2022

Guidance on Stability Studies of In-vitro Diagnostic Medical Device (IVDMD)

Draft for public comment

Central Drugs Standard Control Organization
Directorate General of Health Services
Ministry of Health and Family Welfare
Government of India

Central Drugs Standard Control Organization
(In-Vitro Diagnostic Division)

Guidance Document

Title : Guidance on Stability Studies of In-Vitro Diagnostic Medical Device (IVDMD)

Doc No. : CDSCO/IVD/GD/Stability/01/2022

Date : **07.07.2022**

Notice:

This Guidance document is aimed only for creating public awareness about In-Vitro Diagnostic Devices Regulation by CDSCO and is not meant to be used for legal or professional purposes. The readers are advised to refer to the statutory provisions of Medical Device Rules, 2017 and subsequent amendments and clarifications issued by CDSCO time to time for all their professional needs.

Table of Contents

Preface:

CDSCO is pleased to announce the release of the draft Guidance Document on stability studies of In-vitro Diagnostic Medical Device (IVDMD). It is guidance for Manufacturers in Preparation of a Premarket Review Document for Class C and Class D IVDMD Import or Manufacturing License Applications.

This document is intended to aid manufacturers in the preparation of scientific information to be provided in support of claimed shelf life, in use stability and shipping studies for Class C and Class D IVDMD license applications and Post approval change application filed in pursuant to the Medical Devices Rules, 2017 (MDR-2017). This document has been developed by the CDSCO to encourage and support convergence of regulatory systems for medical Devices among jurisdictions. CDSCO is looking to adopt the use of this Guidance for premarket license applications and Post approval change applications. CDSCO strongly encourages manufacturers to follow this guidance when Submitting Class C and Class D IVDMD license applications and Post approval change applications.

This guidance document integrates global regulatory practices within the Medical Devices Rules, 2017 (MDR-2017) licensing requirements for in vitro diagnostic device license applications.

Please note that once implemented, all premarket in vitro diagnostic device license applications are expected to be prepared as specified in this guidance.

The proposed guidance document is being uploaded for the information of all stakeholders likely to be affected thereby for comments, if any.

Any person interested making any suggestions on the proposed draft guidance documents may do so in writing for consideration of the CDSCO with in a period of 30 days from the date of its uploading, through post to the Drugs Controller General (India), CDSCO, FDA Bhavan, Kotla Road, New Delhi – 110002 and through email at ivd-division@cdsco.nic.in.

1. Introduction

1.1. Key concepts

Stability is the ability of an IVD reagent to maintain its performance characteristics over a defined time interval. The purpose of stability studies is to verify the time period and the storage conditions over which stable performance characteristics of an IVD can be claimed.

1.2. Rationale of stability studies

The stability of an IVD is fundamental for its reliable performance over a defined period of time. It is a regulatory requirement for the manufacturer to provide objective, scientifically sound evidence to support all claims made regarding the stability of an IVD. In addition a manufacturer can use stability studies to show that all lots manufactured during the commercial life of the IVD will meet predetermined user needs.

2. Definitions and abbreviations

2.1. Definitions

The definitions given below apply to the terms used in this document. They may have different meaning in other contexts.

- **Accelerated stability evaluation:**
  Study designed to increase the rate of chemical and/or physical degradation, or change, of an IVD reagent by using stress environmental conditions to predict shelf-life.
  NOTE: The design of an accelerated stability evaluation can include extreme conditions of temperature, humidity, light or vibration.

- **Acceptance criteria:**
  A defined set of conditions that must be met to establish the performance of a system. Numerical limits, ranges, or other suitable measures for acceptance of the results of analytical procedures.

- **Accuracy of measurement:**
  Closeness of the agreement between the result of a measurement and a true value of the measurand.
  NOTE 1: Accuracy of measurement is related to both trueness of measurement and precision of measurement.
  NOTE 2: Accuracy cannot be given a numerical value in terms of the measurand, only descriptions such as 'sufficient' or 'insufficient' for a stated purpose.

- **Arrhenius plot:**
  Mathematical function that describes the approximate relationship between the rate constant of a chemical reaction and the temperature and energy of activation.

- **Batch/Lot:**
  Defined amount of material that is uniform in its properties and has been produced in one process or series of processes.

- **Component:**
  Part of a finished, packaged and labelled IVD medical device.
  NOTE : Typical kit components include antibody solutions, buffer solutions, Calibrators and/or control materials

- **Constituent:**
  Raw materials used to make a component. The physical and performance requirements of an IVD that are used as a basis for IVD design.

- **Drift:**
  Characteristic slow change of a metrological value from a measuring instrument.

- **Environmental factors:**
  Variables that might affect the performance or efficacy of IVD reagents e.g. temperature, airflow, humidity, light, this also includes dust and micro-organisms.

- **Evidence:**
  Information which can be proved true, based on facts obtained through observation, measurement, test or other means

- **Instructions for Use (IFU):**
  Information supplied by the manufacturer to enable the safe and proper use of an IVD
  NOTE: Includes the directions supplied by the manufacturer for the use, maintenance, troubleshooting and disposal of an IVD, as well as warnings and precautions.

- **In vitro diagnostic (IVD):**
  A medical device, whether used alone or in combination, intended by the manufacturer for the in vitro examination of specimens derived from the human body solely or principally to provide information for diagnostic, monitoring or compatibility purposes.
  NOTE: IVDs include reagents, calibrators, control materials, specimen receptacles, used, for example, for the following test purposes: diagnosis, aid to diagnosis, screening, monitoring, predisposition, prognosis, prediction, determination of physiological status.

- **IVD reagent:**
  Chemical, biological or immunological components, solutions, or preparations intended by the manufacturer to be used as an IVD.

- **Metrological traceability:**
  Property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties.
  NOTE:- Each comparison is affected by a (reference) measurement procedure defined in a calibration transfer protocol.

- **Performance claim:**
  Specification of a performance characteristic of an IVD as documented in the information supplied by the manufacturer
  NOTE:- This can be based upon prospective performance studies, available performance data or studies published in the scientific literature.
  "Information supplied by the manufacturer" includes but is not limited to: statements in the IFU, in the dossier supplied to CDSCO and /or other regulatory authorities.

3. MDR-2017 requirements:-

The domestic manufacturer or authorized agent, in case of Import, shall submit the duly signed detailed information pertaining to stability of the product in Device master file as specified in point 15-18 of Appendix III of fourth Schedule part II of MDR 2017; Manufacturer should describe claimed shelf life, in use stability and shipping studies, should provide information on stability testing studies to support the claimed shelf life.

i. Claimed Shelf life:

This section should provide information on stability testing studies to support the claimed shelf life. Testing should be performed on at least three different lots manufactured under conditions that are essentially equivalent to routine production conditions (these lots do not need to be consecutive lots). Accelerated studies or extrapolated data from real time data are acceptable for initial shelf life claim but need to be followed up with real time stability studies. Such detailed information should describe:

(a) the study report (including the protocol, number of lots, acceptance criteria and testing intervals);

(b) when accelerated studies have been performed in anticipation of the real time studies, the method used for accelerated studies;

(c) conclusions and claimed shelf life.

Explanation- Shelf life can be derived from the lot with the longest real time stability data as long as accelerated or extrapolated data from all three lots are comparable.

ii. In use stability:

This section should provide information on in use stability studies for one lot reflecting actual routine use of the device (real or simulated). This may include open vial stability and/or, for automated instruments, on board stability. In the case of automated instrumentation if calibration stability is claimed, supporting data should be included. Such detailed information should describe:

(a) the study report (including the protocol, acceptance criteria and testing intervals);

(b) conclusions and claimed in use stability.

iii. Shipping stability:

This section should provide information on shipping stability studies for one lot to evaluate the tolerance of products to the anticipated shipping conditions.

Shipping studies can be done under real and/or simulated conditions and should include variable shipping conditions such as extreme heat or cold. Such information should describe:

(a) the study report (including the protocol, acceptance criteria);
(b) method used for simulated conditions;
(c) conclusion and recommended shipping conditions.

3.1. Manufacturer responsibility

It is a manufacturer's responsibility to ensure that all claims made regarding the stability of the IVD performance are supported by objective, scientifically-sound evidence.

3.2. Standards

CDSCO recommends the following standards for the use in establishment of stability claims: ISO 23640:2013, CLSI EP25-A and ASTM:D4169-14. It is recommended that manufacturers be familiar with these standards and consider them when designing and planning their stability studies.

3.3. Suitability for use in India

The stability studies submitted to CDSCO should accurately reflect the expected environmental conditions and the normal usage conditions/methods encountered by the users in India's States, such as:

- Extremes of temperature for in-use conditions and during transportation
- Extremes of humidity encountered during in-use conditions, transportation and storage
- Dust
- Light, both the amount required for accurate testing/results interpretation and any affects that light may have on the IVD functionality
- Micro-organisms

3.4. Meeting customer requirements

By undertaking well-designed stability studies including periodic verification activities, the manufacturer can demonstrate that the product meets customer requirements, as required by Fifth Schedule (Quality Management System for medical devices and in vitro diagnostic medical devices) of MDR, 2017. Meeting predetermined user expectations, not merely evaluating the capability of an IVD, is a fundamental aspect of development of IVDs. It is a proactive means for the manufacturer to prevent quality problems at lot release and in the post-production and marketing phase.

4. Basic principles for stability testing

4.1. Critical characteristics of the IVD

A well-designed stability study must generate evidence of stability of each of the critical constituents of the IVD (risk-evaluated critical constituents), each of the claimed analytes, and any particular level of performance including precision, sensitivity and specificity of the kit.

Examples:

1) A hepatitis C virus (HCV) assay containing the critical constituents related to detection of NS3 or core proteins should have the stability of all such constituents proven.
2) For an assay designed to detect both IgG and IgM by use of protein A and protein L, the stability of both protein A and protein L should be proven.
3) For CD4, all the antibodies involved (e.g. anti-CD3 and anti-CD4) must be shown to be stable.
4) For an IVD claimed to detect particular seroconversion specimens, or genotypes, or to have specified precision at particular analyte concentrations, or a particular specificity, each of these claims must be proven over the stated shelf-life.

4.2. Finalized product presentation

During stability testing, all IVD components (including the device, calibrator and / or control material, etc.) must be made and tested to the finalized manufacturing documentation and in the finalized packaging including intended labels and containers. All presentations (e.g. different buffer volumes used for different kit sizes) must be used during stability testing.

4.3. Environmental conditions

The study should subject the IVD to a combination of conditions which define the limits of stability for all lots made during its commercial life. The combinations of conditions, durations of exposure and the number of lots to be used will be driven by a manufacturer's risk assessment for the IVD and data from R&D. The risk assessment should take into account at least:

- the variability of the constituent materials (identifying the most important sources of variability);
- the nature of the users' environments; and
- extreme conditions potentially occurring during transportation to those Users.

Boundary conditions for stability studies should reflect realistic extreme conditions that are consistent with the design input requirements for the IVD. The consequent stability studies will prove the IVD capable of meeting performance requirements at the end of its stated shelf-life, after transport to the users.

4.4. Minimum number of lots

Similarly to clinical performance validation, the design of stability studies should take into consideration lot-to-lot variation, with a risk assessment to identify the most important sources of variability. Lot variability is caused usually more by the biological reagents than by the actual manufacturing process. Although existing standards recommend the use of one lot for certain stability studies, the impact of lot-to-lot variability must be taken into

consideration and use of additional lots may be necessary. To ensure the potential of lot-to-lot variability is addressed, optimally lots containing different batches of critical constituents such as nitrocellulose membranes, recombinant antigens, peptides, nucleic acids and enzymes used in nucleic acid testing (NAT), etc. that are as different as possible should be used.

It is advisable to test in triplicate at each point of testing interval and maintain product, calibrator and external sample 20% extra quantities or needs for extra during the study to accommodate all testing need.

Example: For NAT assays, it is critical to use unique enzyme lots for stability studies. Other components including primer, probe and buffer can also be affected by the manufacturing process (purity, pH, DNase & RNase contamination, etc.). For these, different lots are also highly desired that represent both material and process variability.

4.5. Assessment of liquid components:

It is standard best practice in stability studies to ensure that liquid components are in contact with all the parts of their container – vial, sachet or bottle, such as the stopper, the seal and the body of the container. This is sometimes called "inverted container stability" but is probably best studied by ensuring all containers are on their sides and disturbed by movement during the stability study. This aspect needs particular attention for in-use stability studies of those components that are diluted or reconstituted from freeze-dried before use.

4.6. Specimens for the stability testing panel:

The specimens used in the stability testing panels must reflect all the performance claims related to the IVD. If a variety of specimen types (e.g. serum, plasma, whole blood, saliva) is claimed as being suitable for use in the instructions for use (IFU), the stability plan must be designed to provide evidence that the IVD will maintain each of the claims (e.g. sensitivity, specificity, proportion of valid runs, precision) for each of the specimen types for the whole of the claimed shelf-life including transport to the final users.

Evidence should be statistically valid. The stability testing panel must be validated accordingly and rejection and replacement criteria should be established. Regulatory requirements may also dictate the addition of panel members.

A stored validated stability testing panel is not always feasible. For example, this is often the case for assays requiring fresh and/or whole blood specimens e.g. CD4, assays to detect RNA. When replacing panel members, the accuracy of results generated with the replacement material must be confirmed using an appropriate reference comparator method. Replacement criteria for unstable panel members will include the duration for which a critical member will give valid results.

4.7. Validation of stability testing panel

The validation of the stability testing panel members used is critical. Stability testing panel members themselves must be stable, and they must monitor parameters that are useful to control the component involved.

Stability testing panel members are chosen deliberately to ensure each member has an attribute pertinent to the intended use. As with lot release testing, the goal of stability testing is to ensure that the test method appropriately monitors the functionality of the antigens, epitopes, and antibodies that are relevant to the intended use at the end of the assigned (shelf/in use) life.

For instance, the intended use claim may be that early seroconversion specimens are detected. To show that this claim is true at the end of the product's life, a very early seroconversion specimen is included in the stability panel. This specimen may be a weakly reactive IgM specimen.

An expected value is then assigned to each panel member and this is used to assign the acceptance criteria for that panel member. The value for each member is assigned in a measurable manner relevant to the outputs of the particular methodology. For instance, the acceptance criteria for each panel member may be assigned in terms of sample-to-cut-off ratio, cycle time (CT) values, and band intensity measured semi-quantitatively/quantitatively.

In the example of a weakly reactive IgM seroconversion specimen, the specimen at the start of shelf life may have a reading score on an RDT of 1+ out of 4, assigned by using a semiquantitative value based on band intensity. The acceptance criteria may be that all reactive specimens remain reactive, and all non-reactive specimens do not react in the assay.

As such, panel members must be chosen that not only will be relevant to demonstrate the intended use, but have values that will appropriately detect and therefore monitor any deleterious effects of storage. A strong positive specimen, which has a 4+ out of 4 semi-quantitative reading value, may remain giving this reading despite decay in the assay, whereas a specimen with a reading of 1+ out of 4 (with an assigned acceptance criteria of remaining positive) is more likely to give an indication of the ongoing stability of the assay.

Thus, it is essential to know that where a panel member meets acceptance criteria, this is a true reflection of the stability of the product and not due to the inability of the specimen result to reflect this change.

4.8. Time points

A simple study design requires minimum three testing intervals:

- an initial baseline test and
- a test at the time point beyond the claimed stability limit
- and one point in between.

However, this is a high risk approach that has the potential for wastage of time and resources. If the IVD does not meet the acceptance criteria at the end of testing there is little information about the deterioration of the component or IVD (or lack of deterioration) in the interim period.

A more effective approach is to test at predetermined time point intervals. The manufacturer should decide on practical intermediate test points. The number and length of testing intervals should be determined in advance and form part of the stability plan/protocol. This planning will help to understand the resources required to execute the experiment.

Testing of all panel members is not required at all test/time points. However testing with all panel members is required at the initial, the second last and the last test/time point of any of the study specimen types. The manufacturer should decide on practical intermediate test points at which a smaller minimal number of panel members are tested. There should be a documented rationale for the choice of the panels used at the intermediate test points (e.g. representative members, specimens that are close to the medical decision points and at the extremes of the assay range tested).

4.9. Duration of testing

Testing conducted in stability studies should extend beyond the shelf-life determined from the user needs. The shelf-life should be assigned based on a risk assessment of the lot-to-lot variability in signal change at the end of shelf life. At a minimum, testing should extend at least one time point (one testing interval) beyond the determined user requirement. This provides a safeguard in the event of unexpected IVD failure at the end of the testing period, in which event extrapolation from an earlier time point would not be considered acceptable.

It is recommended to utilize standardized units of measure for the entire study

(e.g. Unopened kit shelf life are always measured in months; opened kit /reagent stability in days or weeks)

5. Shelf-life studies

5.1. Requirements for determination of shelf life

The stated shelf-life of an IVD must be based on real-time experimental results. Accelerated studies or extrapolated data from real time data are acceptable for initial shelf life claim but need to be followed up with real time stability studies. Such detailed information should describe:

(a) the study report (including the protocol, number of lots, acceptance criteria and testing intervals);

(b) when accelerated studies have been performed in anticipation of the real time studies, the method used for accelerated studies;

(c) conclusions and claimed shelf life.

Explanation,- Shelf life can be derived from the lot with the longest real time stability data as long as accelerated or extrapolated data from all three lots are comparable.

5.1.1. Real-time stability studies

Real-time stability is determined using storage temperatures derived from user requirements, over a period longer than the required life of the IVD. Where a range of storage temperature is claimed (e.g. “Store at 4–40°C”), CDSCO expects the studies will provide evidence for stability over the whole of the temperature range for at least the length of the claimed shelf-life. Exceptionally, where claimed stability is restricted to a limited range e.g. “Store at 2-8°C”, it is acceptable that stability studies are conducted at a single temperature within this range.

A sequential approach should be used, in which IVDs are first submitted to stresses simulating transport before they are placed into a shelf-life or in-use study. This approach best simulates the real-life situation, where products will first be transported to the end-user and then stored under the recommended conditions before use, possibly almost until the end of their labelled shelf-life.

5.1.2. Accelerated stability studies

Accelerated stability studies are designed to predict the shelf-life of an IVD from the increased rates of chemical and/or physical degradation caused by extreme environmental conditions (e.g. elevated temperature at higher humidity). If the Arrhenius equation is used to calculate the expected life at temperatures other than those actually used, then the parameters of the equation must be derived from the data and not assumed.

Accelerated stability studies provide results in a relatively short time. However the results of these studies are made using assumptions about the degradation of reagents and IVD components that may not reflect their performance under normal conditions of storage and use.

6. Component stability studies

6.1. General principles

6.1.1. Testing on final specifications

Component stability studies, including antimicrobial and desiccant studies, must be performed using components made according to finalized and approved manufacturing specifications – ideally to validated manufacturing scale – on qualified manufacturing equipment and meeting finalized and approved in- process quality control (QC) specifications.

6.1.2. Considering component stability

Sometimes components of IVDs are prepared in bulk and stored before being used in several different lots of a completed IVD. The design input documentation should define how long components are likely to be stored before use. With that information, component stability studies should be planned to give evidence that component labelled lives will not restrict IVD labelled lives: an IVD cannot have a labelled life beyond that of any of its dependent components.

Shelf-lives of components manufactured in bulk and used in several different lots of an IVD can be verified as for the IVD itself – three lots of the component as a minimum for shelf-life studies and, depending on documented risk assessment related to variability, one or more lots subsequent to change. The evaluated lots of the component must differ in batches of critical constituents but, again subject to documented risk assessment, may all be tested in their final presentation with a single set of the other components which will be used together to constitute the IVD.

Examples of stored components:

Wash solutions and substrates for enzyme immunoassay (EIA), amplification reagents for nucleic acid testing, calibrators for quantitative tests, manufactured and stored in their final labelled vials ready to be put into a kit Component stability can be assessed from the functionality of the lot and also by factors related to the component itself, such as turbidity, colour change, microbial contamination and pH of liquid components changes over time.

Depending on the IVD and the conditions it is subjected to it may be necessary to distinguish between turbidity that arises from heat/cold denaturation and turbidity that arises from microbial contamination.

6.1.3. Considering constituent stability

The plan should also consider whether components made from new constituents (antigens, recombinant antigens, enzymes, antibodies, membranes) will have the same lives as components made from stored raw materials. Although this aspect is difficult to study, some evidence should be provided supporting the use of stored constituents, as well as a plan to evaluate lot-to-lot variance from different critical constituents.

The choice of the reagents to be used to measure the performance of the constituent under study (either materials of proven shelf-life or freshly made) needs substantial consideration.

Examples of stored constituent: Purified recombinant antigens and monoclonal antibodies stored in aliquots ready for dilution and striping onto RDT membranes or other supports

6.2. Stability of control materials

Assay specific control materials provided by the manufacturer are to show that an IVD has performed as intended during use. The manufacturer must be able to demonstrate that the loss of signal of a control does not occur at a different rate from the loss of signal from a validated stability testing panel member or from genuine, critical specimens; otherwise a failed IVD might be regarded as still functional. Thus the stability of the control material must accurately reflect the stability of the assay. A control that is more stable than the IVD and other components, or incorrectly set values for the control material, must be avoided

Example: It is seen in dossiers relating to IVDs submitted by the manufacturer that a positive run control will produce a signal of >2.0 optical

density (OD) in a freshly manufactured lot, and the IFU will state that an OD > 0.8 for the same control qualifies a run. Thus, the IVD could have lost more than half its activity and still appear functional, although some critical specimens are shown in the dossier to have very weak signals on freshly made IVDs. This is not considered appropriate unless data can be provided that demonstrate that the critical specimens will still be detected at the end of shelf life.

6.3. Antimicrobial stability and efficacy

6.3.1. Rationale

IVDs are used in areas which are not necessarily clean and sterile, and antimicrobials are not stable under some circumstances. Bacterial and fungal organisms relevant to the environment of use should be identified in the design input risk assessment, and antimicrobial preservatives should be chosen to avoid contamination of the product. The manufacturer must obtain evidence that the antimicrobial preservative and concentration chosen is stable and effective against the micro-organisms of concern throughout the claimed shelf-life and in-use shelf life.

6.3.2. Study conditions

The studies should reflect expected in-use conditions in opened containers: clean, particle-free laboratories do not usually reflect universal user environment for suggested methods. Examples of bacterial groups to consider are spore-forming bacteria, fungus, indigenous bacteria, bacteria found in the environment of the country of manufacture, as well as use of a negative control. Specific examples include Aspergillus niger, Bacillus subtilis, Candida albicans, Escherichia coli, Salmonella species, Pseudomonas aeruginosa, Clostridium sporogenes and Staphylococcus aureus.

Antimicrobial preservative effectiveness, as measured by the viable microbial species load present in kit components, should be demonstrated during development, during scale-up, and throughout the shelf-life.

Testing for antimicrobial preservative content should normally be performed at release. Under certain circumstances, in-process testing may suffice in lieu of release testing. The acceptance criteria for in-process testing should remain part of the specification.

6.4. Desiccant functionality

Desiccants affect the stability of the entire IVD. Stability studies must show that the desiccant will support the product over the whole claimed shelf-life within the predetermined extremes of transport, storage and in-use conditions.

Note:

1) CDSCO recommends that a self-indicator (a humidity indicator that changes colour upon saturation) be part of the desiccant design. However, CDSCO strongly recommends against the use of cobalt dichloride, the most commonly used humidity indicator, as it is a carcinogenic substance.

2) Sachets are preferable over tablets, since labelling such as “Do not eat” is more visible. There have been anecdotal reports of desiccants in a tablet formulation being mistaken for antimalarial medicine.

7. Stability during transport

7.1. Rationale

Transport stability studies evaluate the tolerance of an IVD to the kinds of environmental conditions (e.g. temperature, humidity, dust) and physical conditions (inversion, vibration, physical handling, stacking) to which it is likely to be subjected in the time between shipping from the manufacturer to its final user. They should provide evidence that there will be no impact on the IVD performance over the whole of its stated shelf-life after recommended transportation methods for the IVD. The manufacturer should assess the potential impact of multiple factors and justify and document whether or not to include them in the evaluation.

CDSCO expects that a transportation challenge should precede the real-time determination of shelf-life This serves to determine that transportation conditions do not reduce the shelf-life of the IVD.

In some cases, it might be acceptable to test the product only over the transport simulation duration, without a subsequent long-term study under normal storage conditions. If that is done, shelf-life must be established under specified storage conditions along with a stringent, evidence-based risk assessment of the probabilities of extreme transport stress affecting the performance at the end of the claimed life.

7.2. Challenge conditions

Determination of the stability during transportation of an IVD should take into consideration the local routes, transport means and transit used to supply the IVD, usually defined in the design input risk assessment. It is not necessary to test the IVDs to the point where it is no longer usable, but merely to validate the window of transport conditions within which the IVD will retain its claimed performance to the end of its stated shelf-life. However, knowledge of the possible limitations of an IVD and at what point the IVD becomes unusable is useful to a manufacturer when trouble shooting post-market problems. CDSCO expects the manufacturer to consider that the product might continue to be subjected to sub-optimal storage conditions at the end-user.

Example: While a static challenge of 45°C for 3 days might represent conditions seen during actual transport of a IVD, a more stringent challenge of cyclical higher and low temperatures (including freezing) for a longer period of time and under vibration might better cover a ‘worst case scenario’ of shipment, storage and subsequent transportation to the end-user.

7.3. Number of lots

For transport studies, at least one lot of the IVD can be used.

7.4. Multiple stress test sequences

Appropriate sequences may be developed on the basis of data from actual product transport studies. Testing multiple stress sequences allow a manufacturer to identify the most cost- and/or resource effective transport conditions from a set of alternatives while ensuring adequate product stability protection.

Note: Environmental conditions investigated as part of a stability study must reflect those likely to be encountered in resource- limited States. Temperatures at some airport tarmacs can exceed 40°C while temperatures encountered during air transport fall below 0°C. Significant delays can be encountered at all times and especially during wet season transport to remote health centres.

7.5. Physical conditions

Physical handing can be both manual and mechanical. The relevant user and commercial factors should be identified as part of the design input risk assessment and the packaging and shipping methods developed accordingly. Reference defines a number of factors to be considered, and their evaluation: drop, impact, compression, vibration, repetitive shock, longitudinal shock, cyclic exposure, vacuum, impact, inversion; along with the size, weight, and composition of the packaging.

7.6. Simulated versus actual challenge

An actual shipping challenge can be used to verify the conditions found in the simulated transportation challenges. However, it should only replace a simulated shipping challenge when there is an appropriate risk evaluation and with experience and data already actively collected from similar products and documented in detail (for example it is insufficient to note “no complaints”). In the R&D phase, actual data from shipping can be used to define the conditions needed for an appropriate simulation of extremes. However, in the post-production phase actual shipping challenges often do not explore the full range of shipping conditions that could be encountered, including extreme values.

8. In-use stability studies

8.1. Rationale

In-use stability of an IVD is the period of time over which components retain adequate performance, after transport to the users, once they are opened, reconstituted and/or diluted and exposed to the environmental conditions in which they will be used.

If a range of conditions for use is stated in the IFU (e.g. use at 15–40°C) evidence should be provided to prove the stability over that range with all the specimen types (e.g. serum, whole blood, oral fluid) claimed. It is considered best practice that the manufacturer extends the stability range by 5°C at the lower and upper end of the proposed acceptable range on the

labelling for all components to ensure that that the claimed stability ranges is acceptable.

8.2. Conditions of use

Determination of the in-use stability of an IVD and/or its components should reflect routine conditions of use of the IVD. Freeze-thaw stability should be considered to address reagents which are exposed to multiple freeze-thaw during use.

Note: In-use stability studies must take into account environmental conditions and usage conditions encountered by users and States, such as exposure to extreme temperatures, humidity, dust, light and micro-organisms.

8.3. Multiple in-use stability claims

Depending on the way in which the IVD is used it may be necessary to have several in-use stability claims. In situations where multiple stability claims are made, a manufacturer must provide evidence from testing that investigates routine use supporting each of the claims.

Examples:

1) A reagent may have a stated period of stability once it has been placed on-board an instrument and another period of stability once it is in active use (i.e. during actual use/testing).

2) Multiple use reagents (e.g. buffers) may repeatedly be exposed to high temperatures during the day while in use and exposed to lower temperatures when not in use and stored in the refrigerator. The actual use of the multiple use reagent – squeezing of bottles, exposure of the lid and tip to working surfaces, hands, exposure to dust and light – also affect stability. Stability studies should take into account all of these factors.

9. Lots used in stability studies

9.1. Considering variability

Stability studies must take into consideration all possible sources of variation within and between manufactured lots. For most IVDs it is likely that differences between batches of the biological reagents will cause the most variance. Factors to consider include apparently minor, technically-uncontrollable differences in culture and purification for recombinant antigens and antibodies; synthesis and purification for primers,

probes and peptides; undocumented production changes of an outsourced buffer component and the lot of nitrocellulose membrane used in lateral-flow IVDs. At a minimum, lots chosen for stability studies should be different in the critical constituents, e.g. different purification and/or culture batches for all recombinant antigens and monoclonal antibodies. If pilot or small scale lots are chosen, special attention must be paid to the potential for variability. However, the sources of variation will depend on the particular process, product and component, and should be identified during product development risk analyses.

Use of different batches of critical components ensures that the stability evidence obtained is more likely to be representative of long-term manufacture. Any variability found can be taken into consideration when assessing the outcome of the studies against the design input requirements and when making claims. This minimizes user problems and hence complaints.

9.2. Testing the final configuration

Shelf-life, in-use and transport stability must be determined for the finalized product configuration, in terms of:

- manufacturing specifications;
- release-to-market QA criteria;
- packaging and labelling; and
- validated manufacturing scale on qualified manufacturing equipment.

Note:

Testing methods should be as included in the IFU of the finalized IVD.

It is important that it can be established that the stability studies were conducted on the IVD as submitted to CDSCO for approval. Even changes perceived as small (e.g. change in production scale, bulk container materials, supplier of a critical biological, change in vial stopper) can have unexpected effects on stability and other performance characteristics. After such changes, a stability plan and study is needed again. Manufacturers should have change control procedures in place compliant with ISO 13485. Stability studies undertaken in the R&D phase of the product lifecycle are important to understand how to design the product so it will meet the final stability requirements in the input documentation. However, these studies are not sufficient for submission to CDSCO since they might not reflect the final design and manufacture of the IVD.

9.2.1. Exceptions

If any of the above criteria are not met (for example if “pilot lots” or small scale lots are used, or if the IFU is not finalized), strong evidence must be provided that the evaluated materials will perform exactly the same as the final product.

Note: In some exceptional circumstances, where it is not possible to sample from actual production lots, samples from pre-production or development lots might be used. If this is the case, manufacturers should justify why production lots were not used, and they should provide robust evidence that the lots chosen are expected to behave identically to the production lots. Data concerning lot-to-lot variability must still be submitted. Although CDSCO will consider the available evidence on its merits, this preliminary information must be followed by stability claims conducted on production lots. A post-Approval commitment may be required to amend this situation when the manufacturer is able to produce fully qualified production lots.

9.3. Number of lots required for testing

As per MDR 2017 Testing should be performed on at least three different lots manufactured under conditions that are essentially equivalent to routine production conditions (these lots do not need to be consecutive lots) be used to verify shelf-life; one lot reflecting actual routine use of the device (real or simulated) be used to verify in-use claims; one lot to evaluate the tolerance of products to the anticipated shipping conditions be used to verify Shipping stability.

Note:

It is not acceptable to sample IVDs from a single manufactured lot but label them so that they appear to have been taken from three separately manufactured production lots. This aspect will be investigated during an onsite inspection by CDSCO. Non-compliance with this requirement may result in a major Non-compliance under the MDR 2017.

9.4. Components of lots required for testing

Stability work is performed using materials in their final packaging, with intended labelling. If there is more than one variant of the IVD (e.g. pack size differences) any potential effects on performance, including stability must be assessed.

In particular, if different reagent-container sizes are used in packs intended for different numbers of use, stability evidence should be obtained on all variants, even if the contents of the containers are identical.

Once component shelf-lives are assigned use relatively fresh components and components which have progressed into their assigned shelf-life in the different production lots used in the establishment of the product shelf-life.

10. Stability protocol

Stability studies should be well designed, scientifically sound, well implemented, well recorded and able to deliver meaningful conclusions about IVD performance.

This will minimize the time and resources taken by the manufacturer to generate appropriate evidence and by the regulatory authority to assess it.

It is good practice to prepare, within the mechanisms of a quality management system (QMS), a plan for the investigation of each aspect of IVD stability. A well-developed study protocol, with clearly defined objectives, responsibilities and pass/fail criteria should be developed, reviewed and internally approved in advance of testing. The protocol should be associated with the design input requirements.

It is essential that the study protocol takes into account the intended use of the product to ensure that these elements are covered within the stability studies. The results of the stability studies support the claims in the instructions for use. Careful forward planning will make a significant contribution to ensuring that sufficient resources are made available, effective experiments are performed and both experimental results and associated documentation are recorded in an appropriate manner.

10.1. Responsibilities

The study protocol should outline responsibilities and applicable training for said responsibilities of all staff involved in the study. The R&D department is usually responsible for set up of the study and testing of newly developed IVDs, monitoring, and any equipment validation if required, and for the documentation of the testing plan and sample selection.

The R&D department should nominate a responsible person for investigating failures. The QA department should nominate a responsible person for conducting risk assessments, if IVD fails to meet the requirements of the design inputs. Performance evaluation is not to characterize an IVD but to show that it meets (or exceeds) predetermined qualities.

10.2. Preparing the testing plan

A complete, detailed description should be prepared that fully documents everything to be done and the expected outcomes. Authorization of the protocol should be obtained internally in advance of starting work. The protocol should include the following details.

- Qualification and training of technical staff performing the work
- Biohazard issues identified with reagents
- The instrumentation, including storage facilities or rooms, validation, calibration, monitoring, servicing
- The batch numbers of kits to be used with justification for any manufacturing anomalies or excursions from documented procedures
- The expected life of the kit from the input documentation
- Any proposal, with justification to launch a kit with a life based on accelerated data, or to launch with a shorter life than in the input documentation while awaiting the conclusion of real-time testing documented.
- The documented nature and extent of in-use testing
- The justification for the choice of lots and components taking into account lot-to-lot variation and the critical characteristics
- The number of units (cassettes, bottles, tablets, etc.) of each component to be collected and stored under each condition
- The nature of the stability testing panel to be used, justifying each panel member's inclusion and defining the volume and characterization of the bulk specimen to be used and the aliquot size and number to be stored for the testing
- The expected criteria for each stability testing panel member at the beginning and end of the product's proposed shelf-life
- The statistical methods to be used for data analysis
- Graphs (paper or electronic) to visualize the performance of each stability testing panel member over the course of testing
- Methods of approval and justification of any deviations from the plan

10.3. Product storage

A sufficient number of product components from the identified lots should be reserved and stored separately to ensure that the study will be completed with identified products. Sufficient volumes should be retained to allow for the predetermined invalid rate.

10.4. Documentation

The Stability protocol should make reference to a study report which will be used to summarize interim, and ultimately, final study findings and conclusions. The study plan, the testing protocol and study report and all associated documentation (worksheets, etc.) should be controlled within the manufacturer's QMS. At the end of the study, the manufacturer should be able to confirm that design input requirements have been met.

Any changes in method must be recorded and undergo risk assessment. It should refer to the development of a detailed and valid testing protocol which includes all information and material relevant to testing.

10.5. Statistical methods

Statistical methods are used to support stability claims by providing estimates of the probability of results being as stated. For example: prior to the stability studies on an EIA it has been documented that if a stability testing panel member has at least a particular optical density (OD) then that device will meet a particular claim. Given the results of the stability study using that stability testing panel member and showing the variability within and between lots of the IVD, the probability of future similar production of the device meeting claims at the assigned life can be estimated. The derivation of valid criteria and the probability of maintenance of all claims can be estimated by appropriate statistical methods.

A fundamental problem is that of how many replicates should be used at each time point and from how many different production lots to produce acceptable overall probability estimates of the likelihood of all future production of similar devices and lots meeting claims (and hence user input requirements) at the end of the assigned life. There are two aspects to this – what is "acceptable" and "how many replicates?" "Acceptability" is a decision critical to quality and must be decided in advance from the user requirements – for example 80% confidence that 95% of all lots will meet the claims. This is in fact a tolerance interval as described in ISO 16269-6:2014. "How many replicates" can then be derived from the tolerance interval required but advice from a professional statistician is strongly advised – after defining the quality critical requirement but before beginning any experimental work.

The statistical methods to be used will be documented in the plans and protocols of any stability study and consideration given to treatment of unexpected and atypical results. In general all results must be used unless there is a documented physical reason (e.g. known operator error, too little volume, incorrect timing, use of an unqualified instrument such as lacking

maintenance or calibration) why a result can be ignored – but even then that result must be recorded and included in the report of the stability work.

10.6. Stability testing protocol

As part of an approved study plan for the determination of IVD stability, a detailed testing protocol should be prepared (examples of stability protocols are provided in Appendix 1: Example stability protocols) including the following as a minimum, as appropriate.

- QMS identifiers (e.g. experiment name, document references, etc.) that allow traceability to both the overarching study plan and to subsequently generated records/documents such as result worksheets
- The name(s) of operator(s)
- The dates and times when the experiment was performed
- Signatures of the operator and supervisor(s)
- The objectives of the study (i.e. determination of shelf-life, determination of in-use stability of a component, etc.)
- The name and lot number of the IVD and/or components being investigated
- How the components will be sampled from the production department
- Stability testing panel members and their characterization to be used, including valid test methods which reflect the IFU claims
- The experimental method that will be used for testing. This must follow the finalized testing method from the IFU. It must describe clearly how the experiment was performed in terms of:
  - required storage and/or challenge conditions;
  - the duration of storage/challenge;
  - the schedule of testing intervals;
  - the stability testing panel; and
  - the numbers of replicate tests performed for each stability testing panel member.
- How and where results are to be recorded
- Acceptance criteria
- How aberrant, discordant or invalid results will be dealt with
- How storage/challenge conditions are to be applied
  Example: For determination of stability during transportation it should be made clear that each IVD will be subjected to a sequence of stated temperatures.
- How actual storage/challenge conditions are recorded
  Example: Recording of temperature not as “room temperature” but as an actual numerical value obtained from calibrated instrumentation

Note: It can be unclear to a CDSCO reviewer from a general statement such as “… Sample buffer was stored at the required temperature and tested each month…” whether (1) the bottles of sample buffer were stored

open at the required temperature for the entire testing period, or (2) the bottles were stored capped and refrigerated, and only reopened briefly at the required temperature at each schedule test point.

10.7. Reading and recording results

10.7.1. Avoiding reader bias

It is good practice to use approaches to make the reading more objective, such as a scoring system. For IVDs where a subjective element forms part of the result, e.g. reading the intensity of an RDT band within a specified time frame, the results should always be reviewed by a first and second reader to avoid operator bias. Both readers must be blinded to the expected results; the second reader must be blinded to the first reader's results. If a validated band intensity scoring tool is to be included in the final RDT kit, this should be used to record results.

10.7.2. Recording actual individual results

The results of a test, not only the test interpretation, should be recorded. An interpretation on its own has insufficient resolving power to allow degradation of a signal over time to be observed. Some IVDs, e.g. line-blots, may require particular band patterns to allow an interpretation to be reached, and several different patterns may yield the same final result. Recording only the final interpretation of a test specimen may cause the failure of particular bands to go unnoticed while allowing the IVD to otherwise "pass". Photographic records of qualitative tests are recommended, as appropriate.

This is particularly important when testing a panel of like specimens, e.g. "20 HIV antibody positive specimens" for which the acceptance criterion is "all 20 specimens must be positive". It is not sufficient to simply record "all 20 positive" or "pass" without first recording the individual test result directly from the IVD for each specimen in the panel.

Example 1: For most enzyme-linked immunoassays (EIAs) if the sample-to-cut-off ratio is > 1 then the result is interpreted as "positive" or "reactive". In this case three pieces of information should be recorded: (1) the numerical value of the assay sample-to-cut-off ratio, (2) the numerical value of the signal for the specimen and (3) the final interpretation.

Example 2: Some rapid diagnostic tests (RDTs) may stipulate that the strength of test band is not correlated with the strength of antibody titre.

Nevertheless, the following should be recorded: (1) the intensity of observed patterns according to a predetermined, validated intensity scoring system with as fine a gradation as possible, and (2) the final result interpretation.

Example 3: A qualitative NAT assay may report "positive" and "negative" for a particular analyte, but the underlying decisional parameter is often quantitative (e.g., a PCR signal-based cycle number). The quantitative parameter should be recorded.

10.7.3. Retention of records

CDSCO encourages retention of photographic records, machine printouts, electronic data or physical retention of membranes from opened cassettes, as appropriate. Records should be retained for a period of time at least one year after the date of expiry of IVD as defined by the manufacturer, but not less than two years from the date of product release by the manufacturer

10.8. Degradation vs deterioration

Testing at more than two time points can be important to avoid confusion between imprecision and stability. For example, if the end testing shows 10% decrease, one may not judge if the difference was due to imprecision or degradation. If tested one or more times in between are used, fluctuation caused by imprecision can be distinguished from drift due to instability. This can be ameliorated by increasing the number of replicates and runs. All studies should support precisely defined periods of in-use stability claims.

Example: An RDT test cassette – may be labelled "Use immediately on opening". In such cases it is still necessary to determine the interval (one hour, one day, etc.) over which the IVD performance remains stable after the component is opened.

10.9. Testing schedule

Testing intervals should be selected to detect any trending activity over the testing period. Concurrent testing of separate types of components may be approached with different intervals. For example, it may be appropriate to test an IVD test cassette against a stability testing panel on a monthly or quarterly basis.

10.9.1. Acceptance criteria for results

The acceptance criteria to establish what is acceptable or not acceptable should be defined according to the stability testing panel criteria for both qualitative and quantitative test methods. Results from failed (invalid) test runs must not be used in the determination of the stability claim. However the invalid results should also be recorded.

11. Stability report

11.1. General

After testing has been completed, the findings should be summarized in a stability study report. The report should clearly identify the IVD that was tested, the objectives of the study, the conditions under which the IVD was tested and conclusions that were drawn from findings. The report should be traceable to the study plan, testing protocol and user needs. It should make clear references to other supporting documentation (e.g. result worksheets).

11.2. Link to claims

The results and conclusions of stability studies presented in the study report must support the claims of IVD stability reported in the IFU and elsewhere in the dossier.

11.3. Consider variability

An overall stability claim (whether for shelf-life, in-use stability, or stability during transportation) must be based on the expected stability when taking into account inter-lot variability.

Example: The manufacturer should evaluate the variability between the different lots studied and assume that any differences in shelf-life are inherent to the manufacturing process. The claimed life should be calculated so that a known and stated proportion of all lots (usually >95%) will meet the claimed shelf-life. Frequently more than three lots are needed to obtain a realistic idea of the variability of the results.

11.4. IVD stability versus component stability

A claim of stability for an IVD as a whole must not exceed any individual component stability.

Example: For an IVD claimed to detect HIV-1 and HIV-2 antibodies – if detection of HIV-1 antibodies is stable to 24 months but that of HIV-2 to only 18 months, then the shelf-life must be based on the shorter time.

12. Changes to a Licensed / Approved IVD

Any major modification to a licensed / approved IVD or to its process of manufacturing will require provision of direct evidence of stability. An appropriate risk analysis and an accelerated stability study comparing the original product and the modified product for usability, performance and lot-to-lot variation may serve to assess the impact of the changes to a product formulation or manufacture. It would be necessary to validate the stability of the modified IVD in at least one lot of the IVD (subject to risk analysis) in order to demonstrate equivalence between the original and modified IVDs. More lots may be appropriate depending on the product nature, variability of components and failure risk. CDSCO expects results of accelerated testing to be confirmed by real-time studies.

If there are different presentations, the stability of each one must be assured.

The following examples seek to illustrate the scope for considering the performance evidence from one IVD as support for performance in another:

Examples:

1) For an HIV RDT which uses an identical cassette and physical components of a manufacturer's existing, fully validated HCV RDT, the reagent formulations are different (antigen/antibodies, buffers, conjugates, etc.). Evidence of stability of the HCV RDT would not suffice for the HIV RDT. Even if the manufacturer claims that both IVDs have been sold in a number of countries for several years and no adverse feedback has been reported, this would not constitute evidence in support of the stability of either IVD.

2) For an HIV RDT that has been fully validated for detection of HIV-1 antibodies; a new product is developed which includes detection of HIV-2 antibodies. The stability of any sample buffers that are identical between the two IVDs would probably not need to be validated. However, other components (conjugates, antigens, antibodies) that are different between the two IVDs would need to be tested; it would not be sufficient to assume that HIV-1 reagents will have the same stability in the new IVD. A modification of this nature is likely to require substantial validation of stability.

3) An HIV RDT IVD previously intended for testing serum/plasma has added to it a claim for detection of HIV-1 in whole blood. The only substantive design change associated with the new claim is the addition of a small pad of some suitable material near the sample port which acts as filter for whole blood specimens.

Depending on the nature of the material it may be reasonable to argue that the material would not be expected to age; that it is not, in any practical sense, chemically labile. Consequently, shelf-life and in-use stability may not necessarily need to be retested in full. However, stability during transportation may need to be determined to provide confidence that the modification is able to withstand likely shipping conditions (e.g. that the extra square of filter paper doesn't dislodge when packages are jostled and bumped in transit).

4) Based on an HIV RDT that has been fully validated for detection of HIV-1 antibodies, a new IVD is developed which includes detection of antibodies to Treponema pallidum (TP). Detection of TP specific antibodies occurs on a completely separate membrane (and associated architecture) to that of HIV antibody detection. Additional handling steps may have an impact on the stability of the HIV-1 antibodies and it may be required to retest. It may be necessary to review evidence of stability during transportation to ensure that new components are not affected by transport (for example a new packaging concept is used).

If a new machine is used for striping of the HIV-1/TP IVD, validation of the new machine (installation qualification, operational qualification and performance qualification) would be required to show that the stability studies are still valid.

If the IVD is designed in a way that HIV and TP detection occurs either on the same membrane and/or using most of the same architecture (and assuming that sample buffers are identical between IVDs) it is likely that this new IVD would need to be fully validated.

It should be noted that these observations pertain specifically to IVD stability. Other aspects of IVD performance should still be validated as appropriate.

APPENDIX

Annexure I	Example stability protocols:
Annexure II	Suggested specimens for stability testing panels
Annexure III	Examples of Stability Study approaches

APPENDIX – I

Example stability protocols:

This appendix contains examples for a wholly fictitious IVD, illustrating the kinds of experimental design to determine the following:

1. Stability of whole kit during transport
2. Stability of whole kits during shelf-life, and
3. In-use stability of whole kits including reagents

The information provided in these examples should not be taken as a checklist of sufficient conditions, but manufacturers are encouraged use as a guide on possible approaches to generate evidence of a standard sufficient to satisfy the equirements of the MDR2017. It is recommended that transportation stress studies are undertaken prior to the shelf-life studies.

Description of fictitious IVD

IVD used for the purpose of the examples is a RDT for the detection of antibodies to HIV-1, HIV-2 and Treponema pallidum in serum, plasma and whole blood. It is recommended that the kit is stored at 8–40°C, but components of the kit must be used at 15–30°C. The product is supplied as a kit with each test cassette sealed in a foil pouch (with desiccant). The pouch must be brought to 15– 30°C. Once opened, it is recommended that the cassette is used immediately. The IVD includes a bottle of specimen buffer/diluent for use with all three specimen types. The specimen buffer is expected to have similar stability as the test cassette in its unopened form. The stability of the opened bottle of specimen buffer is determined below (see Example 3: In-use stability protocol).

The manufacturer of this product proposes to determine the stability of its product and has written a stability plan. As part of this plan a preliminary determination of accelerated stability has been conducted at several extremes of temperature and suggests that the IVD would be stable to an equivalent of 12 months following manufacture. The plan now calls for the development of real-time stability protocols that will form the basis of subsequent testing of the IVD.

Preliminary work has shown that the variability between lots is minimal so that three independent lots (no critical constituents in common) will suffice to enable a reasonable estimation of shelf-life taking lot variation into account.

Example 1: Evaluation of stability during transportation

Objective:

To determine the stability during transportation of the HIV RDT in real-time using simulated shipping conditions and to generate stressed components to be used in real-time shelf-life studies as proposed in Stability Study Plan xxxxx01.

Preparation

Acquire sufficient numbers of kits from three independent production lots using a Predetermined sampling protocol (e.g. random, first X kits in first box, every 100 th kit, etc.). Allow at least 10% for unexpected requirements and re-testing

Note 1: To provide security against unforeseen events, duplicate tests should be performed as a minimum. Testing in triplicate as a minimum provides a level of statistical confidence in the observed test result.

Testing will be conducted at 0, 3, 6, 9, 12 and 13 months.

Note 2: Testing beyond 13 months will allow an understanding of when, in real-time, the IVD is likely to 'fail' and may allow an extension of the proposed shelf-life.

Note 3: For determination of shelf-life a fresh bottle of specimen buffer must be opened at each testing point – although there may be circumstances in which multiple sampling could be taken from the same bottle after it has been opened. Acquire sufficient volume of each stability testing panel member for the duration of the testing schedule.

The protocol for these studies specifies the number of devices to be picked, the statistical sampling plan to be used and the required stability testing panel members and their volumes.

Documentation:

In Worksheet xxxxx01 record the following:

- The lot numbers from which kits were sampled
- The number of kits sampled from each lot
- Details (including manufacturing/lot information) for each of the kit components that will be tested as part of this protocol:

Test cassette:Bottle of Sample Buffer:…

The product kits chosen to be tested are in their final packaging including all labelling.

The IVDs are stored so that the reagents are in contact with all elements of the

packaging (e.g. the bottles in the product kits are stored horizontal lying flat on their sides).

Kits will be divided into two groups. One group will be stored at 40 ± 5°C , the other at 8 ± 2°C . Kits from each group will then be subjected to the following conditions.

Testing schedule: for transport simulation

Condition 1, Temperature and humidity sequence: all kits will be taken through a temperature and humidity sequence consisting of:

i) Ambient humidity (X% RH)

- Put at IFU storage temperature for 24±4 hours followed by
- 30 ± 5°C for 24±4 hours followed by
- 45 ± 5°C for 24±4 hours, followed by
- 8 ± 5°C for 24±4 hours, followed by
- IFU storage temperature for 24±4 hours

Followed by

ii) Desert humidity (30% RH)

- Put at IFU storage temperature for 24±4 hours followed by
- 30 ± 5°C for 24±4 hours, followed by
- 45 ± 5°C for 24±4 hours, followed by
- 8 ± 5°C for 24±4 hours, followed by
- IFU storage temperature for 24±4 hours

Followed by

iii) Tropical humidity (85% RH)

- Put at IFU storage temperature for 24±4 hours followed by
- 30 ± 5°C for 24±4 hours, followed by
- 45 ± 5°C for 72±4 hours, followed by
- 8 ± 5°C for 24±4 hours, followed by
- IFU storage temperature for 24±4 hours

Followed by

iv) Ambient humidity (X% RH)

- Put at IFU storage temperature for 24±4 hours followed by
- 30 ± 5°C for 24±4 hours, followed by
- 45 ± 5°C for 24±4 hours, followed by
- 8 ± 5°C for 24±4 hours, followed by
- IFU storage temperature for 24±4 hours

Note 1: It is important to make clear that the above complete sequence of temperatures will be used, as opposed to separate IVD kits being held at individual temperatures. The actual temperatures, durations and the nature of the sequence

will depend on the IVD and the kinds of conditions expected to be encountered during shipping

Note 2: Freezing temperatures are not considered in this example but should be included if the IVD kits could be exposed to freezing temperatures during transport.

Note 3: If transport by air is anticipated, the effect of reduced pressure should be included in the protocol (3) for a period of time at least 10% longer than the longest anticipated flight, and at a pressure expected in aircraft holds.

Note 4: The protocol should call for testing of at least five individual IVD kits after each stress condition, using the stability panel members giving the most informative results. This approach will enable verification that the IVD kits are sufficiently stable to progress to the next condition, although this should already be known from preliminary experiments and R&D work.

Condition 2, Transport stress conditions - Shaking. Each IVD kit will be placed on a shaking table at X revolutions per minute (rpm) for X hours/days at 42 ± 5°C as defined by ASTM D4169 section 12 (3).

After the simulated shipping challenge, each IVD kit will be returned to its corresponding storage temperature (42±5°C or 8±2°C).

Testing schedule for real time stability studies

Testing will be conducted at 0, 3, 6, 9, 12 and 13 months. At each scheduled time point, the allotted number of IVD kits will be brought to 15 to 30 °C and used to test each member of the panel in triplicate.

Note 1: The test at 0 months will provide evidence that the IVD kit is stable under extreme conditions of shipping (but similar to those likely to be experienced), the testing at later time points will give evidence to support the claimed shelf life after transport, and testing beyond the claimed shelf life will provide evidence that the IVD kit is stable and not close to a failure point.

Documentation for transport stress conditions

In Worksheet XYZ00001 record:

- The lot numbers of the IVD kits used to conduct the test
- The Operator(s) name(s)
- The dates of testing
- Identifying details for each member of the panel being tested
- The temperature at which the IVD kits are stored
- The values of temperature and humidity for each of the challenge conditions
- Instrument settings for the shaking apparatus and duration of operation for challenge conditions.
- The ambient temperature and humidity during testing
- Each test result as an interpretation according to the IFU
- Each test result as a band intensity. Band intensity should be scored using the calibrated scale described in Protocol ZXY0001 (e.g. 0, faint/trace, +1, +2, +3 … +10) (even though the IFU does not give scores to results)

- Any aberrations or deviations from the protocol, the reason for the deviation and any remedial action undertaken. Results from invalid assays must be recorded but not included in calculations of shelf life. Apparently aberrant results, unless the underlying cause can be positively identified as not related to a problem with the IVD, must be included in the calculations of shelf life.

Acceptance criteria

Each panel member should show a band intensity result that matches its expected result at each tested time point. The expected result must be validated so that if the IVD fails to meet the claims (e.g. fails to detect critical specimens, has unacceptable performance at medical decision concentrations, has unacceptable specificity) the panel member would also fail to meet its specified result.

The stability after transportation of the IVD kit will be taken as the time point before the last time point to have met the acceptance criteria, e.g. if the IVD is stable to 13 months, the stability after transportation will be deemed to be 12 months.

The stability after transportation should be identical to the claimed shelf life of the IVD kit, i.e. the extremes of possible conditions to which the IVD kit is likely to subjected during transport must not affect the shelf life of the IVD.

Calculation of results

Detailed statistical instruction must be obtained from a professional statistician with an understanding of the expectations of the stability study plan and outcome. Professional statistical input is particularly recommended when calculating confidence limits for discrete data such as readings from a graduated scale.

Each of the following applies at each time point:

The variance of the results for all replicates within and between all the lots must be calculated for each panel member. From the overall variance between lots, the confidence with which future lots of the IVD kit will detect the panel member at that time point after manufacture and transport can be calculated. If the confidence of the panel member meeting its specification is less than some pre-defined value (normally 95%), it must be deemed to have failed at that time point and the shelf life of the IVD kit should be restricted accordingly.

If regression analysis is used to define the time point at which a panel member would not meet its criterion, then lot-to-lot variability must be included when setting the confidence limits around the regression line. However, real-time data must extend beyond the claimed shelf life so that the intercept of the regression confidence limit and the expected value must be at a time period longer than the claim. It is usually more appropriate to calculate as discussed in the previous paragraph, particularly if the regression cannot be proven to be linear.

Example 2: In-use stability protocol

Objective

To determine the stability of opened bottles of the Specimen Buffer used in the IVD kit in real-time when stored at 15–30°C as proposed in Stability Study Plan XYZ00001.

In this example the manufacturer recommends that the test cassette be used immediately upon opening; this claim should also be validated in a separate experiment, so that it can be confirmed that the IVD will still perform satisfactorily after the test cassette has been removed from its pouch and open at room temperature for 1, 2, 6, 24 hours, etc., as appropriate.

Acquire sufficient numbers of IVD kits from one production lot using a predetermined sampling protocol (e.g. random, first X number of kits in the first box, every 100th kit, etc.).

Acquire sufficient volume of each panel member for the duration of the testing schedule. Establish a method for randomising the panel for testing.

In Worksheet XYZ00001 record the following:

- The lot numbers from which the IVD kits were sampled
- The number of IVD kits sampled from each lot
- Details (including manufacturing/lot information) for each of the IVD kit components that will be tested as part of this protocol (test cassette and specimen buffer).

Preparation

Two lots of specimen buffer are to be tested. One lot of the component must be freshly made, the other should be towards the end of the assigned shelf life of the IVD kit.

The component is to be tested in its final packaging.

The IVD kits are stored so that the reagents are in contact with all elements of the packaging (e.g. the bottles in the IVD kits are stored horizontally, lying flat on their sides, allowing liquids to remain in contact with the bottle closures).

Half of each lot will be stored at 30± 5°C, the other half at 15± 5°C. At the start of testing each bottle will be brought to room temperature (20 ± 2°C), opened, used for testing and then recapped and returned to the stated storage temperature.

Note 1: It is important that the components under test are opened and used under circumstances likely to occur in users' laboratories (i.e. not in rooms with HEPA filtered air) mimicking, as far as possible, genuine use.

Testing schedule

At each subsequent scheduled time point the allotted number of bottles will be brought to room temperature and used to test each panel member in triplicate. Testing will be conducted at 0, 1, 2, 3, 4 weeks, etc., up to the end of the claimed in-use life.

Documentation

In Worksheet XYZ00001 record:

- The lot number of the IVD kit used to conduct the test
- The Operator(s) name(s)
- The dates of testing
- The temperature at which the IVD kits are stored
- The ambient temperature during testing
- Identifying details for each member of the panel being tested
- Each test result as a band intensity. Band intensity should be scored using the calibrated scale described in Protocol ZXY0001 (e.g. 0, faint/trace, +1, +2, +3 … +10)
- Each test result as an interpretation according to the IFU
- Any aberrations or deviations from the protocol, the reason for the deviation and any remedial action undertaken.

Acceptance Criteria

Each panel member should show a band intensity result that matches its expected result at each tested time point. The in-use stability of the sample buffer will be taken as the time point before the last time point to have met the acceptance criteria.

Example: If the IVD kit is observed to be stable to 5 weeks, the in-use stability will be deemed to be 4 weeks.

APPENDIX – II

Suggested specimens for stability testing panels

Examples in this section

Not all of the specimens in the examples that follow will be necessary for all IVDs, nor is the list exhaustive. Panels must be composed according to strict risk management principles, and all decisions must be documented and traceable.
The minimum specimens that are recommended to be included in a testing panel for the different products are outlined below.

1 Specimens to monitor tests for nucleic acid-based testing technology

If a proprietary nucleic acid preparation /extraction system is provided, the recovery must be shown to meet claims for each genotype from each of the specimen types claimed (e.g. dried blood spots, whole blood, plasma). Successful removal of inhibitory substances, if intended, must be demonstrated for appropriate specimen types. Unless potentially variable biological reagents are involved, this system would be expected to be verified in manufacture and not necessarily tested at release.

Specimens	Remarks
Specimens to demonstrate maintenance of sensitivity and/or limit of detection, and/or accuracy, and precision	Traceability is required to one of the standard of NIB or NIBSC or WHO or any other, which are required suitably justified on each of the claimed specimen types.
Specimens to demonstrate specificity and validity of runs	Sufficient negative specimens should be included to ensure that the claims will be met at end of shelf life.
Specimens (or reagents) to demonstrate stability of each of the critical components of the IVD	If more than one part of the genome is to be detected, both systems must be shown to be stable. If both DNA and RNA are measured the complete system must be shown to be stable.

2 Specimens to monitor tests that measure CD4 cells

Rationale

CD4 measurements are quantitative, and accuracy at the clinical decision points is important. The design input should have information on the accuracy and other parameters required, and the panel must be designed to provide evidence that these parameters are maintained over the assigned life of the reagent and measuring IVD.

Parameters

The panel used in stability work must be able to demonstrate the following.

- Stability of all the antibodies used in the IVD (frequently anti-CD4 and anti-CD3 antibodies; any other critical components must also be covered)
- Accuracy and trueness of measurement maintained at the critical level (at least five specimens required)
- Claimed linearity over the required range of CD4 count (at least five specimens required)
- Measure drift

Specimens

Artificial specimens, such as stabilized blood specimens, can be used if a risk assessment based on R&D work indicates that they are effective. Fresh specimens are usually required. Measurements should be compared to an approved reference system.

Examples of approaches

Aged or in-use lots may be compared with a reference, e.g. a new lot. Precision studies can be performed as described in CLSI guidelines.

3 Specimens to monitor tests for HIV antibodies

Specimens	Remarks
IgM first seroconversion specimens and IgG first seroconversion specimens	Possible approaches to obtain samples : • Study the early data from commercial seroconversion panels where the seroconversion was frequently monitored by IgM and IgG blots • Study the responses to second and third generation assays or protein A and protein L assays (this approach is less useful).
All other parts of the HIV proteome included, e.g. reverse	

transcriptase (RT)	
Late stage specimens – usually a high dilution set near the sample-to-cut-off ratio	This might serve to monitor any kit run control. HIV serology is not particularly genotype dependent. It is usually not necessary to include controls for genotype detection unless risk assessment or experiment shows that it is required for a particular IVD.
HIV-2, diluted to near the sample-to-cut-off ratio	Seroconversion specimens are very rare.
HIV-1 (0), if claimed	
Difficult specimens to monitor specificity and invalid rates	100 negatives at release subject to risk analysis and statistical analysis of the allowable (relative to the claimed) false reactive rate and invalidity rate

4 Specimens to monitor tests for antibodies for HIV-1/2 and Treponema pallidum (TP)

Specimens	Remarks
Specimens to detect HIV	See above section 3 Specimens to monitor tests for HIV antibodies
Specimens to detect all the critical epitopes in the IVD, for example TpN47, TpN17 and TpN15	Note: Each of these epitopes play a role in detecting syphilis in different stages of the infection. It is necessary to have a panel member to monitor each epitope system present (and possibly each stage of infection), even if poly-fusion proteins are used. This can be avoided if the manufacturer can demonstrate that each epitope system is equally stable.
Specimens able to show that the invalidity and specificity rates do not fall outside the claims, particularly if whole blood is a claimed specimen type	Note: It would not be sufficient for WHO prequalification to extrapolate to the stability of HIV-2/TP detection by testing only HIV-1 positive specimens.

5 Specimens to monitor tests for hepatitis C virus antibodies

Specimens	Remarks
NS3 first seroconversion specimens and core first seroconversion specimens	
Specimens to monitor any other antibodies claimed (frequently against NS5 and NS4)	Results can be obtained from line immunoassays that differentiate antibody responses to the different proteins.
A late stage dilution near the sample-to-cut-off ratio	Note: Hepatitis C virus serology is not particularly genotype dependent in terms of anti-core and anti-NS3, but it is possible to make serotyping assays based on NS4 that mimic genotyping reasonably well. It is usually not necessary to include controls for genotype detection, unless risk assessment or experiment for a particular IVD show otherwise.
Difficult specimens to monitor specificity and invalid rates	100 negative specimens subject to risk analysis and statistical analysis of the allowable false reactive rate and invalidity rate (relative to the claimed rates)

6 Specimens to monitor for tests for hepatitis B surface antigen (HBsAg)

Specimens	Remarks
Specimens to define sensitivity relative to the claim	Traceability is required to one of the standard of NIB or NIBSC or WHO or any other, which are required suitably justified on each of the claimed specimen types.
Specimens to monitor the maintenance of the claims of a variety of serotypes / genotypes and mutant forms	These will almost certainly be traceable to the “First International Reference Panel 2011, for Hepatitis B virus genotype panel for HBsAg-based assays" PEI code: 6100/09.
Specimens to control against prozone/high	

dose hook effect if found or if theoretically an issue	
If detection of HBsAg in the presence of anti-HBsAg is claimed (current best practice) proof of maintenance of the claim	
Specimens to monitor the critical components of the IVD	If the monoclonal antibodies used have particular function or bias, such as against the ayr or adr serotypes (not controlled by the standards) or to detect mutant forms of the antigen, each must be monitored to ensure viability at end of shelf life. These may be the same specimens as mentioned in the previous paragraphs. If there are critical dissociation chemicals or red-cell capture or rupture agents used, these must also be monitored.
Difficult specimens to monitor specificity and invalid rates	100 negatives subject to risk analysis and statistical analysis of the allowable (relative to the claimed) false reactive rate and invalidity rate.

APPENDIX – III
Examples of Stability Study approaches

1 Accelerated Stability Study: (Three lots)

Product name	Claimed Storage condition	Claimed shelf life	Accelerated condition	Testing interval
HIV 1-2 Ab Rapid qualitative test Rapid qualitative test	2-30°C	24 months	2-8°C, Room Temperature (20-30°C), 37°C and 45°C	0 day, 4thday, 7thday, 14thday, 21stday, 30thday and 41stday

2 Real Time Stability Study: (Three lots)

Product name	Claimed Storage condition	Claimed shelf life	Real Time Stability condition	Testing interval
HIV 1-2 Ab Rapid qualitative test Rapid qualitative test	2-30°C	24 months	2-8°C, Room Temperature (20-30°C),	0 month (Initial Testing) and 3 months, up to 27 months in three months interval

3 In Use Stability Study: One lot

Product name	Claimed Storage condition	Claimed shelf life	Claimed In Use Stability or Open Bottle Stability condition	Claimed Stability after opening	In Use Stability or Open Bottle Stability condition	Testing interval
HIV 1-2 Ab Rapid qualitative test Rapid qualitative test	2-30°C	24 months	2-30°C	upto 24 hours after opening	2-8°C, Room Temperature (20-30°C), 37°C and 45°C	0 hrs (initial testing), then at 0 hrs (initial testing), then at 0 hr, 1 hr, 2 hrs, 3 hrs , 4 hrs, 5hrs, 6 hrs, 24hrs and at 25hrs

4 Shipping stability study: One lot

Product name	Claimed Storage condition	Claimed shelf life	Control group without temperature and Humidity cycle	Test group temperature and Humidity cycle
HIV 1-2 Ab Rapid qualitative test Rapid qualitative test	2-30°C	24 months	2-30°C	Cycle:1 at 40% RH 1-40°C for 24hrs ± 4 Followed 30 ± 5 °C for 24hrs ± 4 Followed 45 ± 5 °C for 24hrs ± 4 Followed1-40°C for 24hrs ± 4 Followed Cycle:2 at 85% RH 1-40°C for 24hrs ± 4 Followed 30 ± 5 °C for 24hrs ± 4 Followed 45 ± 5 °C for 72hrs ± 4 Followed1-40°C for 24hrs ± 4 Followed Cycle:2 at 40% RH Followed 30 ± 5 °C for 24hrs ± 4 Followed 45 ± 5 °C for 24hrs ± 4 Followed1-40°C for 24hrs ± 4