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# **GUIDELINES ON STABILITY EVALUATION OF VACCINES**

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## 1. Introduction

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](http://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 in view of the facts that 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.

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 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, real time and other relevant environmental factors. In addition, the WHO guidelines for nonclinical and clinical evaluation of vaccines, stress a need for stability data to support clinical trial approval (1,2). However, until now there has been no comprehensive guidance document available which deals with the stability evaluation of vaccines at different stages of vaccine development, production, licensing, lot release and post-licensing studies.

At its 51<sup>st</sup> meeting the Expert Committee on Biological Standardization recommended that WHO set up a working group on stability evaluation of vaccines to take this issue forward. 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 a guideline 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 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 the 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 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 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 (ie 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 described. The document effectively gives manufacturers two options for stability testing with respect to the design and data analysis: (a) 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 (b) the "new" method, where statistical evaluation is used to define an expiry date through extrapolation of the data. The manufacturer is encouraged to discuss early in the development process these approaches for the study design and data analysis and their suitability for the product in question with the NRA.

In developing this document, guidelines for stability evaluation of medicines, including biologicals, issued by WHO and other bodies (3,4,5,6,7,8,9) were considered. The present document is not intended to conflict with any of these existing documents but rather to complement them with vaccine specific considerations.

## **2. Scope**

This guideline applies to all vaccines against infectious diseases.

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

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 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 conjugated vaccine is 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** is 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 expected handling and storage conditions. The results are used to recommend storage conditions, and to establish the shelf-life and/ or the release specifications.

**Stability indicating parameters** are 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 the detectable rate of change. Qualitative parameters such as sterility could also be considered but cannot be included in the statistical analysis.

**Shelf-life:** The period of time during which a vaccine, if stored correctly, is expected to comply with the specification<sup>1</sup> 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.

<sup>1</sup> “Shelf-life specifications” are those specifications that should be met throughout the shelf-life of the vaccine (should not be confused with “release specification”).

**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 program, but are used instead to establish protective packaging and container conditions, and to support exclusionary labeling.

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

**Utilization period:** Time 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 indeed in some cases live attenuated microorganisms as active components, as well as stabilizers, adjuvants, preservatives and other substances which together contribute to overall vaccine efficacy and safety. The intention of 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 liquid formulation, and the protective nature of packaging, providing that the closure system of 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 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 susceptible to light in routine stability studies.

Stress testing of extreme environmental conditions such as light or extreme temperatures is not a mandatory test 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, light, and oxidizing agents, freeze thaw, as well as susceptibility to hydrolysis across a range of pH values.

A major problem in assessing vaccine stability is the fact 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 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 of 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 for vaccines. The 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; aggregation of adjuvant etc. 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, specific toxicity etc), are performed prior to or after vaccine exposure to 1) recommended storage temperature (real time real storage conditions studies) or to 2) 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 biological 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 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 mentioned later in the section on the design of studies and statistical considerations (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 simply on a single lot release assay. The adherence to good manufacturing practice is therefore of critical importance in establishing a confidence in 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 in terms of the design, parameters tested, environmental conditions to which vaccines are exposed etc, 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 potential link between biological activity (e.g. toxicity, 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 demonstrating 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 physico-chemical 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, apart from in-vivo/ in-vitro potency, antigen content, appearance, pH, general safety, specific toxicity, antimicrobial agent content, completeness of adsorption, sterility, adjuvant (adsorbent) content, and changes in physico chemical 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.

Current approach for testing frequency (3, 6, 9, 12, 18 and every 6 months afterwards) described for pharmaceuticals does not apply to all vaccines. Therefore, appropriate time points for testing should be set up taking into account 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 NRA 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 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 long proposed storage times/shelf-life periods, full data set 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 giving a license under condition that real-time/ real-condition stability data would be provided on an ongoing basis as data 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 observed and therefore will have significant limitations as to their value to predict real-time stability data. Final acceptance, pre-or post-licensing, of a storage period of an intermediate or of a shelf-life of 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 of 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, shelf-life or approval of their extension. Nevertheless, manufacturers are encouraged to collect such data on an ongoing basis and report them to the National Regulatory Authorities.

The storage conditions and periods of the intermediates should be specified until sufficient evidence has become available demonstrating 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 final product stability 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 of the stability of all intermediates as well as of the final formulation. Therefore, data on stability of the intermediates as well as stability data of 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 data included in the manufacturers 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 section 7 and 8 of this document.

### ***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 vaccine stability 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 and maintaining samples into different positions during a certain period of time. These positions should mimic possible situations that may occur during the transport and storage and that provide contact between vaccine and the closure system (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 recommended storage conditions, in particular, temperature, that guarantee the maintenance of its quality and hence 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 vaccine exposure to different temperatures beyond the recommended ones (1011).

For logistic reasons “short-time excursions” outside the validated cold-chain may at some time 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 of time. The studies would usually involve parameters reflecting vaccine potency (e.g. immunogenicity, antigen content, molecular size distribution etc) 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, 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 have acceptable quality and hence, safety and efficacy profiles, at the end of their shelf lives, or storage periods, at 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 manufacturing or formulation changes. Stability data helps assure that marketed product is within specifications for the entire shelf life.

A stability protocol is an important element of the manufacturer’s dossier and should include all tests performed to support shelf-life of the vaccine in question. Given that stability is

an ongoing process, the dossier submitted for licensing should be completed as the stability studies are ongoing. 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 adequately support the proposed conditions for storage. Changes in manufacturing will necessitate additional stability studies and regulatory approval.

### **6.1. Stability studies for clinical trial approval**

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 (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 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 proposed minimum release specification, when 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 modeling of stability-indicating parameters. 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 this document.

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 final container. This should be assessed on a case-by-case basis taking into account vaccine characteristics and their potential relevance with clinical efficacy and safety. Stability of final lots as well as stability of intermediates should be demonstrated and 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. Modeling of the minimum release specification, however, is highly unreliable with less than 12-months data. The calculated minimum release specification will be artificially high with less data. Pilot scale data may be acceptable providing that manufacturing scale batches are tested following approval and comparability demonstrated. Real time real storage condition data should be required for all vaccines. Stability of final bulk/ final lot should be determined, 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 their replacement.

However, some production processes may have very tight timelines (e.g. seasonal flu 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, ongoing 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 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 to the national regulatory authorities on an annual basis.

### **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. Thermal stability test is not designed to provide a predictive value of real time stability but to test a conformation with defined specification for a tested vaccine.

Thermal stability testing is part of lot release specifications for live attenuated vaccines such as OPV, MMR, 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 such test justified. In principle, if the rate of change has no relevance for safety and efficacy of a vaccine in question, it would be difficult to justify thermal stability test at lot release other than as an indication of lot-to-lot consistency. For example, determination of the antigen content could be detected after vaccine exposure to elevated temperatures but may or may not be directly linked with immunogenicity and subsequent efficacy of the vaccine. Therefore, the appropriateness of such 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 a vaccine's lifecycle. Stability studies are conducted to:

- 1) determine shelf life, storage conditions and to support licensing;
- 2) monitor vaccine stability in the post licensure period, and
- 3) 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 appropriate design of the study and data analysis 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. Vaccine stability study results may either be subject to acceptance criteria, or may undergo statistical analysis to estimate key vaccine stability characteristics.

### **7.1. Statistical considerations in vaccine stability study design**

There are several statistical considerations associated with the statistical design and interpretation of vaccine stability study results. These relate to estimating the risk to the vaccinees associated with receiving unsafe (eg, vaccine with potency higher than demonstrated to be safe in preclinical or clinical studies) or ineffective (eg, vaccine with potency lower than demonstrated to be effective in clinical studies) vaccine, 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 improves the statistical uncertainty in the loss rate of the product, and thus provides better information to assure adequate potency and safety through the shelf life of the product. Due to the increased burden placed upon the analytical laboratories, this may be combined with bracketing and matrixing, 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 are represented by the data generated on the extremes. This may require some data to demonstrate this assumption.

For each sample, a minimum of three lots of vaccine should be included in the study. 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.

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; antigen content in micrograms etc). Descriptive results reported as pass or fail should not be used if the assay can provide a defined value. 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/ fail criteria is an exception and has limited value in stability studies. For example, in the case of 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 outcome reflects that whatever change might occur over time, this did not affect the sterility of the product. However, such result cannot be part of any analysis using mathematical models.

Validation of the assays is yet another issue of critical importance for stability assessment of a vaccine in question. Study design strategies may be employed to mitigate the effect of stability assay variability. Calibration to a standard and the use of stable reference preparations are of critical importance in the stability study and should be carefully considered in the analysis of the data. Comparison to 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 more frequent stability intervals increase the risk that individual measurements will appear not to support 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 dating period. This risk may be mitigated through a carefully documented protocol, describing the study objectives, the proposed data analysis, and the interpretation of stability study results.

#### **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 assure that the product maintains a minimally effective potency throughout its shelf life, and in some cases, to assure that degradation products do not exceed levels shown to be safe in clinical or preclinical studies.

Stability studies on commercially packaged product should support planned exposures of vaccine to temperatures associated with expected temperature excursions, as well as the labeled storage temperature. This includes conditions for labeling, 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 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 characteristic, more 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 employed when vaccine kinetics is linear. Studies may be likewise 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 storage at -70° C might be sampled at 6-month intervals. Data from such a study can be utilized to demonstrate maintenance of a stability characteristic throughout its 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 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.

Data from studies designed to support expiry dating or release criteria are generally analyzed either by comparing individual results to a 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 dating that provide assurance of efficacy and safety throughout the shelf-life of the product.

#### **7.5. Design of ongoing monitoring of post licensure stability**

Post licensure stability studies should be conducted to monitor consistent performance 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 test, pressure/vacuum decay, microbial challenge or immersion tests) should be done periodically. These tests have several advantages, such as the detection of a breach in the container or the closure system during the shelf-life, not time consuming, reduction of 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 to stability study acceptance criterion.

Data from these studies is often analyzed 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 supporting manufacturing changes**

##### **Design of stability studies supporting manufacturing changes**

Accelerated stability studies may be performed to support process changes that may be suspected to impact 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 from 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 utilized 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 analyzed by comparing stability parameters among different lots, including lots manufactured with old and new processes.

### **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 did 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 new process material. One approach to accomplish this is to compare the difference in loss rates at each temperature, to 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;

$t_{\alpha}$  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 percent 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 modeling such as Arrhenius analysis can reveal similarity of loss rates across accelerated conditions. The analysis should not extrapolate from the accelerated conditions to the labeled storage condition, 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 program.

## **8. Data analysis**

The plan for analysis of data from a vaccine stability study should be documented in the stability protocol, prior to initiation of the study. The plan should specify whether individual data points will be compared to acceptance criteria, or subject to statistical evaluation. When the data are to be analyzed 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 to an acceptance criterion**

The analysis of data from a vaccine stability study may require that stability measurements be compared to an acceptance criterion. In such cases, conformance to the criterion is assured 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 modeling such as regression analysis may be used to analyze data from stability studies. Modeling can be performed 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, expiry periods of vaccine products are calculated by testing a pre-defined number of lots, at pre-defined 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 analysis scheme 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 shelf-life.

Statistical modeling such as regression analysis may be used to analyze real time vaccine stability data. This method uses a statistical confidence interval on the regression of stability

study measurements, to determine the maximum time 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 as described for shelf-life determination, and may include factors related to in use conditions in addition to the labeled 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:

$$\text{MinimumReleaseSpecification} = \text{ClinicalMinimum} + \sum t_i \hat{b}_i + z_\alpha \cdot \sqrt{\sum t_i^2 s_{\hat{b}_i}^2 + s_{\text{Assay}}^2}$$

where Clinical Minimum = the lowest dose of vaccine that shows adequate immunogenicity or efficacy, usually reported as percent response or percent protected in the tested population,

$t_i$  = time at the  $i^{\text{th}}$  temperature,

$\hat{b}_i$  = loss rate at the  $i^{\text{th}}$  temperature,

$z_\alpha$  = a statistical constant, associated with 95% confidence,

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

$s_{\text{Assay}}$  = 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 post-marketing stability monitoring study data depends upon the specific goal of the study. One approach is to compare results at each time point to the vaccine end-expiry acceptance criterion. Conformance to acceptance criterion is assured using a fiducial interval or a confidence interval on the estimated stability assay measurement. Alternatively, statistical modeling 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 utilizes 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 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 stability estimates, 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 (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 in the label. Sensitivity of vaccine to some environmental factors (e.g. light, freezing etc) should be stated together with recommended preventive measures (e.g. vaccine should not be exposed to freezing temperatures; should be protected of light; etc.).

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

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