Message

From: Sunny Balwani [/O=THERANOS ORGANIZATION/OU=FIRST ADMINISTRATIVE GROUP/CN=RECIPIENTS/CN=SBALWANI]
Sent: 8/25/2013 12:39:38 AM
To: Elizabeth Holmes [eholmes@theranos.com]
Subject: FW: Immunoassay plan

From: Adam Rosendorff
Sent: Saturday, August 24, 2013 3:44 PM
To: Daniel Young; Surekha Gangakhedkar; Nahal Gharaati
Cc: Sunny Balwani
Subject: Immunoassay plan

Dear all

Please find attached the verification plan for Theranos-modified immunoassays on the ADVIA devices, together with appendix A1 (Acceptance Criteria) and appendix A2 (Patient Questionnaire).

I look forward to your comments.

Nahal, following out conversation last night, can you provide an operational timeline for the verification that I can include as appendix A3?

Thanks,

Adam Rosendorff, MD, FASCP
Theranos, Inc
(650) 856-4412 (Office)
(650) 823-4953 (Mobile)
(650) 852-9594 (Fax)
arosendorff@theranos.com

theranos redefining healthcare	**Standard Operating Procedure**	Document Number: Revision: A
	CLIA Laboratory	Effective Date: 08/23/13

MASTER VERIFICATION PLAN FOR THERANOS-MODIFIED IMMUNOASSAYS ON THE ADVIA 1800

Author(s):

Signature:	Date:
Name: Adam Rosendorff, MD	Title: Laboratory Director

Reviewer(s)

Signature:	Date:
Name: Surekha Gangakhedkar, M.S.	Title: Immunoassay Team Leader

Signature:	Date:
Name: Daniel Young, Ph.D.	Title: Vice President

Approver(s):

Signature:	Date:
Name: Adam Rosendorff, M.D.	Title: Laboratory Director

MASTER VERIFICATION PLAN FOR THERANOS-MODIFIED IMMUNOASSAYS ON THE ADVIA 1800

CONTENTS

MASTER VERIFICATION PLAN FOR THERANOS-MODIFIED IMMUNOASSAYS ON THE ADVIA 1800

1 PURPOSE

1.1 To define a master Validation Plan for the validation/qualification of Theranos modified immunoassays for the ADVIA 1800. The immunoassays will maintain existing stochiometries of R1, R2 (where applicable), sample dilution, however the final read volume will be reduced from 120uL to 80uL. Thirteen (13) assays will initially be validated (Appendix I).

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-1600, associated Appendices, and all associated SOPs.

5 PRINCIPLE

5.1 Immunoturbidometric assays rely on a chance in absobance properties of a sample when antibodies against a specific target analyte are added to a sample. Formation of antibody-antigen complex results in an increased turbidity. The turbidity at the reaction endpoint is measured as the amount of light absorbed at discrete wavelengths. In some cases the antibody-antigen reaction is enhanced in the presence of

polyethylene-glycol, or the agglutination reaction occurs with latex beads attached to specific antiobdies that are cross-linked in the presence of antigen (Microparticle enhanced immunoturbidometric reaction).

5.2 Generally the simple immunoturbidometric assays are read at wavelengths around 400nM while the particle enhanced immunoturbidometric assays area read at wavelengths around 600nM

6 TESTING SYSTEMS

ADVIA 1800 instruments will be used throughout with modifications to accommondate BCD-scale samples involving pre-analytic manual dilution or TECAN automated dilution.

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.

11.1.1

11 CALIBRATION

Calibration will be performed according to the manufacturer's instructions.

12 QUALITY CONTROL

12.1 Quality control procedures will be performed using levels provided by Siemens according to manufacturer's package inserts.

13 PRECISION

MASTER VERIFICATION PLAN FOR THERANOS-MODIFIED IMMUNOASSAYS ON THE ADVIA 1800

13.1 Precision of each assay at at least QC (decision) levels will be determined using decision levels and acceptance criteria specified in Appendix [I].

13.2 Briefly, following calibration and QC, BIORAD QC materials (3 levels) will be run as patient samples to verify the precision claims in the Siemens package insert for each assay.

13.3 Where decision levels do not equal BIORAD assigned values, the closest BIORAD level should be used for comparison.

13.4 Run each QC level 8 times in duplicate per day for a daily total of 16 and a grand total of 80 data points. 4 runs can be performed in an a.m. shift and 4 runs can be performed in a pm shift. Shown below is a table for one shift.

13.5 CLSI document EP5A should be used as a guideline for precision assays.

A.M. Shift Worksheet

Date:

Analyte:

Operator:

Instrument:

Reagent Lot/Source:

Calibrator Lot/Source:

Day	Date	Result 1	Result 2	Mean	Result 1	Result 2	Mean	Shift mean
1								
2								
3								
4								
5								

13.5 Calculate the within-device precision according to CLSI EP05A2E, Section 10.82., "Estimates of within-

Day#	Run#1	Run#2	Mean
	$(Rep1-Rep2)^2$	$(Rep1-Rep2)^2$	$(Mean\ Run\#1-Mean\ Run\#2)^2$
1			
2			
3			
4			
5			
SUMS			

device or within laboratory precision", pgs 11-13.

14 ANALYTICAL SENSITIVITY

14.1 Manufacturer's claims will be accepted for analytic sensitivity.

15 ANALYTICAL SPECIFICITY (INCLUDING INTERFERING SUBSTANCES)

15.1 Manufacturer's claims will be accepted for analytic specificity.

16 BIAS ESTIMATION:

16.1 **Venous blood, Lithium-Heparin Plasma versus EDTA-Plasma.**

16.1.1 Bias estimation will be conducted for all assays shown with a tick mark in Appendix [I].

16.1.2 The abovementioned assays have been validated in Lithium-Heparin Plasma but not in EDTA, therefore verification of test performance in EDTA-plasma from venous blood is required.

16.1.3 20-40 unique clinical specimens, from healthy patients, are required in paired Lithium-Heparin and EDTA vacutainers.

16.1.4 Clinical specimens should be assayed in duplicate assayed by each method.

16.1.5 Bias should be calculated using average bias, using the Bland-Altman method (CLSI EP09A2).

16.1.6 Plot the mean value of all replicates of all methods on the X-axis, and the difference between mean values for each method on the Y-axis.

16.1.7 Mean bias (%) should not exceed the limits for bias in [Appendix I]

16.2.1

16.2 **EDTA-plasma from venous blood versus EDTA-plasma from capillary blood**

16.2.2

16.2.3 Assay 20-40 matched samples in duplicate for each immunoassay

Separate cells from plasma promptly (desireable =30mins-1hr, maximum 2hrs).

Assess bias as described in Section 16.1

17 REPORTABLE RANGE (MEASURING INTERVAL)

17.1 Manufacturer (Siemens) claims for reportable range will be adopted as specificed in the package insert.

17.2 In some instances, reportable ranges may be quite narrow, and it would then be necessary to dilute patient specimens if the result exceed the ULOQ.

18 VERIFICATION OR RECALCULATION OF 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 A patient questionnaire will be given to each study participant (Appendix [II])

18.3 Reference ranges studies will be calculated from Siemens reference ranges for lithium-heparin plasma, using the technique of transference, according to CLSI C28A3cE pgs 28-30 “Transference: Comparability of the Analytic System.”

18.4 Apply correction factors to Siemens reference intervals by transforming low and high cutoffs using the regression equation.

18.5 For a description of the procedure refer to CLSI C28A3cE pgs 28-30 “Transference: Comparability of the Analytic System.”

18.6 Bias estimated in 16.1 and 16.2 may be used to recalculate reference ranges, where applicable.

18.7 In instances where significant bias is discovered in procedures 16.1 and/or 16.2, one or two transference procedures may be applied.

18.8 In instances where bias is discovered in both procedures 16.1 and 16.2, apply a sequential transference procedure.

18.9 Briefly, if the regression equation is Y=1.4X-2, where Y is the test method and X is the predicate method, and the reference limits for analyte Z is 55-155 mg/dL, then the new reference range is calculated as follows.

18.10 Low limit: Y=1.4(55)-2=75, High limit: Y=1.4(155)-2=215.

18.11 The new reference range would therefore be 75mg/dL-155 mg/dL

19 TOTAL ALLOWABLE ERROR PERCENTAGE (%TEa)

19.1 Total allowable error criteria for each assay are specificed in Appendix [I].

19.2 For each assay add percent imprecision to percent bias to calculate total error.

20 CARRYOVER

20.1 Carryover studies are not applicable

21 STABILITY

21.1 Manufacturer (Siemens) claims for reagent stability will be accepted.

22 CALCULATIONS AND STATISTICAL ANALYSES

22.1 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.

23 ALTERNATIVE ASSESSMENT PROCEDURE

23.1 Not applicable

24 TIMELINE FOR VERIFICATION OF THE LDTs

24.1 The timeline is written such that it may be accomplished in 5 days.

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

25 ATTACHMENTS

25.1 CL-PLN-1600a1, Appendix [I], “Immunoassay acceptance criteria for bias, precision, and total error.”

24.2 CL-PLN-1600a2, Appendix [II], “Donor Questionnaire”

26 REFERENCES

26.1 Code of Federal Regulations, Title 42, Chapter IV, Subchapter G, Part 493, Subpart K, Sections 493.1217, 493.1253, and 493.1255.

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.

26.3 Guidance for Industry: bioanalytical method validation. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, 2001.

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

26.5 I/LA21-A2, Clinical Evaluation of Immunoassays; Approved Guideline - Second Edition, 2008, Clinical and Laboratory Standards Institute, Wayne, PA.

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.

26.8 StatisPro (version 1.13.00). Clinical and Laboratory and Standards Institute, Wayne, PA. 07/14/2011.

26.9 Dexter-Immunoassay (version1.0), Theranos, Inc., 2009.

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.

26.19 Thiers RE, Wu GT, Reed AH, et al. Sample stability: a suggested definition and method of determination. Clin Chem 1976; 22:176-83.

27 REVISION HISTORY

REVISION HISTORY			
Revision Level	**Effective Date**	**Initiator**	**ECO Number**
Section Number	**Description and Justification of Changes**		

[ ]

File Produced in Native Format

theranos redefining healthcare	**Standard Operating Procedure**	Document Number: **CL PLN-1650** Revision: A
	CLIA Laboratory	Effective Date: 8/24/2013

Clinical study participant questionnaire for blood draws

Study Title:

Study Description:

Protocol Director:

Time of draw:

Tubes and volume drawn:

Patient Identifiers

(1) Subject ID number:

(2) Sample ID number:

(3) Gender:

(4) Age:

(5) Time and date of last meal:

(6) List Medications/ Vitamins:

(7) Smoker Y/N

(8) Alcohol use Y/N If yes how often per week?

(9) Blood transfusion within the last 2 months?

Please answer yes or no to the following:

Diabetes Y/N	Liver Disease Y/N	Cancer? Y/N
High Blood Pressure Y/N	Heart Disease Y/N	
Sleep Apnea Y/N	Respiratory Disease? Y/N	
Bleeding Disorder Y/N	Kidney Disease? Y/N	
High Cholesterol? Y/N	Thyroid Disease? Y/N	
Anemia Y/N	Oral Contraceptives Y/N	
Ulcer Y/N	Pregnant? Y/N	