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TSH_Master Validation Plan for ELISA Assays on Theranos devices-08-23-13.docx
Theranos_In house TPSA scfv 5D5A5_Assay_Development_Report.doc
Theranos_TSH_Assay_Development_Report_4-4-11.doc

Hi Daniel & Adam,

Attached are the development reports for TSH and TPSA and the validation plan for TSH.

As discussed in a meeting with Elizabeth, please review the data in the development reports for usability towards validation and propose what will be needed for verification for the 3.5 system with the pre-dilutions.

Thanks,

Surekha

MASTER VALIDATION PLAN FOR ELISA ASSAYS ON THERANOS DEVICES

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MASTER VALIDATION PLAN FOR ELISA ASSAYS ON THERANOS DEVICES

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MASTER VALIDATION PLAN FOR ELISA ASSAYS ON THERANOS DEVICES

1 PURPOSE

1.1 To define a master Validation Plan for the validation/qualification of ELISA assays.

2 SCOPE

2.1 This document applies to the validation/qualification of ELISA assays on Theranos devices under CLIA regulations by CLIA laboratory personnel.

2.2 Per 42 CFR 493.1253 the qualification/validation of the ELISA assays specified in attachment CL ATT-14002-A1 on the Theranos devices will include the following performance specifications and other elements, as applicable:

- 2.2.1 Calibration
- 2.2.2 Quality Control
- 2.2.3 Precision
- 2.2.4 Analytical Sensitivity (Limit of Detection)
- 2.2.5 Analytical Specificity (including interfering substances, as applicable)
- 2.2.6 Accuracy
- 2.2.7 Reportable Range (measuring interval; analytical measurement range)
- 2.2.8 Reference Interval
- 2.2.9 Any other performance characteristics or elements required for test performance (e.g., carryover, stability of reagents, stability of samples, proficiency testing, and correlation with clinical findings)

3 DEFINITIONS AND ABBREVIATIONS

The following definitions and abbreviations are used in this document and related documents and attachments:

3.1 **Accuracy**: Accuracy is defined by CLSI as the closeness of agreement between a test result and an accepted reference value. Method accuracy is used in a different sense by the American Association of Pharmaceutical Scientists where it is expressed as percent relative error (%RE). **Trueness**, a related CLSI term, is the closeness of agreement between the average of a number of replicate measured quantity values and a reference quantity value.

3.2 **Analyte**: Component represented in the name of a measurable quantity. The closely related term **measurand** is defined as the particular quantity subject to measurement.

3.3 **Analytical sensitivity**: There are several alternative uses of this term. Most commonly, and for the purposes of this Validation Plan, it is used interchangeably with limit of detection. It is also used to describe the ability of an analytical method to assess small variations of the concentration of an analyte, such as the slope of the calibration curve (IUPAC).

3.4 **Analytical specificity**: Ability of a test or procedure to correctly identify or quantify an entity, including in the presence of interfering substance(s) or phenomena.

3.5 **Calibration**: Set of operations that establish, under specified conditions, the relationship between values of quantities indicated by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values realized by standards. Under CLIA, calibration refers to the process of testing and adjusting an instrument, kit, or test system, to provide a known relationship between the measurement response and the value of the substance being measured by the test procedure (42 CFR 493.1217).

3.6 **Calibrator**: A substance, material, or article intended to be used to establish the measurement relationships of a diagnostic medical device.

3.7 **CLIA**: Clinical Laboratory Improvement Amendments of 1988. Congressional legislation that defined and requires specific quality assurance practices in clinical laboratories.

3.8 **CLSI**: Clinical and Laboratory Standards Institute.

3.9 **Coefficient of Variation**: The ratio of the standard deviation to the average, often multiplied by 100 and expressed as a percentage, abbreviated as %CV.

3.10 **ELISA**: Enzyme-linked immunosorbent assay is an immunochemical method used to detect or quantify the amount of a given analyte/antigen in a sample.

3.11 **Interfering substance**: A substance or quantity thereof that is not the measurand but that affects the result of the measurement.

3.12 **IUPAC**: International Union of Pure and Applied Chemistry

3.13 **LDT**: Laboratory –developed Test.

3.14 **Linearity**: Linearity is the ability of a quantitative analytical method to provide results that are directly proportional to the concentrations of an analyte in test samples, within a given measuring interval. It is an important parameter to confirm when evaluating an analytical method because it verifies correct interpolation of results between points.

3.15 **LMR**: Lower end of the measuring range is the lowest level at which defined conditions, including all stated characteristic of the method, are met.

3.16 **LoB**: Limit of Blank is the highest value in a series of results on a sample that contains no analyte.

3.17 **LoD:** Limit of Detection is the lowest amount of analyte in a sample that can be detected with stated probability, although perhaps not quantified as an exact value.

3.18 **LoQ:** When used without a prefix, the Limit of Quantitation is the lowest actual concentration at which an analyte is reliably detected and at which uncertainty of the test result is less than or equal to the goal set by the manufacturer or laboratory. The term may also be used with prefixes L for lower (LLOQ) and U for upper (ULOQ), respectively. Note: LoB < LoD < LoQ.

3.19 **Matrix**: All components of a material system, except the analyte. A **specimen matrix** is the biological milieu in which an analyte exists (e.g., whole blood, plasma, serum, urine, or other body fluids). A **matrix effect** is the effect of all the other components of a sample, except for the measurand, on the value of the measurand.

3.20 **Measuring Interval (reportable range; analytical measurement range or AMR)**: A measuring interval consists of all numeric values between the lower and upper numeric values for which a method can produce quantitative results suitable for clinical use. Where applicable, a linearity study is frequently used to establish or verify the measuring interval that can be reported for a measurement method. Alternatively, the lower limit of the measuring interval may be assigned as the LoQ (LLOQ).

3.21 **Precision:** Precision is the closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions. It is usually expressed numerically in terms of standard deviation (SD) or percent Coefficient of Variation (%CV).

3.22 **Reference interval**: The interval between and including two reference limits. It is common practice to define a reference limit so a stated fraction of the reference values is less than or equal, or greater than or equal, to the respective upper or lower limit.

3.23 **SOP**: Standard Operating Procedure.

3.24 **Testing System**: The entirety of the testing process, including instrument, sample, reagents, supplies, and procedures. Personnel are sometimes included in the definition.

4 RESPONSIBILITIES

4.1 It is the responsibility of the Laboratory Director to ensure that the ELISA assays indicated in attachment CL-PLN-14002-A1 are qualified and validated on the Theranos systems in accordance with the qualification plan specified in this document.

4.2 It is the responsibility of all Testing Personnel in the CLIA laboratory to follow the steps indicated in this Master Validation Plan, specific validation plans developed for individual analytes indicated in attachment CL-PLN-14002-A1, and all associated SOPs.

5 PRINCIPLE

5.1 ELISA involves at least one binder (typically an antibody) with specificity for a particular analyte/antigen. The sample with an unknown amount of analyte/antigen is immobilized on a solid support phase (such as polystyrene) either non-specifically (via adsorption to the surface) or specifically (via capture by another binder (such as an antibody) specific to the same antigen, as in a "sandwich" ELISA). (Competitive assay formats may also be used.) After the analyte/antigen is immobilized, a detection antibody is added, forming a complex with the analyte/antigen. The detection antibody can be covalently linked to an enzyme, or can itself be detected by a secondary antibody that is linked to an enzyme through bioconjugation. Between each step, the reaction surface is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound. After the final wash step, the reaction surface is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of analyte/antigen in the sample.

6 TESTING SYSTEMS

The Theranos devices and systems consist of an analyzer and multiple single-use cartridges that contain all the chemicals required to perform the assays on that cartridge. The system can be operated with minimal training and performs multiple tests on a variety of sample types, such as whole blood, serum, plasma, urine, feces or respiratory samples. The system enables results in under an hour with precision and accuracy equivalent to traditional clinical laboratory analyzers. The device consists of a touch display for user interface, processor and communication systems, and sample processing modules, such as multichannel pipette, robotics, imaging apparatus, thermal control systems, centrifuge, spectrometer, cytometer and a photomultiplier tube.

7 SAFETY

7.1 Universal/ Standard precautions will apply, including the use of appropriate personal protective equipment.

7.2 All specific safety precautions spelled out in individual SOPs will be followed by personnel carrying out validations.

8 TRAINING AND PROFICIENCY

8.1 Individuals conducting the evaluations must be trained on the analyzers and measurement procedures and demonstrate acceptable proficiency.

9 PREVENTATIVE MAINTENANCE

9.1 The instruments used during the validations must be maintained according to manufacturer's instructions.

10 STANDARDIZATION

10.1 Methods will be traceable to a predicate or reference method which uses reference materials, such as from the National Institute of Standards and Technology (NIST) or a comparable entity as appropriate to the method, via patient correlation. Assigned values of the calibrators and controls will either be traceable to this standardization or to one provided by the manufacturer.

11 CALIBRATION

11.1 The Theranos systems are initially calibrated by the manufacturer at the factory.

11.2 In 42 CFR Part 493.1255, it is required to perform calibration procedures with at least the frequency recommended by the manufacturer, or using criteria specified by the laboratory, or when calibration verification fails to meet acceptable limits.

11.3 The term "calibration verification," as used in CLIA, includes:

11.3.1 Confirming that a calibration meets the method manufacturer's specifications

11.3.2 Verifying that the calibration is suitable for the entire measuring interval (or "reportable range," which is the CLIA term)

11.4 Calibration standards will be provided by the manufacturer (Theranos) within the anticipated assay range in an appropriate matrix. This may be pooled if using a biological matrix. Calibrators will be made in depleted serum with a purified native human TSH analyte. A total of 8 calibrator levels will be used to cover the reportable range from 50.0 uIU/mL to 0.1 uIU/mL. Expected LLOQ is 0.1 uIU/mL and expected ULOQ is 50.0 uIU/mL.

11.5 For the purposes of this Validation Plan, calibration will be carried out for each new lot of reagent cartridges.

11.5.1 At each level 3 cartridge replicates will be tested.

11.5.2 A calibration curve should consist of a blank or zero sample (matrix sample processed without internal standard or a buffer without internal standard) and six to eight non-zero samples covering the expected range, including calibration standards at the LLOQ and ULOQ of the range,

11.5.3 Acceptance criteria: For each run, a minimum of 75% of the back-calculated mean values of the total number of calibration standards in the calibration range should be within 100 ± 20% (100 ± 25% at LLOQ and ULOQ standards) of their nominal values, and a minimum of six unique standard concentrations must be within the assay range.

12 QUALITY CONTROL

12.1 Two to four level quality control samples (e.g., LLOQ, QC low, QC medium and QC high), as appropriate to the assay, will be analyzed in each run on each instrument for each experiment during the validation. QC levels: High = 20 uIU/mL Mid = 2 uIU/mL, Low = 0.2 uIU/mL. The QC levels are not included when generating the calibration curve.

13 PRECISION

13.1 Precision will be evaluated according to CLSI standard EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods.

13.2 Individual, pooled patient, or commercially available specimens are preferred by CLSI. At a minimum, evaluate high and low concentration levels that span a significant portion of the measuring interval of the procedure. Typically, the low QC sample should be within 3x the LLOQ, one QC is near the middle of the standard curve range, and the high QC is near the upper boundary of the standard curve (approximately 75-80% of the highest calibration standard). If possible, include another level near a medical decision value. If levels exhibit large differences in precision based on manufacturer's data or preliminary experiments (see below), add additional intermediate level(s). Alternatively, multi-level QC materials may be used if the aforementioned sample types are not available.

13.3 Preface the full precision experiment with a protocol familiarization period to train personnel and to detect any serious problems. It is recommended that a preliminary evaluation of repeatability is performed at the end of the protocol familiarization period. Analyze 20 aliquots of an appropriate test material in sequence, ideally at two or more concentration levels. These results will be used during the data analysis of the full precision experiment to detect outliers. Alternatively, manufacturer's previously established precision claims may be used for this purpose.

13.4 A minimum of 20 operating days is recommended for a complete experiment to establish precision (as opposed to 5 days needed to verify a manufacturer's previously established precision claim).

13.4.1 During each of the testing days, analyze two separate runs with two to three test samples/replicates at each of at least two levels of analyte concentration (spanning the measurement interval and at important decision or intermediate levels).

13.4.1.1 Change the order of analysis of the samples for each run or day.

13.4.1.2 Separate the runs performed each day by a minimum of two hours.

13.4.2 If "runs" do not constitute an aspect of the devices for the assays under consideration, then four samples at each level will be analyzed as two sets of pairs, under repeatability conditions, at different times during the day. The paired results will be treated the same as two results obtained in the same run.

13.4.3 In addition to the test samples, at least one quality control sample will be analyzed in each run.

13.4.4 The report will include scatter plots, precision summary tables and conclusions about the performance claim(s) regarding within-run (repeatability), between-day, between-lot and within laboratory precision.

13.4.5 Acceptance criteria: The %CV of replicates at each concentration of the samples should not be more than 20% (25% at LLOQ and ULOQ) for ELISA assays.

14 ANALYTICAL SENSITIVITY

14.1 The LoD is often called the analytical sensitivity (e.g.,CLIA term) of a measurement procedure. It is the lowest amount of analyte or measurand in a sample that can be detected with a stated probability, although it may not necessarily be quantified as an exact value. However, it is usually not used to define the low limit of the measuring interval because the precision and bias are often not suitable for clinical use. The results provide an understanding of the level of uncertainty of a method at low concentrations and ensure that low-level samples are interpreted appropriately in a clinical setting.

14.2 CLSI guideline EP17-A will be used to establish the limit of detection (LoD):

14.2.1 Specimens should include blanks and low positive samples similar to the specimens used in clinical testing.

14.2.2 Minimum of 60 measurements of a blank material

14.2.3 Minimum of 60 measurements on samples with a low concentration similar to the manufacturer's claim, if known.

14.2.4 Evaluate claims over several days.

14.2.5 The time period will include a change in reagent (cartridge) lot.

14.3 The analysis in the report will contain the following elements:

14.3.1 A frequency histogram

14.3.2 A statistical summary table showing the mean, median, SD, and CV of each concentration level

14.3.3 LoB and LoD summary tables containing the study results and conclusions.

14.4 Note: The above procedure also may be used to establish the LoQ (ULOQ) if samples similar in concentration to the manufacturer's LoQ claim are evaluated.

15 ANALYTICAL SPECIFICITY (INCLUDING INTERFERING SUBSTANCES)

15.1 Preliminarily, the effect of expected interfering substances may be examined using samples of individually hemolyzed, icteric, and lipemic samples obtained from commercial sources (e.g., Bioreclamation or ProMedDx). Neat samples will be run to obtain endogenous levels. Two to three analyte concentration levels across the assay range will be spiked into matrix and back-calculated against the standard curve. The back-calculated concentrations will be compared to the nominal values of the spiked samples.

15.2 Cross reactivity will be tested for structurally related analytes: FSH at 900mIU/mL, LH at 60 ng/mL and hCG at 10,000 ng/mL.

16 ACCURACY/ TRUENESS/ COMPARABILITY

16.1 Depending on the assay, a method accuracy (AAPS) experiment, as initially performed by R&D during method development, may be repeated in the CLIA laboratory.

16.1.1 Follow the procedure outlined in DeSilva et al

16.1.2 Study at least 2 replicates (5 if the data will subsequently be used for FDA submission) at 3 sample (QC) concentrations

16.1.3 Default acceptance criteria for immunoassays are absolute mean bias (%RE) ≤ 20% (≤ 25% at LLOQ) and total error (sum of the %CV and absolute %RE) ≤ 30% (≤40% at the LLOQ)

16.2 When a clinical laboratory-developed method is evaluated, trueness is best established by comparing results to a reference measurement procedure, if available. A list of approved reference measurement procedures is displayed at the following website for the Joint Committee for Traceability in Laboratory Medicine (JCTLM): [HYPERLINK "http://www.bipm.org/jctlm/"]. If a reference measurement procedure is not available, a best-available comparative method may be used with the following caveat. If the comparative method is not a reference method, one is technically establishing the comparability rather than the trueness of the test method.

16.3 To establish the trueness or comparability of two procedures, the following guidelines will be followed:

16.3.1 40 patient samples will be tested on the predicate method and the test method. (Quality control samples are not suitable because they may not behave the same as patient samples.)

16.3.2 Sample values must span but not exceed the measuring interval.

16.3.3 At least 50% of samples should be outside the reference interval.

16.3.4 Process each sample in replicates of at least two and preferably three.

16.3.5 Spread the collection of patient data over a number of days and runs. Store the samples as appropriate and in the same manner for both procedures. However, analysis of individual samples by the two methods should occur within a time span consistent with the analyte stability, generally not to exceed two hours.

16.4 The analysis in the report will contain the following elements:

16.4.1 Repeatability plots, difference plots, and/or scatter plots

16.4.2 The result of an adequate range test (linear fit with r value) to ensure that the sample results span the measuring interval

16.4.3 The results of the calculations for the predicted bias (or difference) estimates and confidence intervals

16.4.4 Statement of performance claims

17 REPORTABLE RANGE (MEASURING INTERVAL)

17.1 The measuring interval (CLSI term) is also called the "analytical measurement range" (AMR; common chemistry term) or "reportable range" (CLIA term). Laboratories developing test methods need to *establish* the measuring interval. Additionally, by *verifying* the measuring interval, clinical laboratories can ensure that the calibration of the measurement procedure is correctly applicable over the range in which they report patient results and that the measuring interval they are obtaining in their laboratory is comparable to the interval defined by the manufacturer in the product insert.

17.2 A measuring interval consists of all numeric values between the lower and upper numeric values for which a method can produce quantitative results suitable for clinical use.

17.3 Linearity is the ability of a quantitative analytical method to provide results that are directly proportional to the concentrations of an analyte in test samples, within a given measuring interval. It is an important parameter to confirm when evaluating an analytical or clinical laboratory method because it verifies correct interpolation of results between points. A linearity study is used to establish or verify the measuring interval that can be reported for a measurement method.

17.4 CLSI guideline EP06-A will be followed to establish the measuring interval by performing a linearity study:

17.4.1 Assay a minimum of two to four replicates at seven to eleven concentrations spanning the anticipated linear measuring interval.

17.4.1.1 Use specimens with known concentrations or known concentration relationships (relative values). In some instances it may be possible to create midlevel samples by mixing various proportions of high- and low-level samples.

17.4.1.2 Minimum concentration will be at or near the lower limit of the linear measuring interval.

17.4.1.3 Intermediate values will include concentrations throughout the measuring interval.

17.4.1.4 Maximum concentration will be at or near the upper limit of the linear measuring interval.

17.4.1.5 Obtain all results for a single analyte on the same day.

17.4.2 Due to the nature of many immunoassays, the range of quantification of the standard curve may be quite narrow. If so, it may be necessary to show that the analyte of interest, when present in concentrations above the ULOQ, can be diluted to bring it back into the validated range. Depending on the analyte, a dilutional experiment shall also be designed at "extended range", where an analyte level at 2x to 3x the ULOQ is tested to evaluate for the presence of a prozone (hook) effect. This will be performed to ensure that the back calculated value of a concentration above the ULOQ is correctly reported above the ULOQ.

17.4.3 The analysis in the report will contain the following elements:

17.4.3.1 A scatter plot

17.4.3.2 Repeatability summary table showing the repeatability statistics for each concentration evaluated

17.4.3.3 Linearity summary table including the values obtained with a linear fit and the best-fitting nonlinear fit.

17.4.3.4 Conclusion regarding the measuring interval of the study

18 REFERENCE INTERVALS

18.1 A reference interval is the interval between and including two reference limits, which are estimated to enclose a given proportion of the values for a population from which the reference subjects are drawn. The selection criteria for reference individuals are designed to exclude pathological conditions known to affect the concentration values of the analyte under investigation. Reference individuals should also be representative of the population served by the laboratory and of possible partitioning parameters that may influence a result. Examples of partitioning parameters include sex, age, race, or a clinical condition such as pregnancy. The test result is compared to the reference interval to make a meaningful medical diagnosis, therapeutic management decision, or other physiological assessment.

18.2 The reference interval is frequently calculated as the central 95% interval of results for the group of reference individuals. CLSI guideline C28-A3, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory will be followed:

18.2.1 When *establishing*, as opposed to verifying, a reference interval at a reference level of 95% and confidence level of 90%, evaluate a minimum of 120 samples for each partition. For instance, if a reference interval needs partitions for gender, then samples from 120 men and 120 women will be needed.

18.2.2 In some cases only one reference limit is of medical importance, usually an upper limit. If establishing a reference limit at a reference level of 97.5% and confidence level of 90%, evaluate 120 samples for each partition.

18.2.3 Carefully define subject preparation, sample collection and processing, the analytical method, and instrumentation.

18.2.4 Analyze the samples over several days.

18.2.5 Include quality control samples normally used in routine testing.

18.3 The analysis in the report will contain the following elements:

18.3.1 A frequency histogram

18.3.2 An outlier box plot.

18.3.3 A table summarizing the data from each partition, including the range of values, mean, median, and standard deviation.

18.3.4 A summary and conclusion table for each partition that includes the calculated reference interval based on the reference level defined during the study design and the confidence intervals of the lower and upper limits of the interval.

19 CARRYOVER

19.1 Carryover is defined as a discrete amount of reagent or analyte carried by a measuring system from one test into subsequent test(s), thereby erroneously affecting test results. Since the Theranos systems virtually eliminate sample carryover by using a new pipette tip for each sample, sample carryover is highly improbable. Nonetheless, in certain experiments, a blank will be run immediately after running a high to rule out carryover. Analysis shall be carried out as described in CLSI guideline EP10-A3.

19.2 Due to the design of the devices and the fact that the reagents are in single use cartridges, reagent carryover is not applicable.

20 STABILITY

20.1 Stability of Reagent Cartridges

20.1.1 Cartridge stability will be evaluated at 4ºC, nominal (range, 2-8ºC).

20.1.2 A four point standard curve will be used.

20.1.3 Time points to be tested include 0, 1, 2, 4, 8, 12 and 24 weeks.

20.1.4 Once the other experiments in the validation are finished, an initial validation report may be issued including stability to that point; extended stability will be reported in a revision or addendum.

20.2 Sample Stability

20.2.1 The Theranos systems are Point of Care devices which in practice will use freshly obtained specimens.

20.2.2 Nonetheless, stability studies at room temperature, 4ºC, -20ºC and -80ºC (nominal) will be carried out after the methods of DeSilva et al and Thiers et al. Assessments may be made by adding analyte to whole blood or plasma. The experiments should mimic the conditions under which study and subsequent clinical specimens will be stored and handled. A freshly prepared standard calibrator curve and QC samples should be used as the reference for comparison of the stability samples. Since specimens will be aliquoted, freeze and thaw stability after three cycles is not applicable.

20.2.3 Stability for venipuncture samples used in method comparisons will be as described by manufacturer's recommendations or as verified in the Theranos CLIA laboratory.

21 CALCULATIONS AND STATISTICAL ANALYSES

21.1 Necessary calculations will be performed by the Theranos Operating System.

21.2 Statistical analyses will performed using Excel, R, Dexter, and/or StatisPro, as appropriate. Traditional descriptive statistics will be performed in Excel or R. Calibration statistics will be performed using the version/module of Dexter (Theranos developed) appropriate to the assay format. StatisPro will be used to establish performance claims according to applicable CLIA and CLSI guidelines.

22 ALTERNATIVE ASSESSMENT PROCEDURE

22.1 Because of the unique features of the Theranos systems, traditional Proficiency Testing programs are not expected to be applicable.

22.2 Instead, an alternative assessment program (AAP) will be developed as described in CLSI guideline GP29-A2. Briefly,

22.2.1 Acceptable methods include comparison with a reference method, blind retesting of samples, split samples, or analysis of other lots of calibrator and control materials.

22.2.2 Define the acceptability limits for each assay in advance (e.g., use the mean ± 3 SD of the QC data).

22.2.3 Perform AAP for each assay twice yearly

23 OPERATIONAL TIMELINE FOR VALIDATION OF THE LDTs

23.1 Decide on final version of test procedure to be used for method validation studies

23.2 Develop a method validation protocol including the following:

23.2.1 Determine applicable test performance specifications to be established

23.2.2 Identify which reagents and specimens will require stability studies to establish storage and expiration specifications

23.2.3 Determine if validation needs to address the issue of interfering substances in the patient sample; if yes, develop study protocol to assess the impact of these interfering substances on test accuracy

23.2.4 Establish patient specimen acceptability criteria

23.2.5 Develop a study protocol to generate data for the purposes of establishing test performance specifications; clinical trials may be required if clinical significance of test results will be reported.

23.2.6 Develop appropriate maintenance and control methods to properly control all aspects of the test system

23.2.7 If applicable develop a protocol to establish reference ranges (i.e. normal values)

23.2.8 Develop an alternative testing protocol since traditional “proficiency testing” is not available to independently assess test accuracy after approval to test and report patient results (can be done post-validation)

23.2.9 Establish a review and approval process for method validation studies

23.2.10 If test system uses one or more Analyte Specific Reagents (ASR’s), the final report must include an FDA-mandated qualifier

23.2.11 Establish a format for final reporting of test results

23.3 Conduct studies to generate performance specification data

23.4 Analyze study data and determine performance specifications

23.5 Document review and approval of test validation study

23.6 Ensure that the test procedure and effective start date for the new procedure are approved by Laboratory Director

23.7 Document training of all appropriate personnel as required

24 RECORDS

24.1 All study source data, analyses, and approved documentation will be retained for the life of the tests plus two years.

25 ATTACHMENTS

CL PLN-14002-A1 List of Approved ELISA Assays for the Theranos devices

26 REFERENCES

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26.2 DeSilva B, Smith W, Weiner R, et al. Recommendations for the bioanalytical method validation of ligand-binding assays to support pharmacokinetic assessments of macromolecules. Pharmaceutical Res. 2003; 20:1885-1900.

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26.4 Burtis CA, Ashwood ER, Bruns DE (eds.). Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, Fourth Edition. Saunders, Philadelphia, 2006.

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26.6 I/LA23-A, Immunoassay Systems: Radioimmunoassays and Enzyme, Fluorescence, and Luminescence Immunoassays; Approved Guideline, 2004, Clinical and Laboratory Standards Institute, Wayne, PA.

26.7 R (version 2.13.1). The R Foundation for Statistical Computing, 07/08/2011.

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26.10 EP10-A3, Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures; Approved Guideline—Third Edition, 2006, Clinical and Laboratory Standards Institute, Wayne, PA.

26.11 EP15-A2, User Verification of Performance for Precision and Trueness; Approved Guideline—Second Edition. 2005, Clinical and Laboratory Standards Institute, Wayne, PA.

26.12 EP09-A2-IR, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Second Edition (Interim Revision), 2010, Clinical and Laboratory Standards Institute, Wayne, PA.

26.13 EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition, 2004, Clinical and Laboratory Standards Institute, Wayne, PA.

26.14 EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline, 2003, Clinical and Laboratory Standards Institute, Wayne, PA.

26.15 C28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition, 2008, Clinical and Laboratory Standards Institute, Wayne, PA.

26.16 EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline, 2005, Clinical and Laboratory Standards Institute, Wayne, PA.

26.17 EP7-A2, Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition, 2004, Clinical and Laboratory Standards Institute, Wayne, PA.

26.18 GP29-A2, Assessment of Laboratory Tests when Proficiency Testing is not Available; Approved Guideline—Second Edition. 2008, Clinical and Laboratory Standards Institute, Wayne, PA.

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27 REVISION HISTORY

REVISION HISTORY			
Revision Level	**Effective Date**	**Initiator**	**ECO Number**
A	11/04/2011	A. Gelb	CL ECO-00026
Section Number	**Description and Justification of Changes**		
ALL	Initial Release		

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