Draft Guidance for Industry and FDA Staff

Assay Migration Studies for In Vitro Diagnostic Devices

DRAFT GUIDANCE

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Comments and suggestions regarding this draft document should be submitted within [insert] days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. Alternatively, electronic comments may be submitted tohttp://www.regulations.gov. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Devices and Radiological Health
Office of In Vitro Diagnostic Device Evaluation and Safety
Center for Biologics Evaluation and Research

Preface

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Table of Contents

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Assay Migration Studies for In Vitro Diagnostic Devices

This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

This draft guidance presents a least burdensome regulatory approach to gain FDA approval of Class III or certain licensed[1] in vitro diagnostic devices in cases when a previously approved or licensed assay is migrating (i.e., transitioning) to another system for which the assay has not been previously approved or licensed.[2] In this guidance the term "New System" refers to the unapproved/unlicensed system (assay, instrument, and software) to which the assay is migrating from a previously approved/licensed system. Conversely, the term "Old System" refers to the approved/licensed system (assay, instrument and software) from which the assay is migrating to a currently unapproved/unlicensed system.

The focus of this guidance is on the study designs and performance criteria that should be fulfilled in order for a sponsor to utilize the migration study approach in support of the change. FDA will review information from the sponsor, including results of analytical and comparison studies outlined in this guidance, as well as device descriptions and risk analyses, to determine whether the use of the approved/licensed assay with the New System may compromise safety and effectiveness of the assay. The guidance document describes information that we recommend you include in a PMA (premarket approval application) supplement or BLA (Biologics License Application). For devices regulated by OIVD,

[1] This guidance does not apply to immunohematology tests licensed by the Center for Biologics Evaluation and Research (CBER).

[2] This guidance can be used for 510(k) devices where the Replacement Reagent and Instrument Family Policy (http://www.fda.gov/cdrh/oivd/guidance/950.pdf) does not apply (e.g., nucleic acid amplification tests) and devices for which transition to a New System presents specific concerns, either because of the nature of the analyte and indications, or because of the specific technology used.

sponsors may contact OIVD, and for those regulated by CBER, sponsors may contact CBER to obtain feedback concerning study plans.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

The Least Burdensome Approach

This draft guidance document reflects our careful review of what we believe are the relevant issues related to migration studies and what we believe would be the least burdensome way of addressing these issues. If you have comments on whether there is a less burdensome approach, however, please submit your comments as indicated on the cover of this document.

II. BACKGROUND

The FDA believes that the assay migration study paradigm discussed in this draft guidance provides a least burdensome scientific and regulatory pathway for manufacturers to transfer a previously approved or licensed assay with full clinical data from an Old System to a New System (not approved or licensed). The paradigm is suitable in cases when sufficient knowledge can be derived from the documentation of design controls, risk analyses, and prior performance studies on an Old System.

If you make further modifications or iterations of the Old or New System you should compare back to the original Old System that has full clinical data when performing new migration studies. However, if the Old System with full clinical data is no longer available please contact the appropriate FDA CDRH/CBER Division for further discussion.

The migration studies approach is related to the Replacement Reagent and Instrument Family Policy that FDA uses for many Class I and Class II diagnostic devices.[3] Similar to that policy migration studies rest on the premise that as platform changes are made throughout the lifetime of an approved or licensed assay, smaller and more focused analytical and clinical data sets than have traditionally been called for, along with prior knowledge of device design and performance, could allow for credentialing of the safety and effectiveness profile of the modified system.

Use of this review approach is practical, risk based, and consistent with FDA's Critical Path Initiative, which is intended to help bring new medical products to market successfully and efficiently[4]. FDA believes that with proper controls and review, migration studies will meet

[3] http://www.fda.gov/cdrh/oivd/guidance/950.pdf.
4 http://www.fda.gov/oc/initiatives/criticalpath/whitepaper.html

regulatory thresholds for premarket review in a manner that will be least burdensome for both companies and FDA while protecting public health.

III. SCOPE

This draft guidance is intended to be applied, where appropriate, to licensed donor screening tests[5] and approved (Class III) in vitro diagnostic assays, as well as cleared assays for which migration to a New System presents concerns. Possible scenarios for assay migration are when the assay is being transferred from a manual system to an automated or semi-automated instrument system or from a semi-automated instrument system to a fully automated instrument system, or from one automated instrument system to another (and visa versa for all scenarios). A broad variety of methodologies may use the migration studies paradigm depending on what is known about the two Systems involved. Assay transfer may be from one approved or licensed Old System to a New System that has the same technical characteristics. However, if scientific evidence suggests migration studies may not be adequate to predict actual clinical testing performance on the New System, the assay migration paradigm should not apply and FDA will recommend that traditional evaluation studies be performed.

Assay migration studies are ideally suited for test systems for which the assay output (raw signal) is a numerical result or is expressed as a signal to cutoff (S/CO). Devices for which a numerical output is not available might be more difficult to analyze and may not be suitable candidates for use of this approach.

Migration studies would not be applicable to the following devices or to system changes that are generally considered significant, such as:

- systems intended for over-the-counter use,
- systems intended for prescription home use,
- devices intended for point of care use,
- devices that do not meet the Critical Considerations criteria stated below.

[5] FDA does not believe that this guidance is suitable for use in its entirety when immunohematology tests (e.g., blood grouping, blood group antibody detection and/or identification, crossmatching) are being migrated because of the differences in assay methodology and results reading and interpretation as compared to the other assays and systems described in this guidance. If you believe that your immunohematology reagents and system can be evaluated using the criteria outlined in this guidance, contact the responsible review division in CBER. Immunohematology products are reviewed in the Devices Review Branch, Division of Blood Applications, Office of Blood Research and Review, CBER.

The FDA strongly recommends that sponsors discuss proposed migration studies with the Agency early in the product and testing design process in order to determine if the proposed changes are consistent with the parameters that would allow for streamlined and focused testing. For CBER, this may be through an IND, or protocol review, providing preliminary protocols, data, and justifications prior to performing the migration studies. The size, nature, and scope of migration studies we recommend will depend on a detailed evaluation of the Old and New Systems, the level of regulation appropriate for the product (e.g., PMA or BLA), the performance characteristics of the assay, and the design and scope of the analytical testing and clinical trials used to support approval or licensure of the assay on the Old System.

IV. CRITICAL CONSIDERATIONS FOR DETERMINING WHETHER THE MIGRATION STUDIES PARADIGM MAY APPLY TO A PARTICULAR DEVICE

A sponsor should take into account the following critical considerations in determining whether the migration studies approach is appropriate for a particular product, and describe these considerations in the submission:

- The intended use and indications for use for the New System should be unchanged from the Old System, except for inclusion of the New System.

- Reagent and assay parameters (e.g., cutoff) should be unchanged, except for very minor differences in assay parameters (such as small changes in incubation times) in order to optimize the assay on the New System. However, the sponsor should provide evidence that the changes do not compromise the assay's performance.

- Assay and system technologies should remain unchanged. All biochemical (e.g., antibody and antigen interactions or DNA probe construct) and physical detection (e.g., colorimetric, chemiluminescence, or dye binding) technologies should be unchanged from the Old System. Minor differences in hardware instrumentation may be appropriate and will be evaluated on a case-by-case basis.

- There should be no expected change to the assay performance when run on the New System. However, actual changes will be evaluated in the context of their impact on the clinical use of the assay. Due to the limited number of positive and negative samples, the migration studies approach is not appropriate to support changes in clinical performance claims.

V. ADDITIONAL CONSIDERATIONS

In addition to addressing each of the critical considerations noted in Section IV, you should also include the information listed below to demonstrate the applicability of migration studies to the transfer of the assay from the Old System to the New System. The information in your submission should include, but is not limited to, the following:

- Device description, including functional block diagrams and hardware and software components for both the Old and New Systems to allow an evaluation of the changes to the New System when compared to the Old System.

- A similarities and differences table for a side-by-side comparison of assay parameters and hardware/software functions, requirements and design.

- A risk analysis of the New System (software/hardware/assay) using relevant guidance documents.[6]

- Summaries of software data validating functional operation of alerts and alarms in real or simulated circumstances.

- System Operator Manual(s): If the New System was previously approved or licensed with analytes other than the one under consideration, a new review of the Manual may only be called for if changes have been made that impact safety and effectiveness, or if there are assay-specific sections or changes.

- Proposed modifications to the labeling that appropriately describe respective prior data and new migration study information. The sponsor should consult with FDA when determining whether a dual or separate package insert will be appropriate. Inclusion of the Old System's analytical and performance data should be included where appropriate.

In addition, the sponsor should include documentation on software and instrumentation for the New System. When appropriate for the device, this documentation should meet all recommendations for the appropriate Level of Concern.[7] To better control risk, the studies should be performed on the final model and software version of the New System that is intended to be marketed.

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[6] ISO 14971:2007, Medical devices – Application of risk management to medical devices, and Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices.

[7] Guidance for Industry and FDA Staff: Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices, **http://www.fda.gov/cdrh/ode/guidance/337.html**.

VI. ASSAY MIGRATION STUDIES

This section outlines specific studies that may be appropriate to support assay migration for in vitro diagnostic devices. Before preparing to use the migration studies approach, you should determine whether the assay is quantitative or qualitative, according to the definitions in this guidance. Specifically, for the purposes of this guidance, qualitative assays are those that determine numerical values (e.g., signal, S/CO), which are used for categorical determination of assay results (e.g., positive or negative). Quantitative assays determine numerical values which are referenced to a linear range, and standards that allow absolute determination of analyte concentrations. Section VI.A describes studies we recommend for Qualitative assays; Section VI.B addresses Quantitative Assays. Special considerations for blood screening assays are covered in Appendix I, "Migration Studies for Blood Donor Screening Assays."

A. Migration Studies for Qualitative Assays

1. Analytical Studies for Qualitative Assays

The evaluations described below are based on the idea that similar studies were conducted previously for the Old System. If the study design of the analytical studies conducted for the Old System were different from the design of the studies described in this guidance, please contact the FDA for feedback. If you believe that some of these studies do not apply to your particular device, you should present your justification for FDA review.

We recommend that you use fresh clinical specimens for all analytical studies. If this is impractical, in some cases you may substitute or supplement fresh clinical specimens with banked samples. If banked samples are not available, spiked or diluted clinical samples may be used. In some instances, use of otherwise contrived matrix-specific samples may also be appropriate; however these should mimic clinical specimens as much as is feasible. We recommend that you contact FDA if you wish to discuss appropriate sample types for these evaluations. The matrix of any of these alternative specimens should be the same as that specified by the intended use of the Old System.

a. Performance at Low Analyte Levels

You should evaluate the performance of the assay on the New System compared to the Old System at low analyte levels with dilution panels and seroconversion panels (if applicable).

- Where available, assay performance at low positive analyte levels using dilution panels should be based on international standards (e.g., World Health Organization (WHO) standards, Paul Erhlich Institute (PEI) standards) and compared to the Old System.

- If available and appropriate, test well-characterized seroconversion panels similar in number and type to the panels originally used to support approval/licensure. The seroconversion panels should be run on both the Old System and the New System.

b. Within-Laboratory Precision Study

We recommend that you conduct in-house within-laboratory precision studies, (to supplement the external site reproducibility studies described below in Section c). When appropriate and justified, the in-house within-laboratory precision study may not be necessary, such as (i) if you established that the New System only needs to be recalibrated at relatively long time-intervals (e.g., 6 months or more) and any other concerns can be appropriately addressed by the reproducibility study, or (ii) if the New System is recalibrated daily, so that calibration cycle variability is inseparable from day-to-day variability (which is assessed by the reproducibility studies described below) and any other concerns can be appropriately addressed by the reproducibility study

It may be sufficient to perform within-laboratory precision studies only on the New System. However, if the study design or composition of the precision panel of the Old System precision study was very different from that described in this guidance, it may be important to perform the precision study on the Old System as well. The within-laboratory precision study described below is based on modified CLSI document EP5-A2.

Sources of variability we recommend for the within-laboratory precision study are at least 12 days of testing, with 2 runs per day, and 2 replicates of each sample per run. These 12 days are not necessarily consecutive and they should span at least two calibration cycles (the calibration cycles may be non-consecutive). For each cycle, days at the beginning and end of the cycle should be included (e.g., 3 days at the beginning and 3 days at the end of each cycle, for each cycle). You should include other additional sources of variability in the design of the study, if they are important to the specific assay (e.g., operators, lots, etc.). In such cases, overall modification to the variables might be possible (e.g., spreading days of testing between different operators). If analytical and clinical performance is similar across all matrices that are included in the intended use of the Old System, then establishing performance of the New System using the most commonly employed matrix may suffice.

In concept, a cutoff for a qualitative test is established based on acceptable clinical performance (e.g., sensitivity/specificity) for the samples from the intended use population. This cutoff (threshold) is used for defining positive and negative results of the test. When the observed result exceeds the threshold, the result is considered positive (or reactive); when the observed result is below the cutoff, it is considered negative (or not reactive). A useful characteristic of the cutoff is that a sample with an actual *concentration* at the cutoff yields a positive result 50% of the time and a negative result 50% of the time when a large number of replicates of that sample are run under stipulated conditions (see Figure 1 below). We denote this concentration as C_{50}.

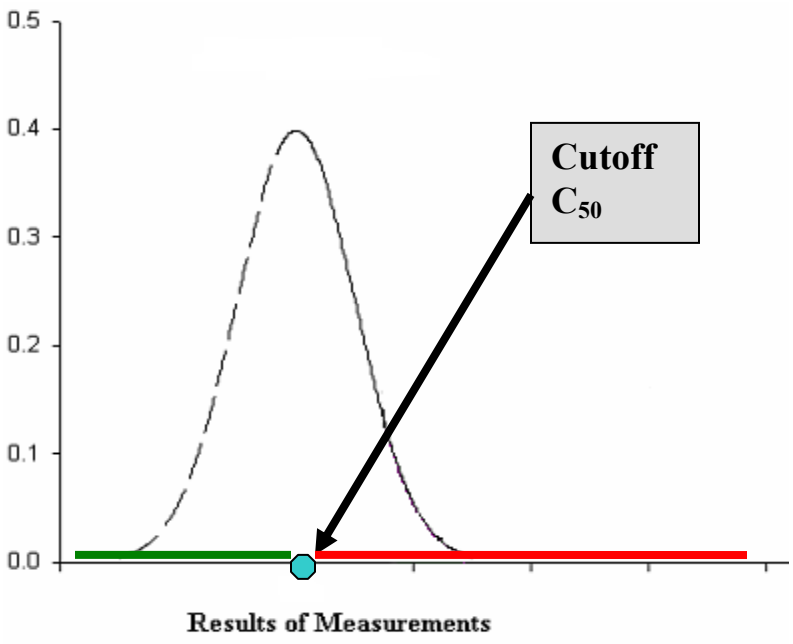


Figure 1. Results of a qualitative test for a sample with a concentration at the cutoff

For samples with concentrations exceeding C_{50}, one expects to see positive results more than 50% of the time and similarly for samples with a concentration below C_{50}, one expects to see positive results less than 50% of the time. In this guidance we refer to an analyte concentration that yields, upon evaluating many replicates, a positive result 95% of the time (and negative result 5% of the time) as a Low Positive concentration (C_{95} concentration). We refer to a sample concentration below the C_{50} which yields a positive result 5% of the time (and negative result 95% of the time) as a High Negative concentration (C_5 concentration). When the limit of blank (LoB) is used as a cutoff, then the concentration C_{95} is the same as the limit of detection (LoD) and zero concentration (no analyte present in sample) is C_5. The LoB and LoD are discussed more thoroughly in Clinical Laboratory and Standards Institute (CLSI) document EP17-A.

Samples with concentrations of analyte close to C_{95} and C_5 as determined by the Old System are recommended for the within-laboratory precision (see CLSI EP12-A2). The panel should consist of at least three members, as described below (also, see Figure 2):

- A High Negative sample: a sample that repeatedly tests negative approximately 95% of the time and a positive result 5% of the time by the Old System. One should expect that the same concentration tested by the New System will produce negative results approximately 95% of the time (C_5 concentration as determined by the Old System).

- A Low Positive sample: a sample with an analyte concentration around C_{95} as determined by the Old System. Repeatedly testing this sample by the Old System should give a positive result approximately 95% of the time and a negative result 5% of the time. One should expect that the same concentration tested by the New System will also produce positive results approximately 95% of the time. Note that if the LoB is used as a cutoff, then the concentration C_{95} is the same as the LoD.

- A Moderate Positive sample: a sample with a concentration close to the cutoff and at which one observes positive results by the Old System approximately 100% of the time (e.g., a sample with a signal approximately two to three times the signal at cutoff if the cutoff=1.0 or a sample with concentration approximately two to three times the 95% LoD if the cutoff is based on LoB).

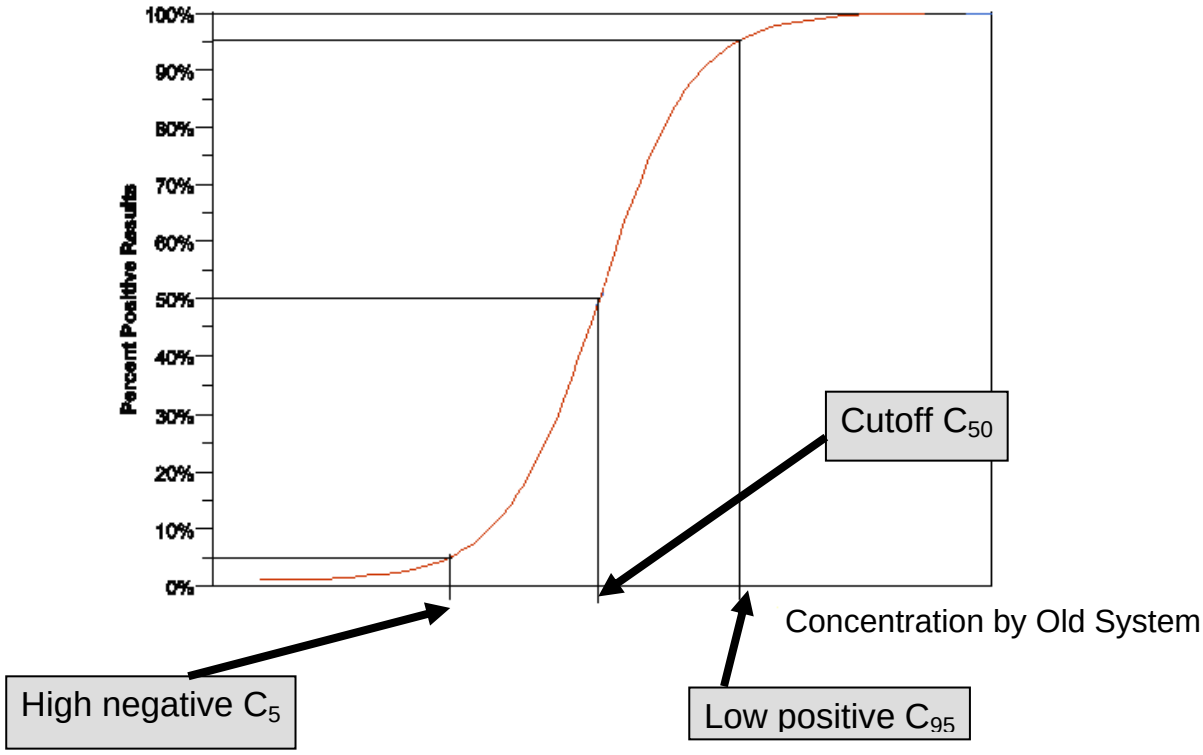


Figure 2. Relationship between percent of positive results and the analyte cutoff concentration.

For details of how the C_{95} and C_5 concentrations can be evaluated from the previous precision studies of the Old System (see Appendix II: Statistical Notes, 1). For the precision study of the New System, it is not necessary to have the high negative and low positive samples at exactly C_5 or C_{95} of the Old System. If the high negative and low positive samples in the precision study of the New System are close enough to the cutoff that the standard deviation (or percent coefficient of variation (CV)) is approximately constant over

the range around the cutoff, the C_5 and C_{95} of the New System can be evaluated from this precision study (see Appendix II: Statistical Notes, 1).

In addition to the high negative, low positive, and moderate positive samples, you should run the appropriate control material (negative and positive controls) and calibrators in the precision study.

c. Reproducibility Study

We recommend you perform the reproducibility study based on a modification of CLSI EP15-A2 on the New System. The panel composition and analyte levels for this study should be the same as described in the within-laboratory precision study (Section A.1.b) and sources of variability should include testing for at least 5 days, 2 runs per day, with 3 replicates of each panel member per run at 3 laboratories (1 in-house and 2 external sites). Other sources of variability might be applicable if relevant to the specific assay (e.g., operators). If analytical and clinical performance is similar across all matrices that are indicated in the intended use of the Old System, then establishing performance of the New System using the most commonly employed matrix may suffice.

For each concentration level, similar information should be available for the Old System. Otherwise, you should perform a new reproducibility study for the Old System with study design and concentration levels as described in this section.

2. Comparison Studies for Qualitative Assays

You should perform comparison studies using comparison panels. However, minor changes to the Old System might not warrant performing all comparison studies. The extent of the utility of these studies can be evaluated on a case-by-case basis in consultation with the FDA.

a. Comparison Panels

For each analyte, the qualitative assay comparison panels should consist of the following:

- Panels of samples known to be positive or negative for a specific assay on the Old System (in order to evaluate the same assay on the New System). We suggest that the positive and negative panels have at least 100 panel members each.

  Positive Panel members should be prepared so that approximately 60%-80% of the samples have analyte levels close to the cutoff. Of these samples, approximately one half should be close to the C_{95} of the Old System and the other half should be Moderate Positive samples. The remaining positive samples should evenly cover the full detection range of the assay.

  Negative Panel members should be prepared so that approximately 30%-40% are High Negatives (close to C_5 of the Old System). Because FDA is assessing clinical effectiveness based upon the result agreement for the assay performed on

the two instruments, these samples can have an analyte concentration below the cutoff such that repeat testing of these samples should be negative approximately 95% of the time. Alternatively, these High Negative samples may be obtained from banked clinical samples giving a signal just below the cutoff.

- It is preferable to use non-contrived clinical samples to create the panel members. However, where positive samples are not available or volumes are low, you should work with the FDA to define acceptable sample types. Positive panel members may be diluted in a clinical matrix in some cases. Preferably, individual negative samples should be used for each dilution. Spiked samples may be acceptable, but only from individual strong positives in a clinical matrix (i.e., the same sample cannot be used repetitively).

- Archived samples are acceptable (if stored in accordance with the package insert instructions). The samples should be randomized and masked in the order that they are run.

b. Testing Venue for the Qualitative Assay Comparison Panels

You should test the comparison panels on the Old System at a minimum of one site. This may be done in-house. However, you may want to use more than one Old System to better assess instrument bias. The New System should be tested at a minimum of three sites (one may be in-house) with at least one reagent kit lot. Each panel member should be tested at least four times: once with the Old System and three times with the New System. You should send the same positive and negative panels to each site, rather than dividing the panel between the three sites. Three different builds of the New System should be tested, one at each of the three sites.

3. Statistical Analysis of Data

We recommend you include the information described in this section for each of the respective studies, in your submission:

a. Within-Laboratory Precision Study

- For each analyte concentration level tested, we recommend you present the mean value with variance components (standard deviation and percent CV) for the New System. In addition, for qualitative assays with relative numerical values, you should include the percent of values above and below the cutoff for each analyte concentration level. You should also provide an estimation of C_5 and C_{95} of the New System (see Appendix II: Statistical Notes, 1).

- For each analyte concentration, you should provide similar information for the Old System. You can obtain this information from the precision study you originally used to demonstrate the performance of the Old System if the study design and the analyte

concentration levels are comparable to those used to assess the New System. You can utilize information about variance components (standard deviation and percent CV) from the precision study found in the Old System labeling. However, if the study design and analyte concentrations for the Old System are different from those described in this document, a new precision study for the Old System should be performed to allow for comparison between systems.

- For each analyte concentration, we recommend you provide repeatability (within-run precision) and within-laboratory precision of the New and Old Systems (standard deviation and percent CV). In addition, you should provide the ratio of the standard deviations of the New and Old Systems along with the 95% confidence interval for this ratio. The confidence interval can be based on the F-statistic for a ratio of variances.

b. Reproducibility Study

- For each analyte concentration level, we recommend you present the mean value with variance components (standard deviation and percent CV) for the New System, for each site separately and for all sites combined. In addition, for each panel member, you should include the percent of values above and below the cutoff, for each site separately and for all sites combined. You should also provide an estimation of C_5 and C_{95} of the New System, for each site separately and for all sites combined.

- For each analyte concentration level, we recommend you present similar information for the Old System. This information can be taken from the precision study originally used to demonstrate the performance of the Old System if the study design and the analyte concentration levels are comparable to those used to assess the New System. The information about variance components (standard deviation and percent CV) can be obtained from the precision study found in the Old System labeling. If the study design and analyte concentrations are different than those described in this document, a new precision study for the Old System should be performed.

- For each concentration level, you should provide repeatability (within-run precision) and within-laboratory precision (standard deviation and percent CV) combined over three sites of the New Systems and Old System. In addition, we recommend you provide the ratio of repeatability standard deviations and the ratio of the reproducibility standard deviations of the New and Old Systems along with 95% confidence interval for these ratios. The confidence intervals for the ratios can be based on the F-statistic for a ratio of variances.

c. Comparison Panels

- You should provide a scatter-plot of S/CO of the Old System (X-axis) vs. S/CO of the New System (Y-axis) with descriptive statistics, for data generated at each site as well as for all sites combined. Both axes should have the same scale, and the line of identity (y=x) should be presented. The same scale on the axes should be applied to the data from each site.

- You should provide an estimation of systematic differences between the relative numerical values generated by the Old System and the New System at each site and for all sites combined (CLSI EP9-A2). You should perform the appropriate regression analysis (Deming regression), which accounts for the random errors associated with the Old and New System measurements and provide the 95% confidence intervals of the slope and intercept from the regression analysis. The emphasis should be placed on estimating the systematic difference between the relative numerical values of the Old and New Systems around the cutoff. You should also calculate the average systematic difference separately for the negative, high negative, low positive and high positive samples of the comparison panel.

- We recommend that you present tables (e.g., with Old System results defined by the columns and New System results defined by the rows), for data derived from each site and for all data combined (see Appendix II: Statistical Notes, 2). Based on these tables, you should calculate positive and negative percent agreements at each site along with the corresponding 95% two-sided confidence intervals (for confidence intervals, see Appendix II: Statistical Notes, 3). In addition, you should provide the positive and negative percent agreements averaged over three sites with the corresponding 95% two-sided confidence intervals. Because the same samples are used at all three sites, we suggest that a bootstrap approach may be the most straightforward for calculating such confidence intervals.

4. Acceptance Criteria for Qualitative Assay Migration Studies

In addition to the acceptance criteria for performance of the New System at low levels of analyte and for seroconversion panels (if applicable), we recommend you apply the following criteria to demonstrate that there are no changes to performance characteristics that could affect the safety and effectiveness of the device:

- The systematic difference between S/CO of the New and Old Systems should be either clinically and statistically insignificant, or, if statistically significant, should not be clinically significant (see Appendix II: Statistical Notes, 4).

- The ratio of standard deviations in the precision studies (reproducibility and within-laboratory precision) of the Old and New Systems should be either clinically and statistically insignificant or, if statistically significant, should not be clinically significant (see Appendix II: Statistical Notes, 4).

- Conceptually, the New System measurements can be considered comparable to the Old System measurements if the New System measurements of a sample are similar to the repeated evaluations of the same sample when run on the Old System. For the Old System measurements, one can expect high agreement between repeated measurements for the samples with high concentrations of analyte giving measurement results far from the cutoff, and some degree of discordance for the samples with concentrations measuring close to the cutoff.

  In the study using comparison panels, the lower limits of the 95% two-sided confidence intervals for the positive and negative percent agreements between the New System and the Old System should be higher than 90% (see Appendix II: Statistical Notes, 5). Discordant results between the Old and New Systems can only occur with samples close to the cutoff and not with moderate or high positive samples by the Old System (similarly, not with moderate or low negative samples by the Old System).

- You should investigate any differences found between the two systems (e.g., in precision testing performance) or any systematic difference, and perform risk assessments to determine the percentage of the intended use population that would be affected by such a difference.

B. Migration Studies for Quantitative Assays

1. Analytical Studies for Quantitative Assays

If you believe that some of these studies do not apply to your particular device, you should describe your reasoning in detail in your application to FDA. If the design of the analytical studies conducted for the Old System were different from those described in this guidance, please contact FDA.

We recommend that you use fresh clinical specimens for all analytical studies. If this is impractical in some cases you may substitute or supplement fresh clinical specimens with banked samples. If banked samples are not available, spiked or diluted clinical samples may be used. In some instances, use of otherwise contrived matrix-specific samples may also be appropriate, however these should mimic clinical specimens as much as is feasible. We recommend that you contact FDA if you wish to discuss appropriate sample types for these evaluations. The matrix of any of these alternative specimens should be the same as that specified by the intended use of the Old System.

a. Performance at Low Analyte Levels

For assays that were previously approved or licensed with a specified LoB (limit of blank) and LoD (limit of detection), the same evaluations should be repeated with the New System. The study should demonstrate that the LoB and LoD are very similar for both systems (a protocol is described in CLSI EP17-A). Specifically, the sample with a concentration at the

LoD (reported as "analyte detected" approximately 95% of the time, measured by the Old System) should also be reported as "analyte detected" approximately 95% of the time, if measured by the New System (see Figure 3 below).

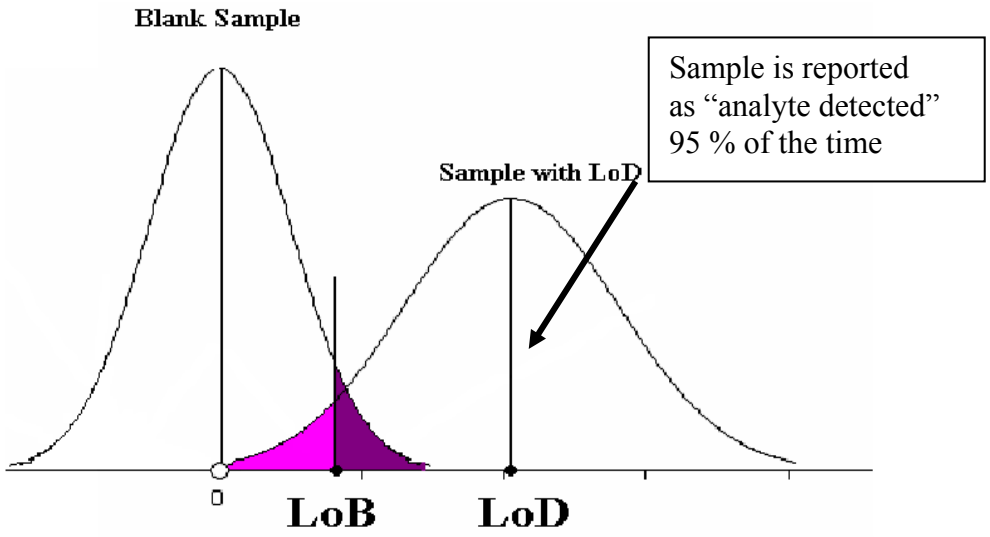


Figure 3. Relationship Between Measurements of the Blank Sample and Limit of Detection Sample.

The limit of quantification (LoQ, or lower limit of measuring range) of the New System should be estimated and compared with the LoQ of the Old System (see CLSI EP17-A) and should be similar to that of the Old System. The specification criteria for the LoQ of the New System should be the same as for the Old System. We also recommend that the LoQ correspond to an analyte concentration level used in the precision studies.

b. Within-Laboratory Precision Study

We recommend that you conduct in-house within-laboratory precision studies (to supplement the external site reproducibility studies described below in Section c). When appropriate and justified, the in-house within-laboratory precision study may not be called for, for example, (i) if the manufacturer established that the New System only needs to be recalibrated at relatively long time intervals (e.g., 6 months or more) and any other concerns can be appropriately addressed by the reproducibility study, or (ii) if the New System is recalibrated daily, so that calibration cycle variability is inseparable from day-to-day variability (which is assessed by the reproducibility studies described below) and any other concerns can be appropriately addressed by the reproducibility study.

It may be sufficient to perform within-laboratory precision studies only on the New System. However, if the study design or composition of the precision panel of the Old

System precision study was very different from that described in this guidance, it may be important to perform the precision study on the Old System as well. The within-laboratory precision study described below is based on modified CLSI document EP5-A2.

We recommend you evaluate samples with the following levels of analyte:

- Lowest limit of the measuring range
- A level of analyte below the medical decision point
- Around the medical decision point
- A level of analyte above the medical decision point
- Upper limit of the measuring range.

In addition, you should run the appropriate control material and calibrators associated with the test kit in the precision study.

If the assay has more than one medical decision point, then samples with concentrations around these medical decision points should be evaluated. It is understood that some assays will not have a specific medical decision point, but rather a range of values; in such cases, the panel should contain samples scattered throughout the measuring range of the assay.

Sources of variability we recommend for the within-laboratory precision study are at least 12 days of testing, with 2 runs per day, and 2 replicates of each sample per run. These 12 days are not necessarily consecutive and they should span at least two calibration cycles (the calibration cycles may be non-consecutive). For each cycle, you should include days at the beginning and end of the cycle (e.g., 3 days at the beginning and 3 days at the end of each cycle, for each cycle). You should include other additional sources of variability in the design of the study, if they are important to the specific assay (e.g., operators, lots, etc.). In such cases overall modification to the variables might be possible (e.g., spreading days of testing between different operators). If analytical and clinical performance is similar across all matrices that are included in the Intended Use of the Old System, then establishing performance of the New System using the most commonly employed matrix may suffice.

c. Reproducibility Study

We recommend you perform the reproducibility study based on a modification of CLSI EP15-A2 on the New System. The panel composition and analyte levels for this study should be the same as described in the within-laboratory precision study (Section B.1.b). We recommend that sources of variability should include testing for at least 5 days, 2 runs per day, with 3 replicates of each panel member per run at 3 laboratories (1 in-house and 2 external sites). Other sources of variability might be applicable if relevant to the specific assay (e.g., operators, etc.). If analytical and clinical performance is similar across all

matrices that are indicated in the intended Use of the Old System, then establishing performance of the New System using the most commonly employed matrix may suffice.

For each concentration level, similar information should be available for the Old System. If this is not the case, a new reproducibility study for the Old System should be performed with study design and concentration levels as described in this section.

d. **Linearity Study**

We recommend that you evaluate linearity for the New System according to CLSI EP6-A. The degree of linearity can be quantified using the maximum deviation from linearity (i.e., the delta described in CLSI document EP6-A). Your linearity study results should demonstrate that the delta of the New System is not greater than the observed delta in the linearity studies of the Old System. You should determine the appropriate number of replicates in the linearity study for the New System based on the precision studies of the New System.

2. Comparison Studies for Quantitative Assays

You should perform comparison studies using comparison panels. Relatively minor changes to the Old System might not warrant all comparison studies. The extent of the utility of these studies can be evaluated on a case-by-case basis in consultation with the FDA.

a. Comparison Panels

For each analyte, the composition of the quantitative assay comparison panels should consist of at least 180 samples,[8] including the following:

- 150 of the 180 samples should span the measuring range of the assay with approximately equal numbers of samples at low, medium and high analyte concentrations.

- If the performance of the assay at low level concentrations is clinically important, the remaining panel members should consist of at least 30 samples (or about 15%-20% of the total) that should be patient samples with concentration at or near zero. The zero-level samples may need to consist of different matrices and/or be from persons with unrelated medical conditions.

- It is preferable to use non-contrived clinical samples. However, where clinical samples are not available, or volumes are low, pooling is a possible strategy. If pooling would not be efficacious, then dilutions, made serially of individual samples with a high analyte concentration diluted into clinical matrices, can be used. Spiked samples are acceptable, if they are prepared from individual samples with a high analyte concentration in clinical matrices (i.e., the same sample cannot be used

[8] For more information on sample size, please refer to Appendix II: Statistical Notes, 7.

repetitively).

- Archived samples are acceptable if stored in accordance with the package insert instructions. Samples should be randomized and masked in the order that they are run.

- The panel should consist of appropriate analyte-specific members. For example, different subtypes or strains of an infectious agent should be included when applicable.

b. Testing Venue for the Quantitative Assay Comparison Panels

You should test the comparison panels on the Old System at a minimum of one site. This may be done in-house. However, you may want to use more than one Old System to better assess instrument bias. The New System should be tested at a minimum of three sites (one may be in-house) with at least one reagent kit lot. Each panel member should be tested at least four times: once with the Old System and three times with the New System. You should send the same positive and negative panels to each site, rather than dividing the panel between the three sites. Three different builds of the New System should be tested, one at each of the three sites.

3. Statistical Analysis of Data

a. Within-Laboratory Precision Study

- For each analyte concentration level tested, we recommend you present the mean value with variance components (standard deviation and percent CV) for the New System.

- For each analyte concentration, you should present similar information for the Old System. This information can be taken from the precision study originally used to demonstrate the performance of the Old System if the study design and the analyte concentration levels are comparable to those used to assess the New System. The information about variance components (standard deviation and percent CV) can be obtained from the precision study found in the Old System labeling. If the study design and analyte concentrations are different than those described in this guidance document, you should perform a new precision study for the Old System.

- For each analyte concentration, we recommend you provide 95% confidence intervals for repeatability and within-laboratory precision (standard deviation and percent CV) of the New and Old Systems. In addition, we recommend you provide the ratio of the repeatability standard deviations of the New and Old Systems and the ratio of within-laboratory standard deviations along with the 95% confidence interval for these ratios. The confidence interval can be based on the F-statistic for a ratio of variances.

b. Reproducibility Study

- For each analyte concentration level, you should present the mean value with variance components (standard deviation and percent CV) for the New System for each site separately and for all sites combined.

- For each analyte concentration level, you should present similar information for the Old System. This information can be taken from the precision study originally used to demonstrate the performance of the Old System if the study design and the analyte concentration levels are comparable to those used to assess the New System. The information about variance components (standard deviation and percent CV) can be obtained from the precision study found in the Old System labeling. If the study design and analyte concentrations are different than those described in this document, a new precision study for the Old System should be performed.

- For each concentration level, you should provide repeatability (within-run precision) and within-laboratory precision (standard deviation and percent CV) combined over three sites of the New Systems and Old System. In addition, you should provide the ratio of repeatability standard deviations and the ratio of the reproducibility standard deviations of the New and Old Systems along with 95% confidence interval for these ratios. The confidence intervals for the ratios can be based on the F-statistic for a ratio of variances.

c. Comparison Panels

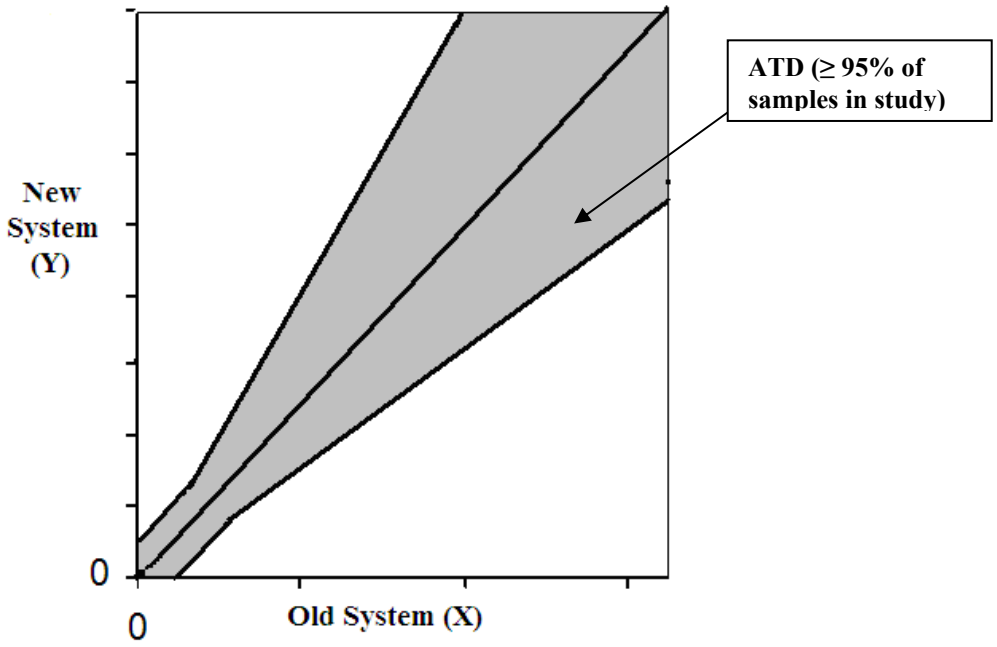
- You should provide a scatter-plot of numerical values of the Old System (X-axis) vs. numerical values of the New System (Y-axis) with descriptive statistics for data at each site as well as for all sites combined. Both axes should have the same scale and the line of identity (y=x), and the same scale on the axes should be applied to the data from each site.

- You should provide an estimation of systematic differences between absolute numerical values by the New and Old Systems (CLSI EP9-A2) for each site and for all sites combined. You should perform an appropriate regression analysis (such as a Deming regression) which accounts for the random errors associated with the Old and New System measurements, and provide the 95% confidence intervals of the slope and intercept from this analysis. We recommend that, for the data from each site and for the combined site data, you draw the regression line on the corresponding scatter plots and plot the fitted lines (for each site and all sites combined) on the same corresponding figures. Using the regression equation, you should calculate the systematic bias at all medically important points along with 95% confidence intervals (see Section 6.1 in CLSI EP9-A2).

- Conceptually, the New System measurements can be considered comparable to the Old System measurements if the New System measurements of a sample are similar

to the repeated evaluations of the same sample when run on the Old System. Using the reproducibility results of the Old System, one can construct limits or boundaries that define where 95% of the differences between two repeated measurements by the Old System are inside of these limits (see Appendix II: Statistical Notes, 6). These limits define an Allowable Total Difference (ATD) zone (see CLSI EP21-A) for differences between New System and Old System measurements. It is anticipated that no less than 95% of the sample results will fall within the ATD zones (see Figure 4, below). For details see Appendix II, Statistical Notes, 6.

Figure 4 Allowable Total Difference Zone



- You should calculate (i) the percentage of the samples at low, medium, and high concentration ranges that fall within the ATD zone at each site and averaged over three sites, (ii) the percentage of samples over the entire range that fall within the ATD zone with a lower limit of 95% one-sided confidence interval at each site, and (iii) the percentage of samples over the entire range that fall within the ATD zone averaged over three sites (using a bootstrap technique, you should provide the 95% one-sided confidence interval for the percentage of the samples over the entire range that fall within the ATD zone averaged over three sites).

4. Acceptance Criteria for Quantitative Assay Migration Studies

In addition to the acceptance criteria for LoB, LoD, LoQ and linearity, we recommend that you apply the following criteria:

- The systematic difference between numerical values of the New and Old Systems should be either clinically and statistically insignificant or if statistically significant should not be clinically significant (see Appendix II: Statistical Notes, 4).

- The ratio of standard deviations in the precision studies (reproducibility and within-laboratory precision) of the Old and New Systems should be either clinically and statistically insignificant or if statistically significant should not be clinically significant (see Appendix II: Statistical Notes, 4).

- The percentage of the observations over the entire range that fall within the ATD zone should be close to 95% with a lower limit of the 95% one-sided confidence interval higher than 90%. The percentages of the observations that fall within the ATD zone for the low, medium, and high ranges should approach 95% for each range (see note in Appendix II: Statistical Notes, 7).

- If applicable, the percent of positive results by the New System among zero-level samples should be consistent with a Type I error of LoB of the Old System across three sites (Type I error is the probability of having truly negative samples, those with zero analyte concentration, give values that indicate the presence of analyte. Usually, Type I error is set as 5% or less).

- You should investigate any differences found between the two systems and perform risk assessment to determine what percentage of the intended use population would be affected by these differences.

VII. Other Studies

Depending upon the unique characteristics of the qualitative or quantitative assay being migrated to the New System, the following studies may be called for. *If not previously conducted for the Old System, they should be performed for the New System*. If you determine that a study described below is not applicable for your system, you should describe your reasoning in detail in your application to FDA. FDA will consider such explanations on a case-by-case basis particularly for manual to semi-automated or automated System migrations.

- Carry-over or cross-contamination studies: The importance of repeating these studies on a New System can be ascertained by a thorough analysis of the New System. As mentioned in section V of this document, block diagrams and side-by-side comparison tables would be beneficial in this determination. Changes to specific physical features such as a change in sample pipettor design or the layout of the New System could indicate the need for new carry-over studies. If a carry-over study for the New System is appropriate and the new design is sufficiently similar to the Old System, the new study can be the same as previously used for the Old System. Samples with high positive concentrations of analyte should be tested alternating with

analyte-negative samples in patterns dependent on the operational function of the instrument. The concentration of analyte in the high positive samples should exceed 95%-99% or more of the results normally obtained in clinical samples from diseased patients in the intended use population. This testing should be done over multiple runs (at least 5 runs are recommended by the Commission of European Communities[9]).

- Matrix equivalency and recovery studies: Presumptively because there are no changes to the assay, there should be no new effects on assay performance due to different matrices unless physical alterations of the New System could create such an effect. Similarly, recovery studies should not be affected by the migration of an assay from an Old to New System.

- Interfering substances studies: Presumptively with no changes to the assay there should be no new effects on assay performance due to interfering substances.

- On-board reagent/calibrator and sample stability studies: Unless there are physical or process changes to the New System, presumptively there should be no effect on assay performance due to on-board reagent or sample stability.

- Cross-reactivity studies: Presumptively, as there are no changes in assay components, migration to a New System should not affect existing cross-reactivity information.

- Hook effect studies: Presumptively because there is no assay change, the parameters of the hook effect should be the same, unless physical alterations in the New System could create such an effect.

- Verification of kit control material and calibrators: Presumptively because there is no change to the assay, the control results and calibration ranges should remain the same, unless physical alterations in the New System could create new effects.

VIII. Molecular Assays

There are specific criteria that are unique to nucleic acid tests (NAT) and therefore NATs present additional specific concerns over serological and antigen assays:

- When appropriate, you should provide testing with panels showing a rise in viral titer over time from serial bleeds (viremic profile). Similar to seroconversion panels, they should have a minimum number of days between bleeds and begin with at least one negative bleed. They should be of clinical relevance to the appropriate individual marker.

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9 Commission Decision 2002/364/EC of 7 May 2002 on common technical specifications for in vitro-diagnostic medical devices [Official Journal L 131 of 16.05.2002].

- Carryover studies: Because of the increased risks of carryover due to the amplification methodologies utilized in molecular testing (e.g., PCR, TMA), you should perform carryover studies for all NAT migration studies.

- Sample stability: Because of the delicate nature of DNA and especially RNA, careful attention should be given to the stability of samples in relation to on-board storage and manipulation.

- Sample processing: The processes of purification and extraction of DNA or RNA from clinical samples is critical to the success of molecular tests. You should evaluate any additions or modifications associated with the New System that could affect these processes.

- Validation of control material and calibrators: You should perform these studies on the New System due to the sensitive nature of molecular assays.

- For molecular assays detecting multiple analytes, please contact the FDA for further discussion.

IX. Regulatory Outcomes

- Should the acceptance criteria noted in Sections VI.A.4 or VI.B.4 be met, it would be appropriate for the sponsor to claim that the New System does not compromise the results as compared to the Old System. It would not be appropriate to claim improved performance characteristics. It would also not be appropriate to compute clinical performance claims for the New System based on the migration studies described here, since these studies are analytical, rather than clinical... Should you wish to develop more extensive claims, the migration studies paradigm would not be an appropriate scientific approach.

- However, if the acceptance criteria are not met and based upon the FDA's best judgment the aberrant performance could affect clinical management, you will be asked to perform a complete clinical study presenting the clinical performance of the assay on the New System.

X. Glossary

For the purposes of this document the following definitions are used. HTD referenced terms are based on the CLSI Harmonized Terminology Database.

Block diagrams: Engineering diagrams that graphically describe the instrument's interior and exterior features, preferably to scale.

C_5: a concentration at which repeated tests of a sample with this concentration under stipulated conditions are 95% negative (or 5% positive) (see CLSI EP12-A2).

C_{95}: a concentration at which repeated tests of a sample with this concentration under stipulated conditions are 95% positive (see CLSI EP12-A2).

C_{50}: a concentration at which repeated tests of a sample with this concentration under stipulated conditions are 50 % positive (or 50% negative). Under ideal circumstances, C_{50} will exactly equal the cutoff established by the manufacturer.

Calibrators: a substance or device that is based on a reference preparation or in which the analyte concentration or other quantity has been determined by an analytical procedure of stated reliability. Calibrators are used to calibrate, graduate, or adjust a measurement [HTD].

Carry-over: amount of analyte carried by the measuring system from one sample reaction into subsequent sample reactions, thereby erroneously affecting the apparent amounts in subsequent samples [HTD].

Control material: a device, solution, or preparation intended for use in the quality control process to monitor the reliability of a test system and to maintain its performance within established limits.

Cross-reactivity: the ability of a drug, metabolite, a structurally similar compound other than the primary analyte, or even unrelated compound to affect the assay [HTD].

Cutoff value (CO): for a qualitative test, the threshold above which the result of the test is reported as positive and below which the result is reported as negative. If a large series of tests were performed for a sample with concentration at the cutoff, 50% of test results will be positive and 50% will be negative; this analyte concentration can be termed C_{50} (CLSI EP12-A2).

High negative sample (C_5): a sample with a concentration of analyte close to the C_5 as determined by the Old System. This term is equivalent to a "weak negative sample" for example as used in the CLIA Waiver Guidance document www.fda.gov/cdrh/oivd/guidance/1171.pdf

Hook effect (high dose hook effect): effect caused by a decreasing signal response at very high levels of analyte. It is used interchangeably with "prozone effect," the result of a suboptimal antigen-antibody reaction in which either the antibody or antigen is in excess resulting in an incomplete, or blocked reaction [HTD].

Interfering substances: endogenous (e.g., blood components, acidic polysaccharides) or exogenous (e.g., talc, anticoagulant) substances in clinical samples that can cause false-positive or false-negative results in a test system [HTD].

Limit of blank (LoB): highest measurement result that is likely to be observed (with a stated probability) for a blank sample (a sample with concentration at or near zero) (CLSI EP17-A; [HTD]).

Limit of detection (LoD): the lowest concentration of analyte that can be reported to be present at a specified level of confidence, although perhaps not quantified to an exact value. Similarly, an amount of analyte in a sample for which the probability of falsely claiming the absence is β (type II error) given a probability α (type I error) of falsely claiming its presence (CLSI EP17-A; [HTD])

Limit of quantification (LoQ): the lowest amount of analyte in a sample that can be quantitatively determined with {stated} acceptable precision and {stated, acceptable} accuracy, under stated experimental conditions (CLSI EP17-A; [HTD]).

Linearity studies: studies to determine the analyte concentration range over which the testing systems results are acceptably linear with the ability (within a given range) to provide results that are directly proportional to the concentration (amount) of the analyte in the test sample.

Low positive sample (C_{95}): a sample with a concentration of analyte close to C_{95} as determined by the Old System. This term is equivalent to "weak positive sample" for example as used in the CLIA Waiver Guidance document, www.fda.gov/cdrh/oivd/guidance/1171.pdf.

Measurand: particular quantity subject to measurement. The term "measurand" and its definition encompass all quantities while the commonly used term "analyte" refers to a tangible entity subject to measurement [HTD]**.**

Measuring range: set of values of measurands for which the error of a measuring instrument is intended to lie within specified limits. The range of values (in units appropriate for the analyte [measurand]) over which the acceptability criteria for the method have been met; that is, where errors due to nonlinearity, imprecision, or other sources are within defined limits (CLSI EP6-A, [HTD]).

Medical decision level (medical decision point): a level or concentration at which a test is critically interpreted for patient care and treatment.

Moderate positive sample: a sample with a concentration close enough to the cutoff and at which one can anticipate positive results by the Old System approximately 100% of the time.

Negative percent agreement: the proportion of samples negative by the Old System for which the results by the New System are negative (see FDA guidance at www.fda.gov/cdrh/osb/guidance/1620.pdf).[10]

[10] The general definition from the cited guidance is adapted in this guidance since the cited guidance refers to clinical subjects, whereas this guidance does not involve subjects, but rather specimens or samples. In addition, the term "non-reference standard" in the cited guidance is analogous to "Old System" in this guidance; the term "test" in the cited guidance is analogous to "New System".

New System: an unapproved/unlicensed system (assay, instrument, and software) to which the assay is migrating from a previously approved/licensed system.

Old System: an approved/licensed system (assay, instrument and software) from which the assay is migrating to a currently unapproved/unlicensed system.

Positive percent agreement: the proportion of samples positive by the Old System for which the results by the New System are positive (see FDA guidance at www.fda.gov/cdrh/osb/guidance/1620.pdf).

Repeatability: closeness of the agreement between the results of successive measurements of the same measurand carried out under the same conditions of measurement (See CLSI EP5-A2, [HTD]).

Reproducibility: closeness of agreement between the results of measurements of the same measurand and carried out under changed conditions of measurement. Reproducibility conditions are conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment and can include additional variables such as days, replicates, and runs (See CLSI EP5-A2, [HTD])

Risk analysis: systematic use of available information to identify hazards and to estimate the risk. Risk analysis includes examination of different sequences of events that can produce hazardous situations and harm [HTD].

Spiked sample: a clinical sample to which has been added exogenous analyte to create specified levels of signal.

Systematic difference: a mean of the measurand on the New System minus a value of the same measurand as performed on the Old System that would result from an infinite number of measurements carried out under the stipulated condition (based on HTD).

Within-laboratory precision: precision over a defined time and operators, within the same facility and using the same equipment; calibration and reagents may vary. Formerly, the term "total precision" was used in CLSI EP5-A2 [HTD].

XI. References

CDRH Guidance Documents:

1. *Guidance for Industry and FDA Staff: Replacement Reagent and Instrument Family Policy*, http://www.fda.gov/cdrh/oivd/guidance/950.pdf.
2. *Guidance for Industry and Staff: Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices*, http://www.fda.gov/cdrh/ode/guidance/337.html**.**
3. *Guidance for Industry and FDA Staff: Recommendations for Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications for Manufacturers of In Vitro Diagnostic Devices,* http://www.fda.gov/cdrh/oivd/guidance/1171.pdf.
4. *Guidance for Industry and FDA Staff: Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests, http://www.fda.gov/cdrh/osb/guidance/1620.pdf.*

Clinical and Laboratory Standards Institute (CLSI) documents:

1. CLSI. *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition*. CLSI document EP5-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2004.
2. CLSI. *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.* CLSI document EP6-A. Wayne, PA: Clinical and Laboratory Standards Institute, 2003.
3. CLSI. *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition*. CLSI document EP9-A2. Wayne, PA: Clinical and Laboratory Standards Institute, 2002.
4. CLSI. *User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline-Second Edition.* CLSI document EP12-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
5. CLSI. *User Verification of Performance for Precision and Trueness; Approved Guideline – Second Edition*. CLSI document EP15-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2006.
6. CLSI. *Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline*. CLSI document EP17-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2004.
7. CLSI. *Estimation of Total Analytical Error for Clinical Laboratory Methods; Approved Guideline*. CLSI document EP21-A. Wayne, PA: Clinical and Laboratory Standards Institute, 2003.

Appendix I - Migration Studies for Blood Donor Screening Assays[11]

1. Introduction

Blood Donor Screening Assays for infectious agents, reviewed under Biologics License Applications, are generally held to stringent standards of sensitivity and specificity. Typically, clinical studies for licensure of products such as HIV assays involve testing of over 1000 known positives and 6000 to 10,000 low risk samples (or pools) collected from the intended use population. Consequently, FDA recommends larger study sizes for migrating blood screening assays to New Systems.

Otherwise, except as specifically noted below, the same considerations apply to blood screening assays as described for qualitative assays in Section VI.A of this document, "Migration Studies for Qualitative Assays."

a. Performance at Low Analyte Levels

For immunoassays, FDA recommends that at least 20 seroconversion panels, or as many as are available (whichever is less) be studied, comparing the New and the Old Systems head to head. For nucleic acid tests (NAT), FDA recommends the head-to-head testing of as many seroconversion panels as were tested for licensure of the Old System (typically 10). Both qualitative results and S/CO ratios should be compared.

b. Precision Study (Within-Laboratory Precision)

FDA recommends that sponsors compare the New and Old Systems in a Precision Study as outlined in Section VI.A.1.b

c. Reproducibility Study

FDA recommends that sponsors compare the New and Old Systems in a Reproducibility Study essentially as outlined in Section VI.A.1.c. However, FDA recommends including in the panel at least one truly negative sample (other than a positive sample diluted to below the cutoff), and that testing be performed at three sites, of which one may be in-house.

2. Comparison Panels

a. Positive Panel Members

Option 1: A positive comparison panel should consist of approximately 100 positive samples, to include 20-30 specimens with signals $\leq$3X the cutoff for an immunoassay or analyte concentrations $\leq$3X the 95% LoD for a NAT. Specimens may be diluted to this range if not clinically available. The panel should be tested on the New System at three sites, one of which can be in-house. This panel should also be tested at least once on the Old System (this can be in-house). The data should be analyzed by S/CO regression and analysis of bias using scatter-plots or similar graphical presentations, as described in Section VI.A.3.c.

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[11] Appendix 1 does not apply to immunohematology tests licensed by CBER.

Option 2: As an alternative option, the sponsor may wish to test head to head on the New and Old Systems at three external sites the lowest 10% of positive specimens from the original clinical trial of the assay on the Old System, if the specimens have been stored under conditions defined in the instructions for use of the assay.

b. Negative Panel Members

Option 1: A negative comparison panel should consist of 3000 known negative samples (or pools), or specimens obtained from a low risk study, with adequate follow-up. The panel should be tested on the New System, distributed over three sites, one of which may be in-house (e.g., 1/3 at site one, 1/3 at site two & 1/3 at site three). The data should be analyzed for agreement of the point estimate of specificity (with the 95% confidence interval) for the New System with the original point estimate of specificity (with the 95% confidence interval) from the original trial of the Old System.

Option 2: As an alternative option, the sponsor may wish to test head to head on the Old and New Systems at three external sites the highest 10% of negative specimens from the original clinical trial of the assay on the Old System, if the specimens have been stored under conditions defined in the instructions for use of the assay.

3. Acceptance Criteria

Except as noted above, the same acceptance criteria as recommended in Section VI.A.4 also apply to blood donor screening assays.

Additionally, sponsors may recommend statistical analysis protocols based on estimating false positive and false negative ratios from the negative and positive comparison panel studies, respectively. Sponsors interested in this approach should determine an appropriate model for the S/CO distribution of each panel (positive or negative) together with a proposed method of analysis.

4. Interfering Substances and Conditions

Only substances and conditions that represent a reasonable risk of interference in the New System should be studied. For instance, interfering conditions such as hemolysis or hyperlipidemia might influence pipetting or washing steps and should be included in migration studies. Conversely, it would seem unlikely that cross-reactivity of, for instance, an HIV NAT assay with HTLV would likely be influenced by migration to a New System.

Each interfering substance/condition may be tested in-house using a panel of approximately 10 low positives with signals $\leq$3X the cutoff for an immunoassay or analyte concentrations $\leq$3X the 95% LoD for a NAT.

Each interfering substance/condition may be tested in-house using a panel of approximately 10 true negatives.

Appendix II - Statistical Notes

1. Evaluation of C_5 and C_{95} using Precision Studies

Consider that an assay (qualitative or quantitative) has a numerical output. If the standard deviations (SD) in the precision studies of the Old System for concentrations around the cutoff value are almost constant, then:

$C_{95} = C_{50} + 1.645 \times SD$, and
$C_5 = C_{50} - 1.645 \times SD$

For example, if the cutoff for optical density (OD) value is 1.00 and the SD around the cutoff is approximately 0.10, then C_{95} is approximately 1.16 OD (=1.00+1.645 x 0.10) and C_5 is approximately 0.84 OD (=1.00 -1.645 x 0.10). In other words, a sample with an actual OD of 1.16 produces positive results (above 1.00) approximately 95% of the time and a sample with an actual OD of 0.84 produces negative results (below 1.00) approximately 95% of the time.

If the coefficient of variation (CV) in the precision studies of the Old System for concentrations around the cutoff value are almost constant, then

$C_{95} = C_{50} + 1.645 \times CV \times C_{95}$ and $C_5 = C_{50} - 1.645 \times CV \times C_5$. From here,

$C_{95} = C_{50} / (1 - 1.645 \times CV)$ and
$C_5 = C_{50} / (1 + 1.645 \times CV)$.

For example, if the cutoff has an OD value of 1.00 and the %CV around the cutoff is approximately 10% (i.e., CV=0.10), then C_{95} is approximately 1.20 OD and C_5 is approximately 0.86 OD.

If the limit of blank (LoB) is used as a cutoff, then the concentration C_{95} is the same as the limit of detection (LoD) and zero concentration is C_5 (see CLSI EP17-A).

2. Examples of Data Tables for Qualitative Assays

Positive panel samples:

	Old System Positive		
	Low Positive (close to C_{95})	Moderate Positive	High Positive
New System Positive	27	30	40
New System Negative	3		
Total	30	30	40

Negative panel samples:

	Old System Negative	
	Low and Moderate Negative	High Negative (close to C_5)
New System Positive		3
New System Negative	70	27
Total	70	30

If the CO of the assay is the LoB, the columns of Low Negative and Moderate Negative can be combined as in the example above.

3. Calculating Score Confidence Intervals for Percentages and Proportions

The following are additional recommendations for performing statistical analyses of percentages or proportions. Confidence limits for positive percent agreement and negative percent agreement can be calculated using formulas for calculating a confidence interval for a binomial proportion. There are several different methods available. We suggest that either a score method described by Altman, et al. (Altman D.A., Machin D., Bryant T.N., Gardner M.J. eds. *Statistics with Confidence.* 2nd ed. British Medical Journal; 2000) or a Clopper-Pearson Method (Clopper CJ, Pearson E. *Biometrika* 1934; 26:404-413) be used.

An advantage with the score method is that it has better statistical properties and it can be calculated directly. Score confidence bounds tend to yield narrower confidence intervals than Clopper-Pearson confidence intervals, resulting in a larger lower confidence bound. So with n=100 samples and 96/100=96% agreement, the score lower confidence bound is 90.2% whereas the Clopper-Pearson lower confidence bound is 90.1%. In this document, we have illustrated the reporting of confidence intervals using the score approach. For convenience, we provide the formulas for the score confidence interval for a percentage. Note that the lower bound of a two-sided 95% score confidence interval is the same as the lower bound of a one-sided 97.5% score confidence interval; and the lower bound of one-sided 95% score confidence interval is the same as the lower bound of a two-sided 90% score confidence interval.

A two-sided 95% score confidence interval for the proportion of A/B is calculated as: $\left[100\%\left(Q_1-Q_2\right)/Q_3, 100\%\left(Q_1+Q_2\right)/Q_3\right]$, where the quantities Q_1, Q_2, and Q_3 are computed from the data using the formulas below. For the proportion of A/B:

$$Q_1 = 2\cdot A + 1.96^2 = 2\cdot A + 3.84$$

$$Q_2 = 1.96\sqrt{1.96^2 + 4\cdot A\cdot (B-A)/B} = 1.96\sqrt{3.84 + 4\cdot A\cdot (B-A)/B}$$

$$Q_3 = 2\cdot (B + 1.96^2) = 2\cdot B + 7.68$$

In the formulas above, 1.96 is the quantile from the standard normal distribution that corresponds to 95% confidence. For calculation of 95% one-sided score confidence interval, use 1.645 in place of 1.96 in the formulas above.

4. Relationship Between Clinical and Statistical Significance

Observed Difference From *Clinical* Point of View	**Observed Difference From *Statistical* Point of View**	**Interpretation**
Small	Non-significant[1]	Acceptable
Small	Significant[1]	Acceptable
Large	Non-Significant	Larger sample size is likely needed
Large	Significant	Not acceptable

[1]Confidence interval is within clinically acceptable differences

5. Acceptance Criteria for Positive and Negative Percent Agreements

For a panel of 100 samples which test positive by the Old System, and of which 96 also test positive by the New System (96 out of 100), the lower limit of the 95% two-sided score confidence interval is above 90%. For 30 samples with values close to C_{95}, the 95% two-sided confidence interval for 26/30 (87%) is 70.3% to 94.7%. If, for example, among the 30 samples with low positive concentrations (concentrations close to C_{95} by the Old System), only 25 samples test positive by the New System, then the percent of positive results by the New System for the samples close to the cutoff is statistically different from 95% (83% (25/30) with 95% CI: 66.4% to 92.7%).

6. Allowable Total Difference

For each sample of the Comparison panel, calculate the differences between the New System result (Y) and the Old System result (X), Y-X (based on CLSI EP21). Also calculate (X+Y)/2. Plot the difference between Y and X, Y-X, against their mean (Y+X)/2 (Bland-Altman plot). On the Bland-Altman plot of (Y-X) vs. (Y+X)/2, provide the Allowable Total Difference (ATD) zone around the axis, (Y+X)/2. The ATD zone is established in such a way that 95% of differences between the Old System result and the repeated result by the Old System fall within the ATD. The ATD zone is expressed as:

$\pm 1.96 \bullet \sqrt{2} \bullet CV \bullet (Y+X)/2 = \pm 2.77 \bullet CV \bullet (Y+X)/2$ for larger values of Old System and $\pm 1.96 \bullet \sqrt{2} \bullet SD = \pm 2.77 \bullet SD$ for the low values of Old System where CV and SD are the reproducibility characteristics of the Old System (see *Establishing SD and percent CV for ATD Based on the Performance of the Old System* below). A hypothetical example of the ATD zone on the Bland-Altman plot is provided below (Figure 5):

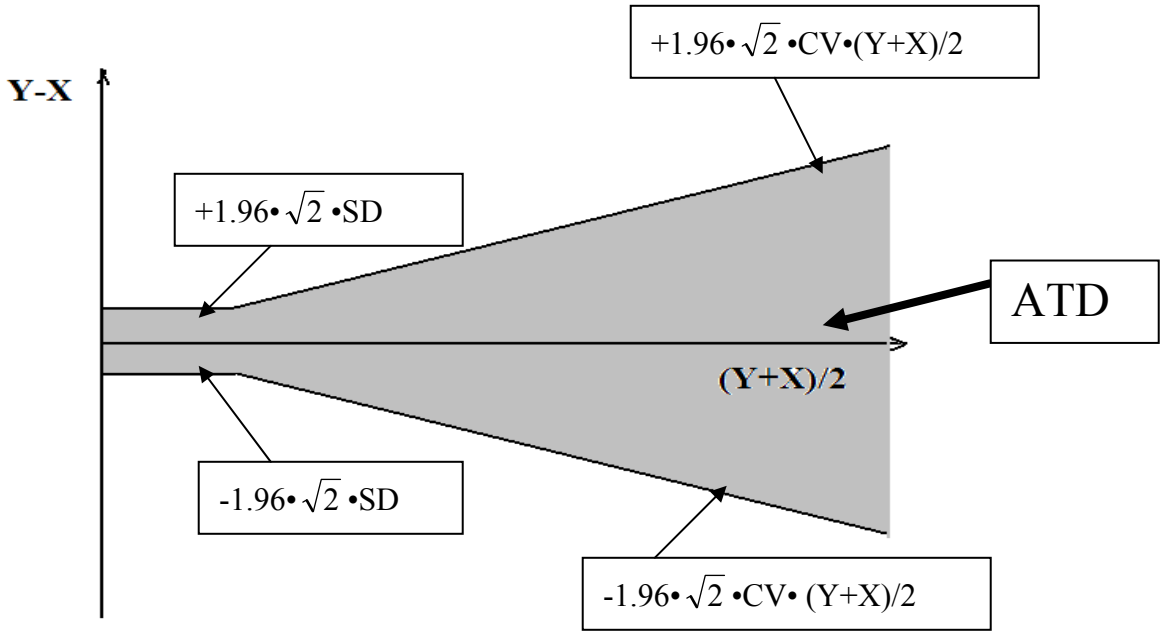

Figure 5. A hypothetical example of the ATD zone on the Bland-Altman plot is provided below.

By the appropriate transformation, a similar ATD zone can be presented on the plane of New System values (Y) against Old System values (X), see Figure 4. The expressions of the lines of the ATD zone on the plane Y vs. X are the following:

Lines parallel to the diagonal of the ATD zone:

$$Y = X \pm 1.96 \cdot \sqrt{2} \cdot SD = X \pm 2.77 \cdot SD, \quad \text{if } 0 \le Y+X \le 2 \cdot A$$

Lines for the "expanding" part of the ATD zone are:

$$Y = X \cdot \left(1 + \frac{1.96 \cdot \sqrt{2} \cdot CV}{1 - 1.96 \cdot \sqrt{2} \cdot CV / 2}\right) = X \cdot \left(1 + \frac{2.77 \cdot CV}{1 - 1.39 \cdot CV}\right)$$

$$Y = X \cdot \left(1 - \frac{1.96 \cdot \sqrt{2} \cdot CV}{1 + 1.96 \cdot \sqrt{2} \cdot CV / 2}\right) = X \cdot \left(1 - \frac{2.77 \cdot CV}{1 + 1.39 \cdot CV}\right) \quad \text{if } Y+X > 2 \cdot A$$

where A is SD/CV.

Establishing SD and percent CV for ATD Based on the Performance of the Old System

For an individual measurement X_i of a given sample by the Old System, there is a following expression: $X_i = X_{truei}$ + Mean-Bias + Random-Bias$_i$ +ε_i where deviation of X_i from the true

value X_{truei} is composed of a mean bias, a random matrix-related interference component, and a random measurement error[12,13]. Because there are no changes in the assay, it is anticipated that the random matrix- related interferences in both systems are the same. Then the difference between New System and Old System measurements of the same sample depends on a random measurement error.

Establishing standard deviation (SD) for the Allowable Total Difference (ATD) zone should be based on consideration of possible variance between the two measurements of the same sample by the Old System obtained at different sites. For each concentration in the precision study, the largest SD among three sites in the precision study may be selected with addition of the between-site component of variance. For example, if for some concentration X1, three sites have SD of precision as 0.111 (site 1), 0.086 (site 2) and 0.118 (site 3) with between-site component of 0.020; then the standard deviation of two measurements by Old System performed in two different sites similar to site 3 (site with highest imprecision) is sqrt(0.118*0.118+0.020*0.020)= 0.120.

Another hypothetical precision experiment for the Old System can produce a slightly higher SD than in this example. In order to address this, the observed SD should be multiplied by the appropriate factor (factor $=(1-1/(4\cdot f)^{-1})\cdot\sqrt{f/\chi^2_{5\%}(f)}$, f is a degree of freedom of the estimated SD).

For example, if the degrees of freedom of the SD in the precision study of the Old System was 40, then the appropriate factor is 1.236 and the expected maximum observed SD can be as high as 0.148 (=0.120 *1.236). After the appropriate SD or percent CV is established for each concentration in the precision study, the ATD zone can be obtained by smooth interpolation**.**

7. Number of Samples in the Allowable Total Difference Zones

For 150 samples with 95% of the observations (143 /150) falling in the ATD zone, the lower limit of the 95% one-sided confidence interval is above 90%.

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