Regulatory on stability studies for In-vitro diagnostic medical device

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INTRODUCTION

The stability of an in-vitro diagnostic (IVD) medical device reagent is the ability to maintain the performance characteristics over a defined time interval. The stability studies are performed to demonstrate that the product remains viable under the specified storage condition until the claimed time period. Since, stability of the IVD cannot be directly assessed through accuracy, performance attributes or customer testing; it is the responsibility of the manufacturer to evaluate the performance of the product by identifying critical factors affecting the stability through developing stability plan, stability protocol and conducting real-time stability studies, accelerated stability studies, in-use stability studies and transport simulated stability studies.

Stability defined as the ability of an IVD reagent to maintain its performance characteristics within the limits specified by the manufacturer. Stability applies to all In-Vitro Diagnostic reagents, calibrators, sample diluents, and controls. The claimed stability is usually based on the data obtained from real-time testing. In order to provide preliminary stability claims or to qualify changes relative to the product with already established real-time stability claim, accelerated stability studies are performed. Subsequently, such claims should be finalized by performing real-time stability studies (Schedule, 2016). A stability evaluation plan for a particular product should involve logistics of performing stability studies, recommend data analyses, document stability claims, assess product transport con-
dition on stability claims, stability verification and perform real-time, accelerated, and in-use stability studies (Establishing component stability of an in-vitro diagnostic medical device, 2019). A stability claim should be developed by demonstrating that the product meets the requirements at the start and end of the specified period (Establishing stability of an in-vitro diagnostic for WHO Prequalification, 2017).

MATERIALS AND METHODS

Need and requirement for stability testing of IVD
Stability testing is performed to provide evidence on how the quality and performance of an IVD varies with time under the influence of various environmental factors such as temperature, humidity and light, and to establish shelf life for the IVD and recommended storage conditions.

IVD reagent manufacturers use stability testing to determine the duration over which their products remain suitable for their intended use, under defined conditions of storage and handling. This assessment encompasses both shelf life and in-use life (Bs/En Iso 23640, 2015).


1. To provide scientifically sound evidence/proof to support claims made by the manufacturer, to meet the regulatory requirement.
2. To demonstrate the probability of the batches manufactured meets the claimed stability until the end of its life cycle with the predetermined user needs.

Parameters considered before conducting stability studies (Establishing stability of an in-vitro diagnostic for WHO Prequalification, 2017)

1. Product for assessment must be available as per the final design.
2. Storage conditions recommended by the manufacturer for the product.
3. Appropriate product handling procedure.
4. Knowledge on recognizing atypical or Out Of Specification (OOS) test results during the study.
5. Knowledge on product instability.
6. Calibrated and well-maintained instruments.


Critical characteristics of the IVD

1. Generate evidence on risk-evaluated critical constituents.
2. Prove stability for each claimed analytes.
3. Performance evaluation including precision, specificity and sensitivity of the kit.
4. Meet specifications for the shelf life of the IVD.

Finished product

1. All IVD components must be tested to the final manufacturing specifications.
2. Intended label and containers should meet finalized packaging specifications.
3. In most of the circumstances, different buffer volumes should be used for different kit sizes.

Environmental conditions

1. The stability of the IVD should be assessed with predefined confidence limits for batches manufactured and marketed during its life cycle.
2. The data from research and development and the manufacturer’s risk assessment plan determines the number of batches to be used and the duration of exposure.
3. The risk assessment should be performed by identifying the sources of variability, user’s environment, extreme conditions occurring during transportation.

Risk assessment plan

The plan should follow certain minimum conditions such as,

1. Identify the different sources of variation of the IVD constituents.
2. Determine and proper understanding of nature of user environment.
3. The extent of potentially occurring conditions such as; temperature, humidity, vibration, ambient pressure etc. during transportation.
**Minimum number of batches**
The important sources of the variation should be identified through risk assessments. The stability testing should be carried out on more than one batch, due to the impact of batch-to-batch variability.

1. Minimum of three batches to verify shelf life (real-time).
2. Minimum of one batch to ensure the in-use claims.
4. Minimum of one batch for extension of in-use claims.
5. Minimum of one batch for simulated transport testing.

**Assessment of liquid components**
For the components, those are diluted or reconstituted from a freeze-dried state before use, needs special in-use stability studies. It is required to ensure that these products need occasional moving, such that the components are in direct contact between the liquid contents and different parts of the container (such as the container closure system).

**Specimens for the stability testing panel**
The stability testing panel includes well-characterized specimens or other components that are used for monitoring, design control, in-process control, and design validation. These specimens must reflect performance claims of the IVD. The specimens are selected, based on where the product is intended to be used in WHO member states.

In case of multiple specimen types, the stability plan should ensure to meet its claims for an individual specimen to reflect the overall performance and shelf-life of the product. The results and decisions must be documented and traceable.

The stability testing panel needs to be validated and establish rejection & replacement criteria.

The validation of stability testing panel members used is very crucial, and storage of validated protocol is not always possible to do.

Each panel member has been chosen to provide attributes relevant to the intended use, where each member assign an expected value, that is used to set acceptance criteria for that panel member. These expected values are relevant to the outputs of the particular methodology. Panel member meets the acceptance criteria; reflect the stability of the final product.

**Time points**
The study design needs a maximum of 3 testing intervals;

1. An initial baseline test.
2. A test at the point beyond the claimed stability limit.
3. One point in between.

This design represents a high risk and waste of time and resources. When the design fails to meet the acceptance criteria but provides some information regarding deterioration/lack of deterioration of the IVD component, thus, it needs still an effective approach, which can be achieved by incorporating additional predetermined intermediate time point intervals.

Manufacturer in prior should determine the no. of test points, length of the testing interval and choose panel members to be tested at these intermediate test points and mention in the stability plan/protocol. In addition, it requires to analyze zero time values and variance.

**Duration of testing**
Stability testing should be performed beyond the shelf-life determined by the user needs. Minimum of one test point beyond the predetermined user requirement should be conducted.

**Length of the time period**
It is based on the risk assessment plan and provides protection, in case of an unexpected failure of the IVD, because the extrapolation from the earlier time point is not acceptable.

**“Zero time” values and variability**
The value of each measured characteristic should be measured independently for each lot during the stability study. Analysis of the data obtained after the study helps to determine if any statistically significant change has occurred to the measured parameters during the study in any batches. These changes are evaluated to decide if they could represent some important changes that are not detected during the stability testing.

**Zero time values**
These values are established by evaluating each characteristic for each batch on five or more occasion, where each occasion may involve a different
day, a different set of equipment’s or different operator to examine potential sources of analytical variation. This value in further evaluates the variance of measured characteristic with freshly made materials.

RESULTS AND DISCUSSION

Shelf-life stability studies

Shelf life is the period of time for which objective evidence exists to demonstrate that a product remains viable under recommended storage conditions prior to the last day of use/expiry date (Evaluation of stability of In-vitro Diagnostic Reagents, 2009), i.e. shelf life is a period of time from the date of manufacture to the expiry date (Bs/En Iso 23640, 2015). Product stability claims are typically based on data from real-time testing. Testing product under more stressful environmental conditions (accelerated testing) may be useful to establish preliminary stability claims or to qualify changes in the product’s formulation or container closure system relative to products with established real-time stability claims. Accelerated stability data is not sufficient to support the claimed shelf-life; hence, the data should be verified and finalized through real-time testing (Evaluation of stability of In-vitro Diagnostic Reagents, 2009).

The shelf –life of the IVD product is claimed by performing:

1. Real-time stability
2. Accelerated stability studies
3. Component stability studies
4. Transport simulated stability studies
5. Open or In-use stability studies

Figure 1 describes the process to establish product stability claims.

Shelf life studies

Real-time stability studies

Real-time stability studies are designed to establish or verify the shelf life of the IVD reagent when exposed to the conditions specified by the manufacturer. Factors that can affect the stability of an IVD reagent include temperature, humidity, light, transport conditions, vibration, etc. Testing should be performed on at least three different lots manufactured under conditions equivalent to routine production conditions (STED, 2011).

For real-time testing, the test components are stored for temperature as per user requirement (Establishing stability of an in-vitro diagnostic for WHO Prequalification, 2017). The study is extended over a period longer than target shelf-life until significant degradation in the performance of IVD can be seen (In-vitro diagnostic reagent, 2000).

The test material is stored in the final container at a recommended storage temperature throughout the complete stability study. The results are predicted through Levy-Jennings plot.

A sequential approach is used in case of real-time stability study, where IVDs before placing into in-use study or shelf-life under recommended storage condition, subjected to stress-simulating transport (Jeffrey et al., 2016).

When IVDs are stored for a long period after manufacture and before shipping, they are first stored in recommended storage condition, then under stressed transport condition and finally back into recommended storage condition during the course of study (Establishing stability of an in-vitro diagnostic for WHO Prequalification, 2017). Figure 2 describes the real-time stability study process.

Accelerated stability studies

Accelerated stability studies are designed to increase the rate of chemical and/or physical degradation, or change, of an IVD reagent by using environmental stress conditions to predict shelf life (New Medical Device Rules, 2017). The design of an accelerated stability evaluation includes stress conditions of temperature, humidity, light or vibration.

For accelerated stability testing, the test material is stored at different elevated temperature, depending on the recommended initial storage temperature for a predefined period of time through increased rate of through increased rates of physical/chemical degradation caused by those extreme environmental conditions (Establishing component stability of an in-vitro diagnostic medical device, 2019).

In further, the study is conducted until a significant deterioration of the product is seen. The results are obtained in a relatively short period of time (In-vitro diagnostic reagent, 2000).

Arrhenius equation is used to predict the shelf-life of the product. These results do not reflect observed performance under recommended actual storage condition but provide information about degradation of reagent & IVD components (Establishing stability of an in-vitro diagnostic for WHO Prequalification, 2017).
Table 1: Difference between Real-time and Accelerated Stability Studies

<table>
<thead>
<tr>
<th>Real time stability study</th>
<th>Accelerated stability study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verify shelf-life over a period longer than shelf-life</td>
<td>Verify shelf-life within a short period of time</td>
</tr>
<tr>
<td>Conditions as per user requirements</td>
<td>Stressful conditions are used i.e. at a elevated temperature</td>
</tr>
<tr>
<td>Product subjected to stress simulating transport before study (sequential approach)</td>
<td>Provide information about degradation of product and the obtained data must be verified by performing real time stability testing</td>
</tr>
<tr>
<td>Results predicted through Levy-Jennings plot</td>
<td>Results predicted through Arrhenius equation</td>
</tr>
</tbody>
</table>

Accelerated stability data is not sufficient to support the claimed shelf-life; hence, the data should be verified and finalized through real-time testing (Evaluation of stability of In-vitro Diagnostic Reagents, 2009).

Component stability studies

These studies involve antimicrobial and desiccant studies for those components that are made as per finalized approved manufacturing specifications on qualified equipment to meet finalized approved in-process quality control specifications (Establishing stability of an in-vitro diagnostic for WHO Prequalification, 2017).

Component stability studies provide evidence to ensure the component shelf-life does not restrict the final IVD product shelf-life. During these studies, the components are prepared in bulk and used in several different lots of a completed IVD.
For minimum shelf-life studies, three batches of components are verified. And based on risk assessment factors related to variability, one or more subsequent batches can be incorporated.

In the case of component stability, there are two shelf-lives to be evaluated for

1. Bulk material stored before transferring to the final packaging.

Component stability can be evaluated from the functionality of the batch and also by factors that might transform over time, such as turbidity, pH, microbial contamination, change in colour, etc. By these studies, one can distinguish between the turbidity occurred from heat/cold denaturation and turbidity occurred from microbial contamination.

It is necessary to ensure whether the shelf-life of the components made from freshly made constituents and components made from stored raw materials will remain same (ISO, 2011).

To ensure that the IVD has functioned as expected and performed as expected during the use, assay-specific control materials provided by the manufacturers are used (ISO:15198, 2004).

Effectiveness of the antimicrobial preservative used in the IVD must be demonstrated throughout its shelf-life to prevent contamination of the product during storage and use (Jeffrey et al., 2016).

Studies to be performed to ensure the desiccant used in the IVD does not affect its stability over the whole claimed shelf-life during transport, storage and in-use condition (Establishing component stability of an in-vitro diagnostic medical device, 2019).

**Stability during transport**

Transport stability studies ensure that the claimed shelf-life is not affected during & after the transport (STED, 2011). These studies are performed by the manufacturer to evaluate the tolerance of the IVD through stress simulated product exposure to different physical conditions such as inversion, physical handling, vibration, stacking and environmental conditions like temperature, humidity, etc. during and after transportation from the manufacturer to final user (Evaluation of stability of In-vitro Diagnostic Reagents, 2009).

**In-Use Stability Studies**

Duration of time over which the performance of an IVD reagent within its expiration date remains within specified limits, after opening the container system supplied by the manufacturer and put into use under proper standard operating conditions (e.g. storage on the instrument) (New Medical Device Rules, 2017).

Open or "In-use" stability studies can be used to evaluate multi-use reagents; this allows a specific recommendation to test the performance after their opening, reconstitution/dilution or exposure to user environmental conditions. These studies are designed to simulate the product in use. They reflect the routine conditions of use (Evaluation of stability of In-vitro Diagnostic Reagents, 2009) e.g. on-board stability, reconstitution, and open-vial/bottle stability (In-vitro diagnostic reagent, 2000).

In order to obtain the best results and confirm the claimed in-use life throughout its shelf-life, the testing should be done both at the starting and end of their shelf-lives and after the simulated transport challenge

**On-board/Open-vial stability studies** (In-vitro diagnostic reagent, 2000)

These studies determine the stability of IVD after first opening of the primary container either in a refrigerator at 2-8°C or in on-board clinical chemistry analyzer in a refrigerated compartment. In both the cases, the testing is done as per a predefined protocol for a period up to 28days by storing the IVD reagent in the final reagent vial. The conclusions are derived from predefined acceptance criteria.

**Reconstituted stability testing** (In-vitro diagnostic reagent, 2000)

These studies are usually applicable for lyophilized product.

The test materials are tested at predefined time intervals after reconstitution. The results are compared to the results obtained from the freshly reconstituted test materials.

The following Table 1 illustrates some of the differences between real-time and accelerated stability studies.

**Stability study design** (Evaluation of stability of In-vitro Diagnostic Reagents, 2009)

The study designs are used to enter and withdraw the testing products from the test storage conditions of the stability study.

1. Classic Design
2. Isochronous Design
Each protocol design has different strengths, associated risks and prerequisites.

**Classic Design**

The classic study design involves placing the test product at testing storage conditions at the beginning of the study and the product is withdrawn for further testing at defined time points throughout the study period. The study does not any prerequisites to be used.

**Merit**

The results are obtained on the given test day itself.

**Demerit**

1. The design involves long-term variability of the study process, sample variability, instrument variability, laboratory environmental fluctuations, etc., over the study period.

2. In case of any instrument failure during the study, shifting test results from one instrument to another has a great difficulty during classic design.

**Isochronous Design**

The isochronous study design is similar to classic design, involving the placing of test product at testing storage conditions at the beginning of the study and withdrawn for further testing and placed into presumed stable storage condition till the end of the study for a predefined time points during the study. The study is either implemented in a staggered start/end condition.

**Merit**

1. Randomized samples testing in a single batch with common test conditions.

2. Shifting of results in case of instrument failure during the study can be achieved significantly.

3. Most suitable for derivative product stability testing and for monitoring stability testing for products of known stability.


**Demerit**

1. Results are not obtained till the end of the study.

2. Early detection of product instability is tough.

3. Involves high risk in case of new products.

**Stability testing plan** *(Establishing stability of an in-vitro diagnostic for WHO Prequalification, 2017; Schedule, 2016)*

The stability testing plan involves experimental design and acceptance criteria to develop stability claims for new, revised and modified IVD reagents.

The study plan should include,

1. Qualification and training of technical staff.

2. Instrumentation process for storage facilities.

3. Selection of no. of lots/batches to be used and justification if any deviations from the documented procedure.

4. Expected shelf-life of the product as per input documentation.

5. Any proposal along with proper justification to launch a product with a shelf-life derived from accelerated stability data, or product with shorter shelf-life than the input documentation, while the results are still pending for real-time testing documentation.


7. Justification for lot-to-lot variability during the selection of batches/lot, if any.

8. No. of units of each component, collected and stored under each condition.

9. Nature of panel members involved and their inclusion criteria.

10. Expected criteria for each panel member at the beginning and end of the proposed shelf-life.


The plan should be properly implemented and documented to derive meaningful conclusions for IVD performance.

The personnel involved in the conduct of the study should be well qualified and well trained for study set-up, monitoring and testing of study, validation of equipment/instruments, process validation, and selection of samples, risk assessment and recording/documentation of results.

**Stability protocol** *(Ds/En Iso 23640, 2011)*

Stability plan should involve a detailed stability testing protocol.

The stability testing protocol should include;
Stability report

Once the study is completed, the results should be summarized in a stability report. The report should reflect the details regarding the product tested, the objective of the study, test conditions, results, and conclusions (Establishing stability of an in-vitro diagnostic for WHO Prequalification, 2017). The report should be properly documented and should be easily traceable along with study plan, protocols and input requirements (Bs/En Iso 23640, 2015).

Criteria's for stability studies to be fulfilled (Establishing stability of an in-vitro diagnostic for WHO Prequalification, 2017)

1. Prior to initiation, studies must be fully documented with risk evaluations, plans and protocols.
2. Studies and risk management must be taken into consideration, the conditions that are likely to be encountered in which the device is intended to be used.
3. Devices must be subjected to stress transport simulation before being used to establish any form of stability data.
4. Transport simulation studies must cover the extreme environmental conditions ascertained during risk evaluations.
5. Devices used in any stability studies must be similar to finalized manufacturing specifications, including packaging, labelling, etc.
6. Sufficient numbers of independent lots of the device must be evaluated to check, inter-lot variability.
7. If critical components of the device are assigned lives irrespective of the life of the device, the various forms of stability of the device must be proven with those reagents at different stages of their assigned lives.
8. If any inter-independent lot variability is found, the manufacturer must provide evidence that subsequent lots will not have worse stability than that claimed.
9. If the analytic function of the device is out of specification from any cause, including stability failure, the control material must claim to prove the functionality of the device, in addition to any other studies.

CONCLUSIONS

IVD's after stability studies should provide information on safety, quality and performance. Their performances are expected to be accurate and reliable till the claimed shelf-life. The article emphasizes on providing IVD manufacturers, the probable ways and design methods to determine stability shelf-life. Additional information includes assessment of product stability claims during transport, component stability testing, creating a stability testing plan, stability protocol and stability report. These guidelines can be used by IVD manufacturers and regulators. In addition, clinical laboratory personnel can use this information to establish stability attributes for laboratory-developed test methods. There is a need to prepare country-specific guidelines for testing stability for IVD.

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