

Annex 5

Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions

| | |
|---|-----|
| 1. Introduction | 238 |
| 2. Scope | 239 |
| 3. Terminology | 239 |
| 4. General considerations for the evaluation of vaccines for use under ECTC | 241 |
| 5. Stability evaluation of vaccines for use under ECTC | 245 |
| 6. Monitoring ECTC | 250 |
| 7. Suggested product labelling information for use under ECTC | 251 |
| 8. Authors and acknowledgments | 252 |
| 9. References | 255 |
| Appendix Product-specific ECTC evaluation of a model monovalent polysaccharide conjugate vaccine | 257 |



Guidelines published by WHO are intended to be scientific and advisory in nature. Each of the following sections constitutes guidance for national regulatory authorities (NRAs) and for manufacturers of biological products. If an NRA so desires, these WHO Guidelines may be adopted as definitive national requirements, or modifications may be justified and made by the NRA.

Abbreviations

| | |
|-------|--|
| CTC | controlled temperature chain |
| ECTC | extended controlled temperature conditions |
| EU | ELISA Unit(s) |
| IFPMA | International Federation of Pharmaceutical Manufacturers & Associations |
| LB | lower bound |
| LL | lower limit |
| MRP | minimum release potency |
| NLT | not less than |
| NMR | nuclear magnetic resonance |
| NMT | not more than |
| NRA | national regulatory authority |
| PS | polysaccharide |

1. Introduction

Vaccines are complex biological products and may undergo degradation during long-term storage under cold chain conditions (for example, 2–8 °C) and this is typically enhanced at higher temperatures. Consequently, establishing the stability characteristics of products is a critical element of the overall evaluation by a national regulatory authority (NRA) to ensure that licensed vaccines remain efficacious at the end of their shelf-life when stored under the approved conditions. In response to the stability assessment needs identified by NRAs, WHO developed guidelines on the stability evaluation of vaccines to assist its Member States (1). While it is well understood that vaccine quality depends on cold chain storage, it is also recognized that immunization programmes in certain regions face substantial challenges in maintaining cold chains in the field, especially during the final stage of distribution in remote areas (2, 3). To address these distribution challenges and expand immunization programmes into specific regions WHO developed a “controlled temperature chain” (CTC) programme. This programme currently requires that a vaccine exhibits a stability profile suitable for a single exposure to at least 40 °C for a minimum of 3 days just prior to administration, while remaining compliant with the approved vaccine specifications. Additionally, the programme requires that the CTC provision should be included in the licensure by the relevant NRA and by WHO prequalification (4).

During the development of these WHO Guidelines, the term “extended controlled temperature conditions” (ECTC) was proposed to distinguish regulatory requirements from WHO CTC programme aspects. This terminology convention is used throughout the following guidance. An ECTC assessment should assure the performance of a vaccine following short-term exposure to temperatures above those of a typical cold chain and could consider any temperature above the traditional 2–8 °C cold chain that might support vaccine distribution. Thus ECTC is independent of the specific programmatic requirements of the current WHO CTC programme. Vaccines licensed for use under ECTC are required to have sufficient information on the approved conditions (such as maximum temperature and time) on the package insert.

An example of an approved ECTC product that is also compliant with WHO CTC programme requirements is the meningitis A conjugate vaccine MenAfriVac. ECTC evaluation and subsequent label approval has made it possible to distribute this vaccine to populations that would otherwise have been difficult to immunize because of the limited availability of traditional cold chains (5, 6). ECTC labelling allows greater flexibility in vaccination campaigns by reducing the burden on health-care workers and, once the vaccine is removed from the cold chain to allow for immunization in remote areas, saving the costs of further refrigeration infrastructure and eliminating the need for wet ice.

Additionally, this on-label NRA-approved approach under the ECTC avoids off-label vaccine administration which is inconsistent with official guidance on best practice (7).

These WHO Guidelines arose from WHO immunization programme requirements (2, 3) and from the resulting discussions of international vaccine stability experts at WHO-sponsored consultations in Ottawa, Canada (8) and Langen, Germany (9). The ECTC guidance provided here is intended to supplement the broader WHO Guidelines on stability evaluation of vaccines (1) and focuses on ECTC-specific issues not covered in existing guidance with as little overlap as possible. The key elements of this document are the applications of the mathematical modelling and statistical concepts in existing stability guidance (1) and related publications (10, 11) to the unique short-term requirements that apply to some cases of vaccine distribution and use (8, 9). Early dialogue between manufacturers and regulators, as well as with public health officials in immunization programmes, is recommended so that those vaccines compatible with ECTC use can be evaluated for licensure by the appropriate NRA.

These Guidelines should be read in conjunction with the existing WHO Guidelines on stability evaluation of vaccines (1). The guidance which follows takes the form of WHO Guidelines rather than Recommendations because vaccines represent a heterogeneous class of agents and the stability testing programme will need to be adapted to suit the product in question. WHO Guidelines allow greater flexibility than Recommendations with respect to specific issues related to particular vaccines.

2. Scope

This document provides guidance to NRAs and manufacturers on the scientific and regulatory issues to be considered in evaluating the stability of vaccines for use under ECTC. Evaluation criteria are provided for the approval of short-term temperature conditions, in addition to those defined for long-term storage of a given vaccine, in situations where the vaccine is exposed to these short-term conditions immediately prior to administration.

This document does not provide guidance on the stability evaluation of vaccines that are inadvertently or repeatedly exposed to temperatures for which they were not licensed.

3. Terminology

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

Accelerated stability studies: studies designed to determine the impact over time of exposure to temperatures higher than those recommended for storage on the physical, chemical, biological, biopharmaceutical and microbiological characteristics of a vaccine. When the accelerated temperature conditions are equivalent to or higher than the ECTC under evaluation, the accelerated stability data can be considered in support of the target ECTC.

Cold chain: a series of storage and transport links used for keeping and distributing vaccines in good condition until use according to the approved long-term storage condition and shelf-life. The typical temperature for the long-term storage condition is 2–8 °C although other approved temperatures can be specified.

Extended controlled temperature conditions (ECTC): approved short-term temperature conditions, above those defined for long-term storage, transportation and use, for a given product immediately prior to administration. Any temperatures above the approved long-term storage temperatures in the cold chain could be considered for ECTC application. The development of CTC terminology and the proposing of the alternative ECTC terminology are described in the Ottawa meeting report (8).

Product-release model: a model that describes the relationship between release and expiry specifications to ensure that the product will meet defined specifications throughout its shelf-life.

Quality attributes: physical, chemical, biological, biopharmaceutical and microbiological attributes that can be defined, measured and continually monitored to ensure that final product outputs remain within acceptable quality limits.

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

Shelf-life: the period of time during which a vaccine, when stored under approved conditions, is expected to comply with the specifications. The shelf-life is determined by stability studies on a number of product batches and is used to establish the expiry date of each batch of a final product.

Stability of vaccine: the ability of a vaccine to retain its physical, chemical, biological, biopharmaceutical and microbiological properties within specified limits to assure clinical performance throughout its shelf-life.

Stability-indicating parameters: quality parameters (direct or indirect indicators of vaccine efficacy or safety) that are sensitive to storage conditions. These parameters are used in stability studies to assure product quality throughout

the shelf-life. Determination of these parameters should result in quantitative values with a detectable rate of change. Qualitative parameters such as sterility may also be considered but cannot be included in the statistical analysis.

WHO controlled temperature chain (CTC) programme: a specific approach to vaccine management that allows vaccines to be kept at temperatures above the long-term storage condition for a limited period of time under monitored and controlled conditions appropriate to the stability of the antigen. Current WHO programme conditions for CTC include a single exposure just prior to administration, tolerating ambient temperatures of at least 40 °C for a limited duration of at least 3 days, with these temperature and time conditions included in the approved label.

4. General considerations for the evaluation of vaccines for use under ECTC

The use of vaccines under ECTC requires an appropriate vaccine stability assessment and consideration of the feasibility of compliance with the approved storage conditions in the field. While the stability evaluation principles described here could potentially be applied to data to support multiple temperature exposures for a vaccine, consideration would then need to be given to how such exposures would be tracked for specific vaccine final containers. When reviewing the potential difficulties in tracking multiple exposures, and ensuring that final containers that have reached the maximum exposure limit are discarded, it was concluded that at this time guidance for an ECTC label should be limited to a single planned exposure of specified duration within the labelled expiry date. Hence, once a vaccine has been stored under ECTC, it should not be returned to normal cold chain storage (for example, at 2–8 °C) in order to prevent the inadvertent administration of vaccine that is potentially out of specification. As experience with ECTC stability assessment and programme implementation expands, this conclusion could potentially be reconsidered in a future guidance update.

An ECTC application could potentially be approved solely on the basis of product-specific stability and other quality data when both of the following conditions are met:

- the approved product specifications, supported by quality attributes of the clinical lots, remain unchanged and the vaccine is expected to be compliant with these specifications following normal storage for the full shelf-life, including the ECTC exposure;
- the battery of tests performed to assess vaccine stability, which may include additional characterization assays in specific cases, has

the capacity to detect changes in potency and/or immunogenicity as well as safety parameters that are predictive of vaccine clinical performance.

Additionally, when a manufacturer has accelerated stability data that bracket the intended ECTC exposure (for example, 40 °C), the potential for interpolation of the data to estimate the decay rate at the target ECTC temperature could be considered on a case-by-case basis. It should be noted that the accelerated stability data must be from lots that represent the current manufacturing process. Vaccines used under ECTC should be capable of withstanding the approved planned exposure conditions regardless of the shelf-life remaining before expiry. These evaluations must involve statistical analysis of stability data to determine the rates of decay under both the approved long-term storage conditions and those of an ECTC exposure. It is essential that adequate potency is available to compensate for any decay over the full approved shelf-life under approved long-term storage conditions, as well as under the planned ECTC exposure (for example, 40 °C for at least 3 days). Consideration of both potency requirements is necessary to address worst-case scenarios where the planned exposure occurs within the shelf-life of a vaccine lot that was filled at or near the minimum release potency (MRP).

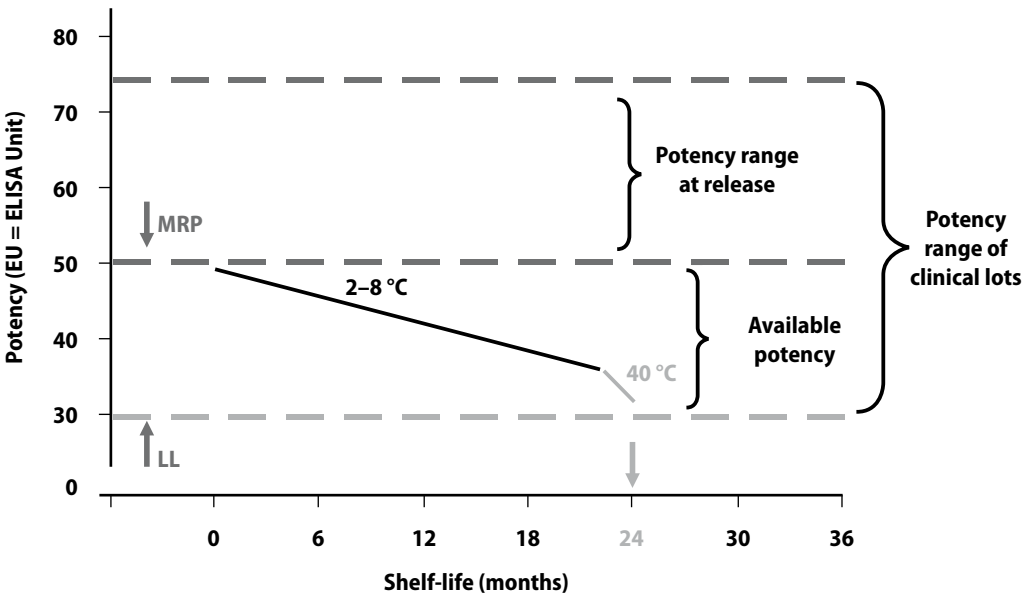
The focus of the current document is on the assessment of potency over the shelf-life of a vaccine given the anticipated loss of potency under ECTC. However, it is also recognized that similar principles may be used to ensure that potentially unsafe degradation products do not exceed approved limits. If exposure to ECTC leads to safety concerns such as undesired degradation products or the potential reversion of toxoids under these accelerated temperature conditions, then such risks should be explicitly evaluated.

Product-specific potency evaluations should be based on decay rates, MRP and an appropriate end-of-shelf-life lower limit (LL) supported by clinical data or experience. This is described both in existing stability guidance and subsequent papers (10, 11) and is critical for ECTC applications, to which the same principles apply. A product-release model (Fig. 1) should be developed on the basis of studies using the manufacturer's assays, along with quality data and other essential information. Labelling a vaccine for ECTC use will require the support of the manufacturer and the approval of the appropriate regulatory authorities.

Fig. 1 illustrates the relationship between the MRP specification of 50 ELISA Units (EU) and the shelf-life (24 months) given the rate of decay (slope) of the potency over both the long-term storage temperature (2–8 °C) and the maximum ECTC temperature (40 °C), and indicates that the vaccine is above the approved LL for the potency (30 EU; supported by clinical lots) at the end of shelf-life. The potency decay assessment should be based on appropriate

statistical analysis of multiple lots with a given degree of confidence (for example, 95%) and should include assay variability (not shown in the figure). As noted below in section 5, logarithmic transformation (log-transformation) of potency data typically permits analysis of stability data by linear regression.

Fig. 1
Graphic representation of a product-release model for an ECTC application



Potency assays used in the assessment of stability should be fit-for-use and are typically validated as accurate, sensitive, robust and stability-indicating. Given the greater chance of product failure after exposure to the ECTC temperatures being considered, the ability of an assay (and even of existing approved assays) to detect quality-related outcomes associated with an ECTC exposure should be re-evaluated. In some cases, supplementary potency assays or key stability-indicating tests linked with the clinical performance of the vaccine should be used. For example, use of the test to ascertain the percentage of free polysaccharide (free PS) in glycoconjugate vaccines should be considered, if not already performed. Other assays, such as those typically performed during product stability testing, should also be considered for use in an ECTC context. These may include assessments of quality attributes that may themselves affect stability (for example, moisture and pH), as well as tests of container integrity under the ECTC (for example, sterility and specific container integrity tests). It is not possible to perform decay modelling on products with potency assays that

have binary outputs (for example, pass/fail). In such cases, supplemental potency assays that are capable of showing the decay of the product's active ingredient (or that can provide a worst-case estimate of that decay) may be considered for use in ECTC evaluation, recognizing the need for conservatism in interpreting the analysis and its results. In the absence of adequate stability-indicating assays for a vaccine, approval of an ECTC label would not be possible solely on the basis of a quality data assessment.

While ECTC approval is not a recommendation for shipping or storage, and unplanned excursions are not within the scope of this document, an approved ECTC label could potentially be taken into account when making decisions on product use in cases of temporary temperature excursions. However, given the finite nature of available potency over the shelf-life of any given vaccine, potency lost through unplanned excursions with specific final containers earlier in the shelf-life would not be available to support the use of those same containers of vaccines during a planned ECTC exposure later in the shelf-life. This highlights the importance of maintaining the cold chain prior to the extreme temperature conditions that the vaccine would be subjected to during a planned ECTC exposure.

For multivalent vaccines, ECTC evaluations must consider all antigens in the product. If one antigen is known to be less stable than other antigens within a specific vaccine then the suitability of the product for ECTC should be based on the least-stable antigen. Potential interference between vaccine components, including adjuvants, stabilizers and preservatives, may also need to be considered, as applicable. To simplify the discussion here, with the exception of comments related to free PS, the focus of this and subsequent sections will be on how to evaluate and manage the available potency of a monovalent vaccine.

At release, a product must possess sufficient potency to ensure clinical effectiveness throughout its shelf-life and to account for assay variability as well as any product decay. If there is insufficient potency available to permit ECTC use, or if it is desirable to extend the time of the ECTC storage condition, several strategies could be considered for enhancing the ECTC potential of a vaccine. For example, the shelf-life under the approved long-term storage conditions could be reduced in order to increase the available potency that could be applied to an ECTC exposure. An example of the use of shelf-life reduction to extend the ECTC storage time is shown in the Appendix below. If such an approach were used, it would be important to assign a unique product name to the ECTC version of the vaccine to avoid confusion in the field. The case study provided in the Appendix also illustrates that the implementation of a lower release specification for free PS would create a larger differential between the release and end-of-shelf-life specifications, which would enhance the ECTC potential of the product. Enhanced ECTC potential could also be achieved by reducing

assay variability, thus reducing the amount of potency required to account for potential errors in initial potency assignment. Finally, with a more accurate characterization of vaccine stability, a manufacturer could reduce the amount of potency required to account for errors in the estimation of shelf-life.

In general, additional clinical assessment for a previously approved product being considered in an ECTC application should be required only where a planned ECTC exposure results in a change of product specifications. For example, a lower end-of-shelf-life potency or a higher release specification that is not supported by clinical experience would potentially require further clinical evaluation. If additional clinical studies could demonstrate that lower potencies were still effective, or that higher-than-approved target release potencies would not result in new or more-frequent adverse events, a manufacturer could submit a regulatory amendment and use the broader potency ranges gained through these approaches to extend the ECTC potential of a product. Field studies in which the clinical evaluation of a vaccine has been performed on a product that has been exposed to temperatures higher than the approved conditions, but without potency and quality testing using the manufacturer's assays, are not considered acceptable from a regulatory perspective. Clinical studies that are intended to support ECTC applications should be performed using a vaccine with known (or modelled) potency at the time of the ECTC use, as determined by the manufacturer's assays.

Finally, when a lyophilized vaccine is being considered for ECTC applications, a stability analysis should be performed for the reconstituted product using the rigorous statistical evaluation principles described in this document. In situations where exposure to an ECTC storage condition could result in changes to the visual appearance of the lyophilized product that do not impact on its clinical performance, the manufacturer should provide relevant supportive data to the NRA considering the ECTC label change. If approved, a description of the potential change(s) in visual appearance should be included in the product leaflet and/or package insert. For liquid multi-dose vials, additional data would be required to demonstrate the antimicrobial effectiveness of the preservative under ECTC.

5. Stability evaluation of vaccines for use under ECTC

Stability evaluation of a specific vaccine planned for use under ECTC must generate sufficient scientifically valid data to support regulatory approval of labelling for such use. This requires assurance that there is sufficient potency available, even with lots near to expiry, to allow for an exposure under ECTC. The best prediction of actual end-expiry potency of any given lot depends on a variety of factors, including release potency of the specific lot, accuracy and precision of the potency assay, and the results of stability studies. Consequently,

statistical evaluation is needed to be able to state that, with a given (usually 95%) degree of confidence, the potency after ECTC exposure at expiry will still be above the minimum threshold needed for product efficacy. It is only through the use of statistical analysis that it is possible to obtain an indication of the level of confidence in results reported at release or in potencies delivered to the vaccine recipient. Therefore, statistical analysis is required to assure the quality of vaccines intended for delivery in the context of ECTC. Because the major ECTC-related concern is usually that the temperature exposure will reduce potency to unacceptable levels, the following guidance focuses on ensuring that the minimum required potency is maintained.

Even when products are sufficiently stable to tolerate an ECTC exposure, poorly designed studies or inappropriate statistical analyses can reduce the likelihood that an ECTC exposure is justified. This section describes study-design and statistical approaches that will improve the likelihood that ECTC exposure can be justified using sound scientific principles.

Although additional clinical studies will not be required for an ECTC approval for most licensed products, it is essential to establish the minimum potency specification for a specific vaccine, through initial licensing studies, for all stability assessments. Changes in specifications (including lowering of end-expiry potency specifications) may require supporting clinical data. Thus, the data package for ECTC applications should include summaries of the initial clinical studies, including the quality data for the clinical lots, to support the end-of-shelf-life potency specification.

The data package should also include stability studies that formally demonstrate that the minimum potency is achieved throughout the time to expiry, including the ECTC exposure. Estimates of the rate (or slope) at which the potency decays (hereafter, referred to as “stability estimates”) at the normal and ECTC temperatures – and an understanding of potential errors in those estimates – are the most important outcomes of the stability studies. The reliability of these stability estimates, and the extent to which the release potency of any lot can be reliably determined, depend in turn on the potency assay.

As mentioned above in section 4, stability studies to support vaccine use in an ECTC context should use the manufacturer’s potency assay in order to preserve a connection between the released product proposed for ECTC use and the original clinical material used to support product efficacy. It is likely that key parameters of the potency assay will already be known from assay validation, including assay accuracy and precision. More reliable estimates of in-use assay precision may sometimes be obtained by other means (for example, by comparing actual with modelled results in the stability analyses). Other data may also be relevant to the estimation of in-use assay precision. Because there are several possible estimates of assay precision that could be used for ECTC-related calculations, the choice of estimate should be scientifically justified; if

a clear justification cannot be made, the more conservative estimate (from the perspective of the ECTC label) should be used.

Statistical analysis of vaccine stability is normally based on a mathematical model and supported by data that describe the kinetics of potency changes at different temperatures for different periods of time. Statistical release models must support the conclusion that the mean potency of final containers in a given lot will, given all stability losses, meet specifications throughout the shelf-life with a given level of confidence (usually 95%), including permitted storage periods outside the long-term storage conditions. Typically, the rate of change (generally loss) of vaccine potency is not a simple linear function of time. Log-transformation of potency data usually leads to a more predictive model that permits analysis of stability data by linear regression. Thus, in most cases, potency data should be log-transformed before analysis. When log-transformation is not used, scientific justification for the use of a more relevant model should be provided. Log-transformation usually provides a preferred model of the biological process (since for most substances the rate of decay at any point in time depends on the quantity of substance present at that time) as compared with direct analysis of non-log-transformed potency data.

Moreover, empirical observation supports the conclusion that potency decay for many vaccines follows first-order kinetics, which are linear following log-transformation – though low precision of the potency assay may make it more difficult to determine whether stability results follow the decay model. In addition, potency measurements are often log-normally distributed; when this is the case, log-transformation may be required to satisfy the statistical assumptions of the modelling, and can further improve the precision of the stability estimate. In all cases, the decay model used should correspond to actual product decay kinetics as observed in stability studies, and this may support the use of non-log-transformed decay models (including linear models) if these models can also be justified as biologically relevant. It should be noted that log-transformation is not always the best approach for stability-indicating assays. For example, increases in degradation products over time usually cannot be modelled on a log scale. Visual examination of the plot of transformed and untransformed stability data can provide an indication of whether mathematical transformations can linearize the decay curve. Sometimes no biologically meaningful model can be identified that fits the data, as may occur when there are multiple phases in the decay kinetics. In this case, decay estimates during ECTC exposures can be estimated by using only the beginning and end results of the stability testing. If the most appropriate choice of model is unclear, selection of the most conservative option is appropriate.

Stability studies should properly evaluate the kinetics of decay, and should indicate that decay rates (after any transformation) are not higher at the end of the observation period than at the beginning. These studies should

include a sufficient number of time points to determine the adequacy of the decay model, while also providing robust stability estimates. Linearity can theoretically be supported by using at least three time points – the starting point, the end-point (corresponding to the desired ECTC exposure time) and ideally the midpoint. However, when assay precision is not high, additional time points will probably be needed to increase the degree of assurance that the change in ECTC-related stability parameters truly follows a linear model. Inclusion of additional time points can also improve estimates of the true rate of change in potency. If linearity has already been established, more precise estimates of decay rates under ECTC can be obtained by testing sufficient numbers of samples at post-ECTC exposure time points as compared to pre-ECTC exposure time points. When decay kinetics are linear, testing at time points beyond the proposed ECTC use can also improve the precision of the stability estimate. It is often assumed that decay rates under ECTC will be similar near the time of release and at expiry, but (as with the rate of decay over the normal storage period) this assumption should be verified. If the rate of decay does vary depending on the time from release then modelling may need to take this into account, along with consideration of the potential uncertainties added by any assumptions that are made. Testing larger numbers of independent samples (batches/lots) can further improve this precision and can potentially increase the likelihood that these studies will support ECTC use, but in all cases a minimum of three lots should be tested.

Typical stability evaluations often include an analysis for “poolability”. The presence of one or more outliers in a stability-indicating assay may indicate unacceptable manufacturing variability and/or could cause a combined decay slope calculated using a small number of lots to be inaccurate. Previous guidance advocated using the worst-case lot for the decay slope estimate when analysis suggested that data from tested lots may not be poolable. However, using the worst-case lot can inappropriately penalize expected variability (over which the manufacturer has no control) and can be a disincentive to conducting more complete testing. It is reasonable to include all tested lots in the stability analysis so long as these lots are considered representative of the licensed (and “in-control”) manufacturing process, and so long as a sufficient number of lots are included to address random variability. Pooling of data from a sufficient number of representative lots should be statistically justified and agreed with the NRA.

Stability testing normally provides information on expected rates of decay (for the linear model, the decay slope) and standard error of the decay slope at “n” different temperatures of exposure (modelling storage, shipping, post-reconstitution and so on) as well as under ECTC (for time t_{ECTC} with decay slope b_{ECTC}). Modelling of stability test results can also provide an estimate of the precision of the potency assay.

A first approximation of the impact of an ECTC exposure is the 95% confidence bound on the expected loss in potency L_{ECTC} as a result of the ECTC exposure. This can be estimated as:

$$L_{ECTC} = -t_{ECTC} \cdot b_{ECTC} + z_{1-\alpha} \cdot (t_{ECTC} \cdot s(b_{ECTC}))$$

...where t_{ECTC} is the time at ECTC temperatures; b_{ECTC} is the decay slope (a negative number) at ECTC temperatures; $z_{1-\alpha}$ is the one-sided z statistic at the confidence level associated with the desired degree of confidence ($\alpha = 0.05$ for 95% confidence bounds); and $s(b_{ECTC})$ is the standard error of the decay slope. If this amount of additional decay in potency beyond that considered by the product-release model is considered acceptable, the product can be accepted for ECTC use.

A more accurate and less conservative estimate can be obtained by calculating the aggregate error associated with all assumptions in the decay model. The product-release model shown above in Fig. 1 defines the needed potency of the product at the time of release. From this information, it is possible to calculate the statistical lower bound (LB) on the mean potency of a product that is released at the minimum release potency and that is exposed to multiple temperature conditions. This is shown as follows:¹

$$LB_{1-\alpha} = MRP + t_1 \cdot b_1 + t_2 \cdot b_2 + \dots + t_n \cdot b_n + t_{ECTC} \cdot b_{ECTC} - U$$

...where $1-\alpha$ describes the statistical confidence level associated with the lower bound ($\alpha = 0.05$ for 95% confidence bounds); MRP is the manufacturer's minimum allowable release potency (usually log-transformed); t_i is time at temperature i (where i is a positive integer used to represent the series of temperatures to which the vaccine may be exposed); b_i is decay slope (a negative number, or zero if positive) at temperature i ; and U is the combined uncertainty associated with the independent estimation of the numbers on the right side of the equation. Typically:

$$U = z_{1-\alpha} \cdot \sqrt{(s_{\text{assay}})^2 + (t_1 \cdot s(b_1))^2 + (t_2 \cdot s(b_2))^2 + \dots + (t_n \cdot s(b_n))^2 + (t_{ECTC} \cdot s(b_{ECTC}))^2}$$

...where $z_{1-\alpha}$ is the one sided z statistic at the confidence level associated with the desired degree of confidence ($\alpha = 0.05$ for 95% confidence bounds); s_{assay} is the assay precision; and $s(b_i)$ is the precision (standard error) of the

¹ As noted, the equations listed are for the general case that could include modelling storage, shipping, post-reconstitution and so on. However, when only considering the normal storage condition and a single ECTC exposure, an example of a simplified form of the equations would be: $LB_{1-\alpha} = MRP + t_1 \cdot b_1 + t_{ECTC} \cdot b_{ECTC} - U$.

decay slope at temperature i . Thus, the expected end-expiry potency is expected to be (with 95% confidence) as low as $LB_{0.95}$ – which accounts for the manufacturer's MRP, the estimated potency losses at the various temperatures and the associated uncertainty.

Without an ECTC exposure, and with omission of the ECTC-associated terms, the above equations yield the minimum potency that an already-licensed product is expected to maintain based on the manufacturer's release model, throughout its time to expiry. Inclusion of the ECTC term allows a reviewer to determine the degree to which potency is affected by the ECTC exposure and whether or not that is acceptable on the basis of clinical experience with the vaccine at that level of potency.

When the LL of potency, defined as the minimum potency below which there is concern about product efficacy (considering the potency results from the clinical lots and post-market experience) has been defined, it is preferable to rearrange the terms of the above equation to determine the MRP required to maintain potency through to expiry, including the ECTC exposure. In essence, this means calculating the minimum amount of potency that must be added to that minimum potency (LL) in order to assure product quality throughout normal storage and handling, including ECTC exposure, as follows:

$$MRP = LL - t_1 \cdot b_1 - t_2 \cdot b_2 - \dots - t_n \cdot b_n - t_{ECTC} \cdot b_{ECTC} + U$$

The product may be released at a higher potency within the approved specification which provides a convenient way to ensure that the release model will support ECTC labelling. If ECTC exposure potential cannot be established then several options could be considered, as outlined above in section 4 (see also the definition of **Product-release model** in section 3 and the related Fig. 1 in section 4). It should be noted that the analytical principles represented in the equations above are the same as those in existing WHO vaccine stability guidance (1) and that they have been expanded to include ECTC exposure. It should also be noted that the equations here are not the only ways to represent these calculations and that other approaches that encompass similar statistical principles could potentially be acceptable where justified.

6. Monitoring ECTC

All vaccines should be kept under the recommended long-term storage conditions with appropriate oversight prior to ECTC exposure. Use of vaccines under ECTC requires specific monitoring of temperature exposure (for example, peak threshold indicator) and time, as well as formal procedures to ensure that the approved maximum temperature and time are not exceeded. Unused vaccines that exceed the maximum approved temperature or time should be

disposed of by suitable procedures. ECTC temperature-monitoring systems need to be able to distinguish vaccines that are still appropriate for use from vaccines that have exceeded the limits imposed by the data supporting ECTC use. The monitoring requirements for ECTC differ from those for long-term storage and transport. The respective monitoring systems for long-term and ECTC storage should be consistent with product stability characteristics. In order to allow an ECTC exposure, the monitoring system should assure that approved long-term storage conditions, especially with respect to temperature, are not exceeded. Prior to vaccine approval for use under ECTC, the relevant stakeholders, which may include the manufacturer, the NRA and the immunization programme, should work together to ensure that an appropriate monitoring system is in place.

7. Suggested product labelling information for use under ECTC

ECTC should be described in the product leaflet and/or package insert in order to provide information to medical practitioners. The statement on ECTC should appear in a separate paragraph in the appropriate section of the label (for example, Storage and Handling).

The ECTC information in the product leaflet and/or package insert should be clear, concise and specific. If a vaccine consists of two or more components (for example, lyophilized vaccine and diluent) then ECTC information should be given for all of the components of the vaccine.

Information to be included in an ECTC statement should take account of the following, if applicable:

- maximum allowed temperature
- maximum time allowed at a specific temperature
- in-use shelf-life after opening (or reconstitution or mixture, if applicable)
- advice on unopened vials exposed to ECTC (for example, on disposal)
- that only a single ECTC exposure directly prior to use or disposal, and within the shelf-life, is permitted.

The following may serve as a model text for the product leaflet and/or package insert text for ECTC use: *The vaccine [and its diluent/solvent or other component] may be kept for a single period of time of up to [x days or x weeks or x months] at temperatures of up to [x °C] immediately prior to administration. At the end of this period, the vaccine [must be disposed of]. This is not a recommendation*

for storage but is intended to guide decision-making when exposure to higher temperatures is planned. [After opening [or reconstitution or mixture], the vaccine can be kept for [x hours or x days] at temperatures of up to [x °C] at which point it must be disposed of].

8. Authors and acknowledgments

The first draft of these WHO Guidelines was prepared by Dr C. Conrad, Paul-Ehrlich-Institut, Germany; Dr E. Griffiths, Consultant, Kingston-upon-Thames, the United Kingdom; Mrs T. Jivapaisarnpong, Department of Medical Sciences, Thailand; Dr J. Kim, World Health Organization, Switzerland; Dr I. Knezevic, World Health Organization, Switzerland; Dr P. Krause, United States Food and Drug Administration Center for Biologics Evaluation and Research, the USA; Dr J. Shin, WHO Regional Office for the Western Pacific, Philippines; Dr D. Smith, Health Canada, Canada; Dr J. Southern, Adviser to the Medicines Control Council of South Africa, South Africa; Dr T. Wu, Health Canada, Canada; taking into account comments received from: Dr B.D. Akanmori, WHO Regional Office for Africa, Congo; Dr S.C. Da Silveira, Agência Nacional de Vigilância Sanitária, Brazil; and Ms A-L. Kahn, Dr A. Meek, Dr C.A. Rodriguez-Hernandez and Ms S. Zipursky, World Health Organization, Switzerland. Acknowledgement is also given to Mr M. Walsh, Health Canada, Canada, for providing expertise on the mathematical modelling and statistical approaches used for ECTC evaluations, and for his contribution to the appendix on evaluation of a model monovalent polysaccharide conjugate vaccine.

The first draft was based on the Ottawa and Langen CTC consultation reports (8, 9) prepared by Dr M. Baca-Estrada, Health Canada, Canada; Dr C. Conrad, Paul-Ehrlich-Institut, Germany; Dr E. Griffiths, Consultant, Kingston-upon-Thames, the United Kingdom; Dr J. Kim, World Health Organization, Switzerland; Dr I. Knezevic, World Health Organization, Switzerland; Dr P. Krause, United States Food and Drug Administration Center for Biologics Evaluation and Research, the USA; Dr M. (Ferguson) Lennon, Consultant, Horning, the United Kingdom; Dr H. Meyer, Paul-Ehrlich-Institut, Germany; Dr V. Oeppling, Paul-Ehrlich-Institut, Germany; Dr M. Pfleiderer, Paul-Ehrlich-Institut, Germany; Dr J. Shin, World Health Organization, Switzerland; Dr D. Smith, Health Canada, Canada; Dr R. Wagner, Paul-Ehrlich-Institut, Germany; Dr T. Wu, Health Canada, Canada; Ms S. Zipursky, World Health Organization, Switzerland.

Acknowledgments are extended to the following participants in the consultations held in Ottawa, Canada, 4–6 December 2012 and Langen, Germany, 4–6 June 2013: Dr M-C. Annequin, Agence nationale de sécurité du médicament et des produits de santé, France; Dr M. Baca-Estrada, Health

Canada, Canada; Dr K. Brusselmans, Scientific Institute of Public Health, Belgium; Dr M. Chultem, Health Canada, Canada; Dr C. Cichutek, Paul-Ehrlich-Institut, Germany; Mr W. Conklin, Merck, the USA; Dr C. Conrad, Paul-Ehrlich-Institut, Germany; Ms D. Doucet, GlaxoSmithKline Biologicals SA, Belgium; Dr W. Egan, Novartis, the USA; Dr L. Elmgren, Health Canada, Canada; Dr D. Felnerova, Crucell Switzerland AG, Switzerland; Dr S. Gairola, Serum Institute of India Ltd, India; Dr E. Griffiths, Consultant, Kingston-upon-Thames, the United Kingdom; Dr D.A. Hokama, BioManguinhos, Brazil; Dr W. Huang, Xiamen Innovax Biotech Co., China; Mrs T. Jivapaisarnpong, Department of Medical Sciences, Thailand; Dr J. Korimbocus, Agence nationale de sécurité du médicament et des produits de santé, France; Dr P. Krause, United States Food and Drug Administration Center for Biologics Evaluation and Research, the USA; Dr H. Langar, WHO Regional Office for the Eastern Mediterranean, Egypt; Dr A. Laschi, Sanofi Pasteur, France; Dr C. Lecomte, GlaxoSmithKline Vaccines, Belgium; Professor H. Leng, Consultant, Somerset West, South Africa; Dr M. (Ferguson) Lennon, Consultant, Horning, the United Kingdom; Ms A. Lopez, Biológicos y Reactivos de México S.A. de C.V., Mexico; Dr A. Luethi, Crucell Switzerland AG, Switzerland; Dr W. Matheis, Paul-Ehrlich-Institut, Germany; Dr A. Merkle, Paul-Ehrlich-Institut, Germany; Dr H. Meyer, Paul-Ehrlich-Institut, Germany; Dr B.L.M. Moreira, Agência Nacional de Vigilância Sanitária, Brazil; Dr K-T. Nam, Ministry of Food and Drug Safety, Republic of Korea; Dr R. Nibbeling, Institute for Translational Vaccinology, Netherlands; Dr V. Oeppling, Paul-Ehrlich-Institut, Germany; Dr D.M. Pascual, Centro para el Control Estatal de la Calidad de los Medicamentos, Cuba; Dr M. Pfleiderer, Paul-Ehrlich-Institut, Germany; Dr M.L. Pombo, Pan American Health Organization, the USA; Dr T. Prusik, Temptime Corporation, the USA; Dr M. Reers, Biological E Ltd, India; Mr T. Schofield, MedImmune, the USA; Ms F.A. Setyorini, PT Bio Farma, Indonesia; Dr S. Shani, Food and Drug Administration, India; Dr I.S. Shin, Ministry of Food and Drug Safety, Republic of Korea; Dr S.C. Da Silveira, Agência Nacional de Vigilância Sanitária, Brazil; Dr D. Smith, Health Canada, Canada; Dr M. Vega, Centre for Genetic Engineering and Biotechnology, Cuba; Dr R. Wagner, Paul-Ehrlich-Institut, Germany; Ms S. Wong, Merck, the USA; Dr T. Wu, Health Canada, Canada; Dr M. Zeng, National Institutes for Food and Drug Control, China; and Dr J. Kim, Dr D.G. Maire, Dr C.A. Rodriguez-Hernandez, Dr J. Shin and Ms S. Zipursky, World Health Organization, Switzerland.

The second draft document (WHO/BS/2015.2268) was prepared by Dr C. Conrad, Paul-Ehrlich-Institut, Germany; Dr I. Feavers, National Institute for Biological Standards and Control, the United Kingdom; Dr K. Gao, World Health Organization, Switzerland; Dr E. Griffiths, Consultant, Kingston-upon-Thames, the United Kingdom; Mrs T. Jivapaisarnpong, Department of Medical

Sciences, Thailand; Dr J. Kim, World Health Organization, Switzerland; Dr I. Knezevic, World Health Organization, Switzerland; Dr P. Krause, United States Food and Drug Administration Center for Biologics Evaluation and Research, the USA; Dr J. Shin, WHO Regional Office for the Western Pacific, Philippines; Dr D. Smith, Health Canada, Canada; Dr J. Southern, Adviser to the Medicines Control Council of South Africa, South Africa; Mr M. Walsh, Health Canada, Canada; Dr T. Wu, Health Canada, Canada, taking into account comments received from: Dr B.D. Akanmori, WHO Regional Office for Africa, Congo; Dr A. Alsalhani, Médecins Sans Frontières, France; Dr M-C. Annequin, Agence nationale de sécurité du médicament et des produits de santé, France; Dr B. Bolgiano, National Institute for Biological Standards and Control, the United Kingdom; Dr J. Bridgewater, United States Food and Drug Administration Center for Biologics Evaluation and Research, the USA; Dr A. Chang, Johns Hopkins University, the USA; Dr A. Cheung, United States Food and Drug Administration Center for Biologics Evaluation and Research, the USA; Ms D. Doucet, GlaxoSmithKline Biologicals SA, Belgium; Dr J. Du, National Institutes for Food and Drug Control, China; Dr W. Egan, Novartis, the USA; Dr S. Gagnet, United States Food and Drug Administration Center for Biologics Evaluation and Research, the USA; Dr D. Garcia, Agence nationale de sécurité du médicament et des produits de santé, France; Dr E. Griffiths, Consultant, Kingston-upon-Thames, the United Kingdom; Dr T. Guo, National Institutes for Food and Drug Control, China; Mr K. Hicks (*International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) representative*), Sanofi Pasteur, France; Ms A. Juan-Giner, Médecins Sans Frontières, France; Dr B-G. Kim, Ministry of Food and Drug Safety, Republic of Korea; Dr J. Korimbocus, Agence nationale de sécurité du médicament et des produits de santé, France; Dr T-L. Lin, United States Food and Drug Administration Center for Biologics Evaluation and Research, the USA; Dr M. Ramos, Public Health England, the United Kingdom; Mr T. Schofield, MedImmune, the USA; Dr J. Shin, WHO Regional Office for the Western Pacific, Philippines; Dr S.C. Da Silveira Andreoli, Agência Nacional de Vigilância Sanitária, Brazil; Dr D. Smith, Health Canada, Canada; Dr T. Prusik, Temptime Corporation, the USA; and Ms A-L. Kahn, Dr U. Kartoglu, Dr D. Petit and Ms S. Zipursky, World Health Organization, Switzerland.

Acknowledgments are extended to the following participants in a consultation held in Geneva, Switzerland, 24 March 2015: Mr F.S. Adeyemi, National Agency for Food and Drug Administration and Control, Nigeria; Dr M. Allin, Pfizer, Belgium; Ms Y. Bai, China Food and Drug Administration, China; Dr J. Bergers, National Institute for Public Health and the Environment, Netherlands; Ms P. Carneiro, Instituto Butantan, Brazil; Dr C. Conrad, Paul-Ehrlich-Institut, Germany; Ms D. Doucet, GlaxoSmithKline Biologicals SA, Belgium; Ms N. Dubois, Pfizer, Belgium; Dr A. Fauconnier, Federal Agency for Medicines and Health Products, Belgium; Dr I. Feavers, National Institute

for Biological Standards and Control, the United Kingdom; Dr S. Gairola, Serum Institute of India, India; Mr K. Gopinathan, Biological E Ltd, India; Dr E. Griffiths, Consultant, Kingston-upon-Thames, the United Kingdom; Dr K. Hicks, Sanofi Pasteur, France; Mr Y. Kaushik, Bharat Biotech International, India; Dr B-G. Kim, Ministry of Food and Drug Safety, Republic of Korea; Dr J. Korimbocus, Agence nationale de sécurité du médicament et des produits de santé, France; Dr P. Krause, United States Food and Drug Administration Center for Biologics Evaluation and Research, the USA; Mr A. Kukrety, Food and Drug Administration, India; Dr J.S. Lloyd, Immunization Supply Chain, France; Dr A. Luethi, Crucell Switzerland AG, Switzerland; Dr B.L.M. Moreira, Agência Nacional de Vigilância Sanitária, Brazil; Dr D.M. Pascual, Centro para el Control Estatal de la Calidad de los Medicamentos, Cuba; Mr C. Perrin, Médecins Sans Frontières, France; Dr T. Prusik, Temptime Corporation, the USA; Mrs J. Rogers, Food and Drugs Authority, Ghana; Ms P.P. Said, PT Bio Farma, Indonesia; Ms C. Schmit, GlaxoSmithKline Biologicals SA, Belgium; Mr T. Schofield, MedImmune, the USA; Dr D. Smith, Health Canada, Canada; Dr J. Southern, Adviser to the Medicines Control Council of South Africa, South Africa; Ms S. Wong, Merck, the USA; Dr T. Wu, Health Canada, Canada; and Ms A-L. Kahn, Dr U. Kartoglu, Dr J. Kim, Dr I. Knezevic, Dr A. Meek and Dr C.A. Rodriguez-Hernandez, World Health Organization, Switzerland.

Further changes were subsequently made to document WHO/BS/2015.2268 by the WHO Expert Committee on Biological Standardization.

9. References

1. Guidelines on stability evaluation of vaccines. In: WHO Expert Committee on Biological Standardization: fifty-seventh report. Geneva: World Health Organization; 2011: Annex 3 (WHO Technical Report Series, No. 962; http://who.int/biologicals/vaccines/Annex_3_WHO_TRS_962-3.pdf?ua=1, accessed 11 December 2015).
2. Meeting of the Strategic Advisory Group of Experts on Immunization (SAGE), Geneva, 10–12 April 2012 [website]. Geneva: World Health Organization; 2012 (http://www.who.int/immunization/sage/meetings/2012/april/presentations_background_docs/en/index.html, accessed 11 December 2015).
3. WHO Immunization Practices Advisory Committee (IPAC), Geneva, 17–18 April 2012. Final meeting report and recommendations. Geneva: World Health Organization; 2012 (http://www.who.int/immunization/policy/committees/IPAC_2012_April_report.pdf?ua=1, accessed 7 February 2016).
4. Vaccine management and logistics: Controlled Temperature Chain (CTC) [website]. Geneva: World Health Organization (http://www.who.int/immunization/programmes_systems/supply_chain/resources/tools/en/index6.html, accessed 14 December 2015).
5. Butler D. Vaccines endure African temperatures without damage: anti-meningitis campaign in Benin delivers more than 150,000 doses with no losses from excess heat. *Nature*. 19 February 2014 (<http://www.nature.com/news/vaccines-endure-african-temperatures-without-damage-1.14744#/ref-link-1>, accessed 14 December 2015).

6. Zipursky S, Djingarey MH, Lodjo J-C, Olodo L, Tiendrebeogo S, Ronveaux O. Benefits of using vaccines out of the cold chain: delivering meningitis A vaccine in a controlled temperature chain during the mass immunization campaign in Benin 2014. *Vaccines*. 2014;32:1431–5 (https://www.researchgate.net/publication/260373472_Benefits_of_using_vaccines_out_of_the_cold_chain_Delivering_Meningitis_A_vaccine_in_a_controlled_temperature_chain_during_the_mass_immunization_campaign_in_Benin, accessed 14 December 2015).
7. Use of MenAfriVac™ (meningitis A vaccine) in a controlled temperature chain (CTC) during campaigns. Geneva: World Health Organization; 2013 (WHO/IVB/13.04; http://apps.who.int/iris/bitstream/10665/86018/1/WHO_IVB_13.04_eng.pdf?ua=1, accessed 7 February 2016).
8. WHO/Health Canada drafting group meeting on scientific and regulatory considerations on the stability evaluation of vaccines under controlled temperature chain. Ottawa, Canada, 4–6 December 2012. Meeting report. Geneva: World Health Organization; 2012 (http://who.int/biologicals/areas/vaccines/CTC_FINAL_OTTAWA_Web_Meeting_report_25.11.2013.pdf?ua=1, accessed 14 December 2015).
9. WHO/Paul-Ehrlich-Institut informal consultation on scientific and regulatory considerations on the stability evaluation of vaccines under controlled temperature chain (CTC). Paul-Ehrlich-Institut, Langen, Germany, 4–6 June 2013. Meeting report. Geneva: World Health Organization; 2013 (http://who.int/biologicals/vaccines/CTC_Final_Mtg_Report_Langen.pdf?ua=1, accessed 7 February 2016).
10. Krause PR. Goals of stability evaluation throughout the vaccine life cycle. *Biologicals*. 2009;37(6):369–78 (abstract available at: <http://www.sciencedirect.com/science/article/pii/S1045105609001201>, accessed 14 December 2014).
11. Schofield TL. Vaccine stability study design and analysis to support product licensure. *Biologicals*. 2009;37(6):387–96 (abstract available at: <http://www.ncbi.nlm.nih.gov/pubmed/19717312>, accessed 14 December 2014).

Appendix

Product-specific ECTC evaluation of a model monovalent polysaccharide conjugate vaccine

The model vaccine and the stability data presented in this appendix were developed on the basis of Health Canada's overall experience with conjugate vaccines and do not represent characteristics or data from any specific product. The analysis presented is also applicable to other stability-indicating parameters, such as vaccine potency. The vaccine example under evaluation is a monovalent conjugate vaccine composed of purified capsular polysaccharide (PS) covalently attached to diphtheria toxoid protein. The final vaccine product is a non-adjuvanted liquid formulation presented in single-dose vials. The normal storage temperature for this model conjugate vaccine is 2–8 °C, with a time to expiry of three years; the temperature under consideration for the ECTC application is 40 °C. The quality attributes monitored in routine stability studies intended for licensure included total PS, free PS, molecular size distribution, free protein, pH and sterility. Free PS is considered a key stability-indicating attribute for polysaccharide conjugate vaccines since in general this parameter is linked to the clinical performance of this type of vaccine. The specification for free PS for this model conjugate vaccine was set as “not more than (NMT) 15%” at release and “NMT 25%” at the end of the shelf-life. A review of manufacturing data indicated that, at release, 90% of the commercial lots contained less than 10% of free PS and 10% of lots contained free PS in the range 10–13%. In addition, vaccine lots containing 5–25% free PS were shown to be safe and immunogenic in clinical studies.

Stability data

Real-time and real-condition stability studies were conducted to establish the shelf-life under normal storage conditions (2–8 °C) and to support the ECTC application. Although a minimum of three lots is required for statistical modelling, analysis of a larger data set (more lots) leads to more-precise estimates. In this example, routine stability-monitoring tests were performed for four commercial vaccine lots stored at 2–8 °C and for an additional four commercial lots stored at 40 °C. In addition, O-acetyl content, nuclear magnetic resonance (NMR) spectrum and immunogenicity (rabbit complement source serum bactericidal assay and immunoglobulin G) in a mouse model were also evaluated to characterize vaccine lots exposed to the 40 °C condition. Analysis

of routine monitoring data revealed that total PS, molecular size distribution, free protein and pH were stable for all lots stored under the 2–8 °C and 40 °C conditions. However, an increase of free PS was observed for all lots, as summarized in Tables 1 and 2.

Table 1
Summary of free PS content at 2–8 °C

| Lot # | Free PS (NMT 25%) | | | | | | | | |
|-------|-------------------|------|-------|-------|-------|-------|-------|-------|-------|
| | 0 | 3M | 6M | 9M | 12M | 18M | 24M | 30M | 36M |
| 1 | 7.53 | 9.58 | 10.73 | 11.17 | 12.54 | 13.51 | 16.07 | NT | 16.05 |
| 2 | 7.01 | 9.36 | 10.77 | 10.32 | 10.59 | 11.92 | 14.60 | 14.56 | 15.03 |
| 3 | 2.38 | 6.01 | 8.13 | 7.46 | 8.94 | 9.37 | 10.08 | 11.09 | 10.88 |
| 4 | 5.71 | 6.15 | 7.85 | 7.77 | 9.02 | 10.87 | 14.37 | 12.21 | 13.77 |

NMT = not more than; M = month; NT = not tested.

Table 2
Summary of free PS content at 40 °C

| Lot # | Free PS (NMT 25%) | | | | | | | | |
|-------|-------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 0 | 1W | 2W | 3W | 4W | 6W | 8W | 10W | 12W |
| 5 | 2.01 | 2.38 | 4.81 | 6.18 | 9.39 | 11.39 | 13.55 | 13.67 | 13.88 |
| 6 | 1.74 | 5.71 | 4.64 | 5.37 | 8.16 | 9.08 | 9.98 | 11.77 | 14.37 |
| 7 | 5.43 | 10.48 | 10.49 | 10.59 | 13.94 | 15.35 | 15.66 | 15.57 | 16.88 |
| 8 | 5.21 | 8.05 | 9.45 | 9.05 | 12.71 | 15.10 | 15.72 | 15.73 | 17.26 |

NMT = not more than; W = week.

Statistical analysis

An initial analysis (1) demonstrated that the free PS data did not fit a linear regression, with or without log-transformation (plots not provided). Because the increase in the stability-indicating free PS is due to the hydrolysis of bound PS, the rate of increase of free PS is the same as the rate of decrease of bound PS. Therefore, the bound PS at each test point can be calculated from the free and total PS on the basis of mass balance. The hydrolysis of bound PS can be analysed as a first-order reaction at a decay rate that is proportional to the concentration

of bound PS. Consequently, the rate of increase of free PS was analysed indirectly through the modelling of bound PS, and log-transformation of the bound PS content at different test points yielded data that were more amenable to linear regression analysis (1). Thus, free PS data obtained in stability studies were converted to percentage bound PS (Tables 3 and 4) and then subjected to log-transformation. A release model was developed to characterize the relationship between bound PS at release and end-expiry, thus permitting evaluation of potential ECTC use.

Table 3
Summary of bound PS content at 2–8 °C

| Lot # | Bound PS (NLT 75%) | | | | | | | | |
|-------|--------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 0 | 3M | 6M | 9M | 12M | 18M | 24M | 30M | 36M |
| 1 | 92.47 | 90.42 | 89.27 | 88.83 | 87.46 | 86.49 | 83.93 | NT | 83.95 |
| 2 | 92.99 | 90.64 | 89.23 | 89.68 | 89.41 | 88.08 | 85.40 | 85.44 | 84.97 |
| 3 | 97.62 | 93.99 | 91.87 | 92.54 | 91.06 | 90.63 | 89.92 | 88.91 | 89.12 |
| 4 | 94.29 | 93.85 | 92.15 | 92.23 | 90.98 | 89.13 | 85.63 | 87.79 | 86.23 |

NLT = not less than; M = month; NT = not tested.

Table 4
Summary of bound PS content at 40 °C

| Lot # | Bound PS (NLT 75%) | | | | | | | | |
|-------|--------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 0 | 1W | 2W | 3W | 4W | 6W | 8W | 10W | 12W |
| 5 | 97.99 | 97.62 | 95.19 | 93.82 | 90.61 | 88.61 | 86.45 | 86.33 | 86.12 |
| 6 | 98.26 | 94.29 | 95.36 | 94.63 | 91.84 | 90.92 | 90.02 | 88.23 | 85.63 |
| 7 | 94.57 | 89.52 | 89.51 | 89.41 | 86.06 | 84.65 | 84.34 | 84.43 | 83.12 |
| 8 | 94.79 | 91.95 | 90.55 | 90.95 | 87.29 | 84.90 | 84.28 | 84.27 | 82.74 |

NLT = not less than; W = week.

Statistical analysis was performed using R version 3.1.1 (2) to estimate the loss of bound PS under both the 2–8 °C and 40 °C storage conditions with 95% confidence, and the key results are summarized in Tables 5 and 6. The analysis was undertaken in the following steps:

1. For each stability lot, the percentage of bound PS at each test point was log-transformed and the slope was calculated using a linear regression model. Plots of the linear regression fit for all stability lots are presented in Fig. A1.
2. Lots 1, 2, 3 and 4, monitored at 2–8 °C, were assessed with respect to slope variability, which was considered acceptable for use of the linear regression model with a pooled (mean) slope for all four lots. The same analysis was also applied to the data set (lots 5, 6, 7 and 8) at 40 °C, which supported the use of a pooled slope.
3. The assay precision (s_{assay}) was estimated by the residual error from the regression analysis using the pooled slope for the corresponding data set.
4. The uncertainty was calculated using formulae described above in section 5. Two examples are:
 - the uncertainty (U) at 2–8 °C for 36 months = $z_{0.95} \cdot \text{sqrt} [(s_{\text{assay}})^2 + (t_{2-8} \cdot s(b_{2-8}))]^2$
 $= 1.644854 \cdot \text{sqrt} [0.01272811^2 + (36 \cdot 0.0001845235)^2] = 0.02361$.
 - U at 2–8 °C for 36 months followed by 3 days at 40 °C
 $= z_{0.95} \cdot \text{sqrt} [(s_{\text{assay}})^2 + (t_{2-8} \cdot s(b_{2-8}))^2 + (t_{\text{ECTC}} \cdot s(b_{\text{ECTC}}))^2]$
 $= 1.644854 \cdot \text{sqrt} [0.01272811^2 + (36 \cdot 0.0001845235)^2 + (0.1 \cdot 0.008625)^2]$
 $= 0.02366$.
5. The change in bound PS was estimated using the linear regression model and the formula provided above in section 5. An example is provided below to illustrate the calculation of the total change in bound PS at 2–8 °C over 36 months, plus 3 days at 40 °C. This is based on the worst-case lots which contain 85% bound PS at release.
 - First step: the log-transformed total decay of bound PS:
 $= (t_{2-8} \cdot b_{2-8}) + (t_{\text{ECTC}} \cdot b_{\text{ECTC}}) - U$
 $= 36 \cdot (-0.002430492) + 0.1 \cdot (-0.07429892) - 0.02365824 = -0.1185858$.
 - Second step: the log-transformed bound PS at end of storage (2–8 °C plus ECTC):
 $= \log_e (\text{bound PS at release}) + [(t_{2-8} \cdot b_{2-8}) + (t_{\text{ECTC}} \cdot b_{\text{ECTC}}) - U]$
 $= \log_e 85 - 0.1185858 = 4.324065$.
 - Third step: bound PS at the end of storage (2–8 °C plus ECTC) = $e^{4.324065} = 75.4949$.

- Fourth step: the decay of bound PS at 2–8 °C over 36 months plus 3 days at 40 °C:
 = bound PS at release – bound PS at the end of storage (2–8 °C plus ECTC)
 = 85 – 75.4949 = 9.5051.

Table 5
 Summary of statistical analysis of bound PS data at 2–8 °C

| Data set | Shelf-life (months) | Pooled slope (per month) | $s(b_{2-8})^a$ | s_{assay}^b | U^c | Decay of bound PS (%) |
|----------------|---------------------|--------------------------|----------------|----------------------|---------|-----------------------|
| 4 lots (0–24M) | 24 | –0.003311 | 0.0002765 | 0.01146 | 0.02178 | 8.1851 |
| 4 lots (0–36M) | 36 | –0.002430 | 0.0001845 | 0.01273 | 0.02361 | 8.9388 |

^a standard error of slope.

^b assay variability, estimated as standard deviation of residuals.

^c combined uncertainty, calculated using the formula described above in section 5.

Table 6
 Summary of statistical analysis of bound PS data at 2–8 °C and 40 °C

| Data set | Pooled slope (per month) | $s(b_{40})^a$ | Months at 2–8 °C | Days at 40 °C | s_{assay}^b | U^c | Decay of bound PS (%) |
|----------------|--------------------------|---------------|------------------|---------------|----------------------|----------|-----------------------|
| 4 lots (0–12W) | –0.04482 | Not done | Not done | Not done | Not done | Not done | Not done |
| 4 lots (0–4W) | –0.07430 | 0.007766 | 24 | 7 | 0.01146 | 0.02199 | 9.5207 |
| | –0.07430 | 0.008625 | 36 | 3 | 0.01273 | 0.02366 | 9.5051 |

^a standard error of slope.

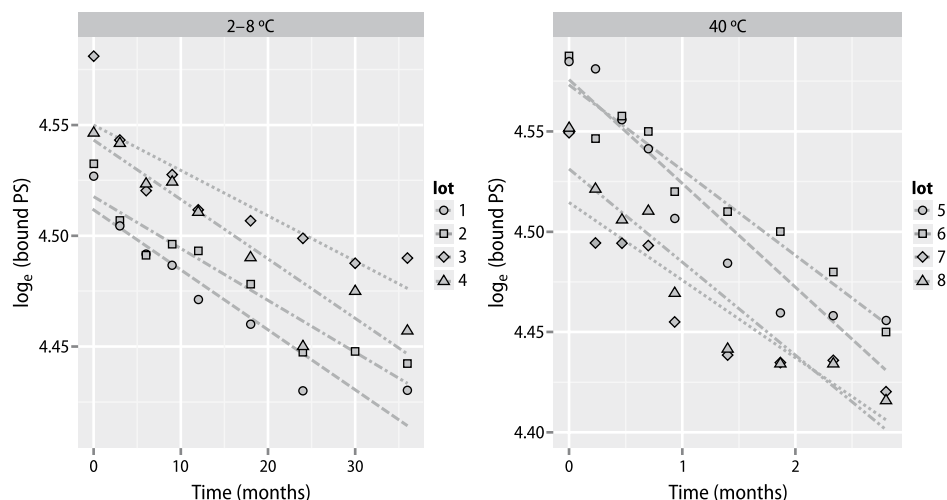
^b assay variability, estimated from the regression analysis residual error.

^c combined uncertainty, calculated using the formula described above in section 5.

W = week.

Fig. A1

Plot of bound PS data for four conjugate vaccine lots



Examination of the plots presented in Fig. A1 indicates that at each of these temperature conditions the decay of bound PS for each lot appears to be better modelled by a straight line when compared to the modelling of free PS. This supports the use of a linear regression model of bound PS in this case. However, it is also noted that the estimated slope of bound PS decay differs over different study periods, especially under 40 °C. As shown in Table 6 the decay rate is higher over 4 weeks (–0.07430/week) compared to that over 12 weeks (–0.04482/month). Differences in rates of change for key quality attributes over a product's shelf-life are not uncommon; therefore it is important to highlight the need to characterize trends when modelling the data and the need to estimate the rate of change based on real-time data over the full study period.

Owing to limited data points at 40 °C, the rate of bound PS decay used for ECTC application was based on the modelling of a 4-week data set, and a conservative approach was taken to limit the total decay of bound PS to slightly below 10%. The statistical analysis summarized in Table 6 revealed an estimated 9.5% loss of bound PS (equal to the increase of free PS) after 36 months storage at 2–8 °C followed by 3 days at 40 °C, or 24 months storage at 2–8 °C followed by 7 days at 40 °C.

Conclusion

The application of the “product release model” to the analysis of free PS can be summarized as:

Release specification (15%) = End of shelf-life specification (25%) minus estimated combined increase at 2–8 °C and 40 °C (upper bound with 95% confidence level)

On the basis of statistical modelling and product-related information, the following can be concluded:

- Different specifications for release and end of shelf-life should be established for free PS for this model conjugate vaccine. A specification of “NMT 25%” at the end of shelf-life is considered acceptable on the basis of clinical lots shown to be safe and immunogenic in clinical studies. A release specification of “NMT 15%” was considered appropriate on the basis of manufacturing capability, which ensures a high compliance rate for commercial lots at release.
- A 36-month shelf-life at 2–8 °C was determined to be appropriate for this model conjugate vaccine. This conclusion ensures that worst-case lots, which contain the highest level of free PS permitted by the release specification (NMT 15%), plus the accumulation of free PS during the storage period (approximately 8.94%), comply with the end of the shelf-life specification (NMT 25%).
- A single storage period of 3 days at 40 °C, prior to immunization, was considered acceptable because the worst-case lots, which contain 15% free PS at release and are stored for almost 36 months at 2–8 °C followed by an exposure of 3 days at 40 °C, were expected to contain approximately 24.51% free PS.
- If a period longer than 3 days at 40 °C is needed, shortening the shelf-life at 2–8 °C from 36 months to 24 months would allow for a single storage period of 7 days at 40 °C. Alternatively, the release specification of “NMT 15% free PS” might be tightened (for example to “NMT 13% free PS”) on the basis of additional manufacturing experience to allow for longer than 3 days of ECTC exposure while maintaining a 3-year shelf-life at 2–8 °C.

As explained above in section 4, clinical testing of a vaccine stored under ECTC would not be necessary as long as a battery of stability-monitoring tests provided sufficient assurance that the critical quality attributes of the vaccine (such as potency) met the specifications supported by clinical experience.

Other than routine stability-monitoring assays, the following characterization tests were also performed to assess quality attributes related to vaccine clinical performance after this model conjugate vaccine was stored at 40 °C for 12 weeks: (a) O-acetyl content remained stable; (b) PS structure was confirmed using NMR; and (c) carrier protein integrity was confirmed by the results of an in vivo immunogenicity test. It was noted that the antigen dose used to immunize the mice was within the dose–response curve, indicating that the in vivo test was of acceptable sensitivity. In conclusion, the available stability data sets assessing critical quality attributes were considered sufficient to support the application of a single storage period of 3-day ECTC (40 °C) for this model conjugate vaccine, within the approved 3-year shelf-life at 2–8 °C.

References

1. Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag New York, 2009.
2. R Core Team. *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing; 2014 (available at: <http://www.R-project.org/>, accessed 22 July 2015).