early evidence of product stability.

Stability studies on process intermediates such as bulks are performed to establish a storage period for the intermediate. These may be performed at regular intervals throughout the proposed intermediate storage period and should support a reliable characterization of the kinetics of the intermediate. For example, samples from a bulk that is intended for 3 years of storage at -70°C might be sampled at 6-month intervals. Data from such a study can be used to demonstrate maintenance of a stability characteristic throughout the proposed storage period or can be evaluated by statistical methods such as regression analysis throughout the course of the study.

A minimum of three lots of vaccine should be included in the study. If fewer than three lots are used, this should be justified in the stability study protocol. This may result from constraints on availability of stability study material. More than three lots may be used to obtain a more reliable estimate of stability loss.

Data from studies designed to support expiry dating or release criteria are generally analysed either by comparing individual results with minimum or maximum quantities known to be clinically effective or safe, or by calculating parameters associated with the kinetics of the vaccine attribute, and using that information to set release criteria or expiry dates that provide assurance of efficacy and safety throughout the shelf-life of the product.

7.5 Design of continuous monitoring of post-licensure stability

Post-licensure stability studies should be conducted to monitor consistency of vaccine stability. One or more lots are placed on long-term stability monitoring. Stability parameters that should be included are attributes that relate to safety and efficacy of the product. Some parameters that relate to container closure, or to the integrity of the stability study, such as sterility, may be tested at the end of the study period. A physical, chemical or microbiological integrity test (e.g. dye penetration, decay under pressure or in a vacuum, microbial challenge or immersion) should be done periodically. These tests have several advantages, such as:

- detecting a breach in the container or the closure system during the shelf-life;
- not being time-consuming; and
- reducing the potential for false-positive results of the sterility test.

The number and spacing of stability time-points should be justified in a stability study protocol. Strategic statistical designs may be utilized in conjunction with a prescribed data analysis plan, which documents the interpretation of statistical results, as well as actions that will be taken upon nonconformance with the stability study acceptance criterion.

Data from these studies are often analysed by comparison either with a previously set lot-specific acceptance criterion or a stability-parameter-specific (e.g. slope) acceptance criterion. When stability parameters are calculated from stability monitoring studies, these data may also be used to update stability estimates for the product.

7.6 Design of stability studies and analysis of the data to support manufacturing changes

7.6.1 Design of stability studies supporting manufacturing changes

Accelerated stability studies may be performed to support process changes that may be suspected to have an impact on vaccine stability. Multiple lots (at least 3) of vaccine manufactured by the new process should be studied side-by-side with multiple lots from the current process, at several (at least 3) different temperatures. The temperatures and times should be selected according to knowledge of the stability characteristics of the particular vaccine product. Accelerated stability studies should be designed to obtain reliable estimates of change in the stability characteristic. Two time-points (initial and final time) may be tested at each accelerated temperature to obtain a loss rate at each temperature. Two time-points are statistically optimal when kinetics are known to be linear. More than two time-points should be used when there is evidence of nonlinear kinetics

at the accelerated temperature. Loss rates can be compared statistically across temperatures, between processes, to establish acceptability of the process change.

The plan for analysis of the data, as well as the acceptance criterion for equivalence, should be documented in a stability study protocol.

Long-term stability of the vaccine manufactured after process change may be characterized through the ongoing monitoring of post-licensure stability.

Data from these studies are generally analysed by comparing stability parameters among different lots, including lots manufactured with old and new processes.

7.6.2 Analysis of data from stability studies supporting process changes

When process changes are made, short-term studies at accelerated temperatures may support the conclusion that the process change does not influence vaccine stability. Loss rates from accelerated studies on current product and product manufactured after a process change may be compared to establish acceptable performance of the material manufactured using the new process. One approach to accomplish this is to compare the difference in loss rates at each temperature with a predefined acceptance criterion. A confidence interval on the difference in natural log loss rates at each temperature can be calculated as:

$$\bar{y}_N - \bar{y}_O \pm t_\alpha s \sqrt{1/n_N + 1/n_O}$$

where \bar{y}_N and \bar{y}_O are the average natural log loss rates across lots, for the old and new process materials;

t_α is a statistical constant related to the degree of confidence (usually 95%);

s is the pooled variability in natural log loss rates for old and new process materials;

n_N and n_O are the number of new and old process lots in the study.

The difference in natural log loss rates is used because this is approximately equal to the percentage difference in losses between the new and old process materials. The stability of the new process material can be judged satisfactory if the confidence interval meets the predefined acceptance criterion. If equivalence in stability between the new and the old process materials is postulated, then the confidence interval must fall within the two-sided acceptance criterion. Thus, for example, if the acceptance criterion on the difference is -0.10 to 0.10 and the confidence interval is $(-0.02, 0.08)$, one can conclude that the stability of the new process material is equal to that of the old process material at that temperature. If noninferiority in stability of the new

material relative to the old material is postulated, then the confidence interval must fall above the one-sided acceptance criterion.

Mathematical modelling such as Arrhenius analysis can reveal similarity of loss rates across accelerated conditions. The analysis should not extrapolate from the accelerated conditions to the storage condition on the product label, but rather be a direct comparison of the loss rates at the accelerated temperatures. Consistency of long-term stability of a process change, to the current process, can be monitored through the post-licensure stability programme.

8. Data analysis

The plan for analysis of data from a vaccine stability study should be documented in the stability protocol before initiation of the study. The plan should specify whether individual data points will be compared with acceptance criteria or subject to statistical evaluation. When the data are to be analysed statistically, the type of analysis as well as the interpretation and/or use of the statistical results should be specified.

8.1 Comparing stability study measurements with an acceptance criterion

The analysis of data from a vaccine stability study may require the comparison of stability measurements with an acceptance criterion. In such cases, conformity with the criterion is ensured using a fiducial interval or a confidence interval on the estimated stability assay measurement. This approach is not warranted when the measurement error has been incorporated into the stability acceptance criterion.

8.2 Estimation of stability parameters, including variability in stability estimates

Statistical modelling such as regression analysis may be used to analyse data from stability studies. Modelling can be performed with data collected after three or more stability time-points have been obtained. Early analyses, however, are less reliable than analyses performed later in the shelf-life, and thus should be interpreted carefully. Larger numbers of lots and stability time-points yield more precise estimates of vaccine stability.

8.3 Calculation of expiry period and/or minimum release potencies

In many countries, the expiry periods for vaccine products are calculated by testing a predefined number of lots at predefined intervals and designating the

expiry period as the first time at which a stability measurement falls below an acceptable threshold. This approach has the advantage of simplicity, but may yield spurious results due to assay variability. Data obtained using this type of analysis are not amenable to further statistical analysis, although statistical methods may be used to estimate minimum release potencies that are predictive of satisfactory material at the end of the shelf-life.

Statistical modelling such as regression analysis may be used to analyse real-time vaccine stability data. This method uses a statistical confidence interval on the regression of stability study measurements to determine the maximum time for which a batch is likely to conform to the expiry acceptance criterion.

Alternatively, a manufacturer may wish to calculate a minimum release acceptance criterion, which assures that the batch will remain within the expiry acceptance criterion throughout the vaccine shelf-life. This employs similar methods to those described for shelf-life determination, and may include factors related to in-use conditions in addition to the labelled storage temperature. The loss rates and their associated uncertainties (standard error of the slope) obtained by statistical analysis can be combined together with release assay variability to calculate a minimum release acceptance criterion. The formula used to estimate the minimum release acceptance criterion is illustrated for the case of vaccine potency:

$$\text{Minimum Release Specification} = \text{Clinical Minimum} + \sum t_i \hat{b}_i + z_\alpha \cdot \sqrt{\sum t_i^2 s_{\hat{b}_i}^2 + s_{\text{Assay}}^2}$$

where clinical minimum = the lowest dose of vaccine that shows adequate immunogenicity or efficacy, usually reported as percentage response or percentage protected in the tested population;

t_i = time at the i^{th} temperature;

\hat{b}_i = loss rate at the i^{th} temperature;

z_α = a statistical constant, associated with 95% confidence;

$s_{\hat{b}_i}$ = standard error of estimate of \hat{b}_i ; and

s_{Assay} = release assay variability, expressed as standard error.

Commercial lots are compared to the minimum acceptance criterion upon manufacture, and released to the market if they exceed the minimum release acceptance criterion.

8.4 Analysis of post-licensure stability study data

Analysis of postmarketing stability monitoring study data depends upon the specific goal of the study. One approach is to compare results at each time-point with the predefined acceptance criterion valid until the expiry date.

Conformance with the acceptance criterion is assured using a fiducial interval or a confidence interval on the estimated stability assay measurement. Alternatively, statistical modelling such as regression analysis can be used to estimate the vaccine stability as shown in the stability monitoring study. The predicted value from the regression analysis uses all of the data collected on the lot to estimate the lot characteristic at the specified stability time-point. As with individual stability time-point measurements, conformance to the acceptance criterion is assured using a confidence interval on the predicted value from the regression. When stability parameters are calculated in stability monitoring studies, these data may also be used to update product-specific estimates of stability, normally reducing the uncertainty inherent in these estimates.

9. Stability evaluation of combined vaccines

Each vaccine component (after combination) should be tested to support initial licensure of combined vaccines. Determination of the shelf-life of a combined vaccine should be based on the shortest shelf-life component. Data generated on monovalent vaccines should support stability of a combined vaccine. However, stability of a combined vaccine should not be based on extrapolation of the stability data of the individual components alone.

The issue of cumulative age of intermediates and its potential impact on the vaccine quality and stability of the final product of combined vaccine should be carefully considered.

10. Labelling

Labelling should be adequate for the proposed storage (suitable quality of label) and in general should meet national requirements for labelling. With respect to stability, recommended storage conditions and expiry date should be clearly indicated on the label. Sensitivity of vaccine to some environmental factors (e.g. light or freezing) should be stated together with recommended preventive measures (e.g. the vaccine should not be exposed to freezing temperatures or should be protected from light).

If vaccine vial monitors (VVM) are to be used, adequate stability data should be generated to support selection of appropriate VVM for a specific vaccine. Further details on the use of VVM for different types of products are available elsewhere (11).

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The concept for the guidelines was developed by the WHO Working Group on Stability Evaluation of Vaccines at the meeting held at the Paul Ehrlich Institute,

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Taking into account comments and suggestions provided by the participants at the consultation held in June 2006, the guidelines were finalized.

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