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Total 25-OH Vitamin D on Edison 3.5 Theranos System

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REFERENCE USE

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1 PURPOSE

1.1 This document describes the standard operating procedure for running Vitamin D samples using the Theranos System: Edison 3.5 on manufactured cartridges in the reader with pre-dilutions using the Tecan Freedom Evo.

2 SCOPE

2.1 This document applies to CLIA laboratory personnel running calibrators, controls, or other samples using the ELISA Theranos System: Edison 3.5 reader and cartridges, with pre-dilutions performed by the Tecan Freedom Evo.

3 CLINICAL RELAVANCE

Vitamin D is important in overall health and wellness. It is produced naturally when the body is exposed to sunlight, and is also available in supplements and many fortified foods. Vitamin D is converted into a hormone in the body and helps regulate the amounts of calcium in the blood. A deficiency in vitamin D can lead to fatigue, and generalized pain. It is also linked to cancer, asthma, type-II diabetes, high blood pressure, Alzheimer's and autoimmune diseases. Vitamin D toxicity also occurs when too much of the supplement is taken, and can cause high blood calcium.
In healthy adults the normal range is between 9.3ng/mL and 47.9ng/mL. Toxicity occurs above 150ng/mL and levels lower than 9.3ng/mL are considered deficient.

The Theranos Total 25 OH Vitamin D (VitD) assay determines the total concentration of 25-Hydroxyvitamin D3 and 25-Hydroxyvitamin D2 in human whole blood (automatically processed into plasma by the Theranos system), plasma or serum. The assay has a reportable range of 5 ng/mL to 150 ng/mL.

4 RESPONSIBILITIES

4.1 It is the responsibility of all operators to adhere to the testing procedure in accordance specified in this document when running any calibrators, controls, or samples on the Theranos system.

4.2 It is the responsibility of all operators to read and understand all related documents that are referred to in this procedure.

4.3 It is the responsibility of all operators to adhere to the safety procedures specified in this document and the guidelines outlined for BSL2 laboratory work.

5 RELATED DOCUMENTS

5.1 VitD manufacturing documents located in the VitD assay folder [we need a location and DCN for this]

5.2 BSL2 laboratory safety docs

6 SAFETY

6.1 Universal/Standard precautions will apply, including the use of appropriate personal protective equipment (PPE) in accordance to BSL2 regulations when handling any blood products and potentially infected samples.

6.1.1 This includes any unknown samples to be tested.

6.1.2 Minimum PPE consists of a lab coat, gloves, and safety glasses with side-shields.

6.2 All biohazardous material must be disposed of safely and completely into the appropriate bins following completion of each test.

6.2.1 This includes any materials that may have been contaminated with biohazardous materials. (Example: pipette tips, used cartridges, lab bench absorbents, etc. This is not a complete list of all possible biohazardous materials)

7 MATERIALS AND EQUIPMENT

7.1 Materials

7.1.1 Pipettes (sizes as needed)

7.1.2 Disposable Pipette Tips (sizes as needed)

7.1.3 Lab Bench Absorbents

7.1.4 Biohazard Bin

7.1.5 Sharps Bin

7.1.6 Calibrators, controls and/or samples to be tested

7.1.7 Personal Protective Equipment

7.1.8 Metal Nanotainer Rack for Tecan Freedom Evo machine.

7.1.8.1 Stickers indicate the proper orientation of the rack in the machine.

7.1.8.2 Slots are numbered as positions 1-24 starting at the front right corner, working by column to the back left corner.

7.1.9 PN: 51-00120 Vessel, NT

7.1.10 PN: 51-00119 Holder, NT

7.1.10.1 Flat side is considered the front face of the holder.

7.1.10.2 When oriented with front facing, the slots are named B and A from left to right.

7.2 Equipment

7.2.1 Tecan Freedom Evo Machine with labware and carriers set up to accommodate Edison Cartridge protocols

7.2.2 Theranos System

7.2.2.1 Manufactured Edison 3.5 cartridges

7.2.2.2 Edison 3.5 Readers

8 THERANOS SYSTEM

The Theranos devices and systems consist of reader and multiple single-use cartridges that contain all the required reagents for performing the assay(s) 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.

8.1 Edison Readers

8.1.1 Edison Readers were calibrated, validated and quality tested internally in accordance to the MOP-00302, Reader Release: Final QC_Assay QC, Edison 3.

8.1.2 Quality tested daily in accordance to "(Edison 3.5 Daily QC doc)"

8.2 Edison 3.5 Cartridge

8.2.1 Edison Cartridges are validated and quality tested in accordance to SOP-00089, Cartridge 060 Acceptance Criteria.

8.2.2 Reagent filled cartridges are stable up to 2 weeks when stored properly

8.2.2.1 Individually packaged in foil pouch to protect from light

8.2.2.2 Store upright at 2-8°C.

8.2.3 Cartridge Onboard Reagents

8.2.3.1 Wash Buffer: TBST pH 8.0, Sodium Azide

8.2.3.2 Theranos AP Substrate Solution

8.2.3.3 Capture Tips

8.2.3.4 Extraction Buffer: 100mM Citrate Buffer pH 2.6

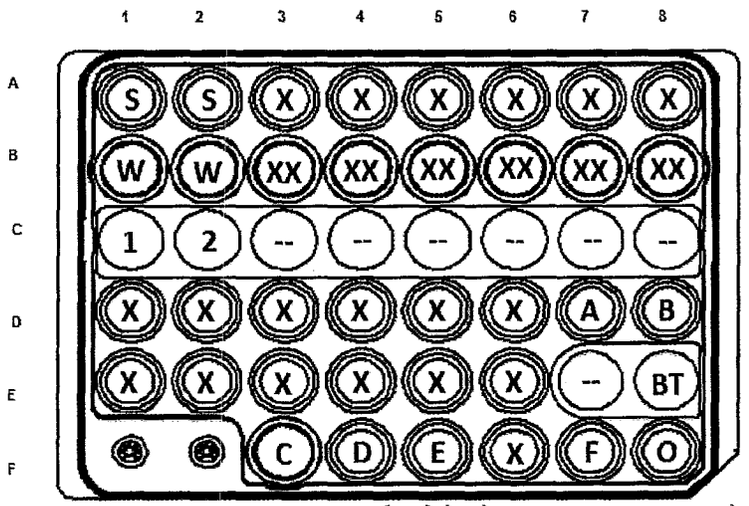
8.2.3.5 Neutralizing Buffer: 50mM TRIS buffer pH 8.0

8.2.3.6 Conjugate solution in Stabilzyme AP

8.2.3.7 Extraction Reagent: Lyophilized Pepsin

Figure 1: Cartridge Layout

Position	Reagent	Conc	Volume (uL)
A1 - A2	In house Alkaline Phosphatase Substrate	--	80
B1 - B2	1x Wash Buffer	--	400
D7	Reagent A	VitD Neutralizing Buffer	150
D8	Reagent B	VitD Extraction Buffer	100
F3	Reagent C	50 mM TBS pH 8.0	400
F4	Reagent D	Capture Ab Solution	60
F5	Reagent E	AP Conjugate Solution	60
F7	Reagent F	Lyopholized Extraction Reagent	
E8	Blood Tip	--	--



Legend	
Symbol	Description
BT	Blood Tip
X	Sealed Empty PCR Tube
XX	Sealed Empty Matrical Tube
O	Unsealed Empty PCR tube
--	Empty
#1-8	Coated Test tips
A	Reagent A
B	Reagent B
C	Reagent C
D	Reagent D
E	Reagent E
F	Reagent F
W	Wash Buffer
S	Substrate

9 TEST PRINCIPLE

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.

A biotin-labeled anti-sheep antibody coated on UltraAvidin serves as the capture surface for the competitive ELISA. The sample is diluted and mixed with pepsin in a low pH buffer to remove Vitamin D Binding Protein (VDBP) and other interfering proteins. The mixture is then further diluted into a buffer with pH 8.0 to inactivate the pepsin, and the sheep anti-25-Hydroxyvitamin D3/2 antibody mixture is added and incubated with the sample for 10 minutes. After this incubation, an alkaline phosphatase-labeled 25-Hydroxyvitamin D3 conjugate (25OHD3-AP) is added to the mixture. The reaction mixture is incubated on the capture surface, then the surface is washed and the alkaline phosphatase substrate is incubated on the surface, and then the resulting chemiluminescence is read in Relative Light Units (RLU).

A greater amount of total 25-Hydroxyvitamin D in the sample results in lower binding of the 25OHD3-AP to the capture antibody. Thus the signal generated by the assay is inversely proportional to the concentration of 25-Hydroxyvitamin D in the sample.

10 SPECIMEN COLLECTION

10.1 EDTA Plasma, Li Heparin Plasma and Serum samples are acceptable for testing.

10.1.1 Samples are stable up to 12 hours when stored at 4C or room temperature.

10.1.2 Minimal volume is 20uL.

10.2 Hemolyzed samples with up to 500 mg/dL of hemoglobin does not affect assay precision

10.3 Lipemic samples with up to 1000mg/dL triglycerides do not affect assay precision.

10.4 Icteric sample with 40 mg/dL bilirubin affect assay precision and Icteric samples should not be used for testing.

11 CALIBRATION

11.1 Factory calibration is performed with each new lot of reagents in accordance with QCP-00003 Calibration of Manufactured Cartridges, and is valid until expiration date (either on packaging or documented)

11.1.1 Dexter analysis

11.1.1.1 The Dexter calculations will be provided as a calibration per lot in accordance with and SOP-00081 Theranos Software for Standard Calibration Curve for Immunoassays

11.1.2 Corrections are performed on the Dexter result. The Excel file will automatically calculate this result in the "Bias Correction." Reader bias corrections are performed internally by the Theranos System.

11.2 Calibrator Formulation

11.2.1 For general guidelines refer to SOP-00101.

11.2.2 8 levels of VitD calibrators were used to calibrate this assay

11.2.3 The Theranos VitD assay has a reportable range of 10 – 150 ng/mL

12 QUALITY CONTROL TESTING

12.1 Quality control testing should be performed daily and before testing any patient samples according to CL SOP-19005.

12.1.1 Test with BioRad Immunoassay QC material levels 1 and 2 by following procedures outlined in section 13.

12.1.2 A new mean and SD for each BioRad Immunoassay QC material should be determined monthly, using the triplicate measurement for each level for each EDISON instrument used.

12.1.3 Ensure that the mean measurement for each level is within 1SD of the mean QC level (using the Immulite SD) published for the Siemens Immulite 2000 method in the BioRad package insert.

12.1.4 Calculate the acceptable range for each QC level as the mean plus or minus 2SD (all values calculated from values obtained on the EDISON readers)

12.1.5 Before each 8hr laboratory shift, run both QC levels in duplicate on all EDISON instruments

For any single EDISON instrument, reject QC if >1 level is >2SD or 1level >3SD

12.1.6 The QC Standards should fall within the ranges below:

Table 1: Expected Range of QC Standards

Daily QC	Mean	Low	High	%CV
Level 1	17.46	8.96	25.96	24.35
Level 2	58.81	40.55	77.06	15.52

12.1.7 The excel file will automatically determine if the tested QC standard falls within the expected range and either pass or fail the run. If both QC standards pass, continue with patient testing.

12.2 In the case of Failed QC standards, follow the procedure outlined below.

12.2.1 Retest the QC standards. If the second test passes, continue with patient sample testing.

12.2.2 Retest the QC standards with a different cartridge/reagent lot.

12.2.3 Retest using a different set of readers.

13 PROCEDURES

13.1 Set up laboratory bench top to maximize access to biohazard bins, biohazard sharps containers, pipette tips, and their corresponding disposable tips. Also, cover bench top with lab bench absorbents to collect any spills.

13.2 **Cartridge Preparation**

13.2.1 Pre-made cartridges are stored upright in 4°C to 8°C when not in use. These packaged cartridges will be pre-loaded with all necessary reagents onboard.

13.2.1.1 Remove packaged cartridges from cold storage and allow them to adjust to ambient temperatures for at least 10 minutes but not more than 24 hours.

13.2.1.2 Once cartridges have reached ambient temperatures, remove from foil sealed pouch.

13.2.2 In some cases cartridges without onboard reagents may be used. These "dry generic" cartridges must be loaded by hand with all reagents in the appropriate wells as outlined in the manufacturing "cartridge layout" in figure 1.

13.2.2.1 Cartridges are stored at room temperature.

13.2.2.2 Barcodes must be added manually before testing.

13.2.2.3 Add reagents immediately before using the Tecan machine to add the pre-diluted sample to the cartridge.

13.3 Organizational Preparation

13.3.1 In the Alchemist Data Retrieval System set up the experimental information to allow for easier data acquisition after tests.

13.3.1.1 Log on to Alchemist with the appropriate credentials.

13.3.1.2 Select the appropriate "Assay ID" from the list or "Create New Assay…" where applicable and select it once loaded.

13.3.1.3 "Create New Experiment…" and select it once loaded or select the appropriate experiment from the available list.

13.3.1.4 Select the proper "Condition Data" and "Sub-Condition Data" for the assay the same as the previous two commands.

13.3.1.5 Scan the barcodes of each cartridge to be tested in the experiment. A range can be selected by scanning the first and last barcodes of the set.

13.3.1.6 Select "add" and confirm that barcodes load properly.

13.3.2 An Edison protocol must be selected for each barcode or range of barcodes. *(Pre-loaded cartridges should be linked to specific protocols, and these steps may not be necessary.)*

13.3.2.1 Open the Theranos Virtual Analyzer (TVA) app and log on with the appropriate credentials

13.3.2.2 Select the "protocols" button

13.3.2.2.1 Locate the "VitD_3ul_100x_level 3" protocol and select "view barcodes".

13.3.2.3 Scan or enter the barcodes that will use this protocol.

13.3.2.3.1 Barcodes can be scanned individually or as a range.

13.3.2.3.2 Select "save" to confirm.

13.4 Sample Preparation

13.4.1 Spin the whole blood patient sample at 2000 rpm for 5 minutes.

13.4.2 Link the SCU to the cartridges by scanning the SCU barcode at the bottom of the Vessel and Holder (51-00120, 51-00119).

13.4.3 The Theranos TSH test uses an automated system to pre-dilute samples.

13.4.3.1 Refer to ELISA Tecan start-up and maintenance protocols outlined in CL SOP-19003

13.4.3.1.1 For the first run of the day perform all Tecan Start-up Protocols

13.4.3.1.2 Follow the Tecan checks and system flush protocols where necessary.

13.4.3.2 Find the EVOware program is located on the computer desktop that corresponds with the Tecan machine.

13.4.3.3 Upon program startup, enter credentials and select

13.4.3.3.1 Username: elisa

13.4.3.3.2 Password: ELISA

13.4.3.4 From the Start-up prompt, select "Run an existing script" and select START YOUR SELECTION

13.4.3.4.1 Find and highlight the appropriate protocol from the available list and select .

13.4.3.4.1.1 For left arm (LiHa1) only "Ed_VitD_3_3_LiHa1"

13.4.3.4.1.2 For right arm (LiHa2) only "Ed_VitD_3_3_LiHa2"

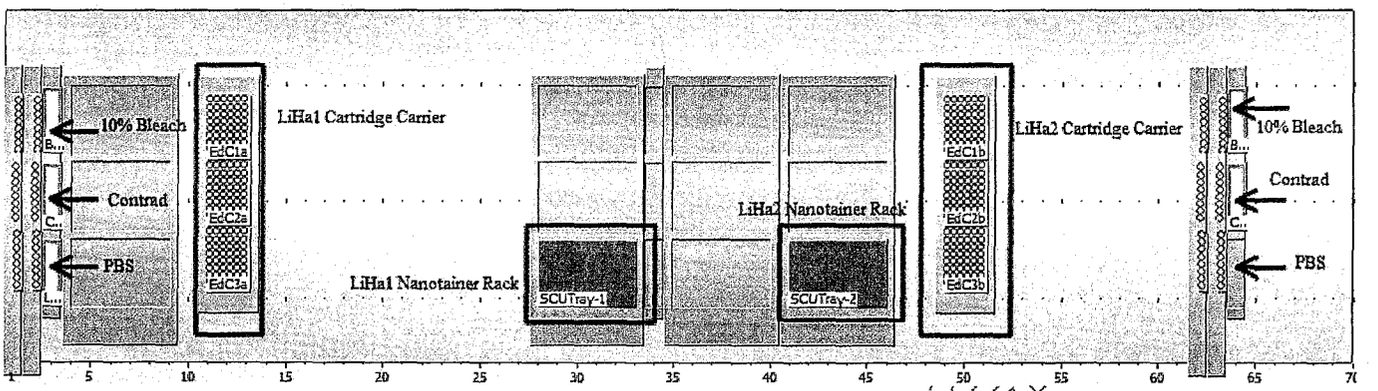
13.4.3.4.1.3 For both arms simultaneously "Ed_VitD_3_3_BothArms"

13.4.3.5 If the EVOware program is open to the protocol editing window, select Open from the taskbar and select the appropriate protocol.

13.4.3.6 Select Run from the taskbar.

13.4.4 Refer to the Tecan Worktable Layout for positioning of labware.

Figure 2: Worktable Layout



13.4.5 Place the (7.1.8) metal nanotainer rack in the appropriate position on (7.2.1) Tecan Machine.

13.4.6 Place (51-00120) Vessel, NT into the (51-00119) Holder, NT in position A.

13.4.7 Manually extract plasma using a pipette, and add it to a new Vessel and Holder (51-00120, 51-00119) taking care not to introduce any bubbles.

13.4.8 Place the Vessel and Holder (51-00120, 51-00119) onto the (7.1.8) metal nanotainer rack at position 1.

13.4.8.1 Holder with Vessel (51-00120, 51-00119) should only fit into the (7.1.8) metal nanotainer rack with the front face forward.

13.4.9 Place three cartridges loaded with reagents (either mfg or hand loaded reagents) into their respective positions on the cartridge carrier.

13.4.9.1 Barcodes facing forward

13.4.9.2 Cartridges should fit snuggly

13.4.10 In EvoWare program select ▷ to start the protocol.

13.4.10.1 A green light above the (7.2.1) Tecan machine will indicate if the protocol is running.

13.4.10.2 Allow pre-dilution protocol to finish (approximately 6 minutes)

13.4.11 Discard Holder with Vessel (51-00120, 51-00119) in the appropriate biohazardous waste container.

13.4.12 Remove cartridges from the (7.2.1) Tecan machine and transfer them to the (7.2.2.2) Edison 3.5 readers immediately following the conclusion of the Tecan protocol.

13.4.13 Select "cancel" to finish the Tecan protocol

13.5 **Reader Use**

13.5.1 Make sure that the (7.2.2.2) Edison 3.5 Reader is powered on and check the temperature icon, network connection, working icon then follow the prompts on the touch screen.

13.5.1.1 Temperature indicator

13.5.1.1.1 A Green bar in the center of the range indicates that the reader is at the appropriate temperature.

13.5.1.1.2 A red bar at the top of the range indicates that the reader temperature is too high. Do not use this reader.

13.5.1.1.3 A blue bar at the bottom of the range indicates that the reader temperature is too low. Do not use this reader.

13.5.1.2 Network Connection

13.5.1.2.1 A green cloud icon indicates that the reader is connected to the server.

13.5.1.2.2 A white cloud icon indicates that the reader is not connected to the server, and data will not be accessible until this connection is fixed.

13.5.1.3 During the run the tear drop Theranos logo will be spinning to indicate that it is working. If there is any need to abort the run before completion of the test press "press to abort"

13.5.2 Press "press to open" and insert the cartridge

13.5.3 Press "close door"

13.5.4 Select "VitD_3ul_100x_level 3" from the available protocol list, which was pre-loaded using the Theranos.TVA app in section 8.3.2, then select "run selected test".

13.5.4.1 If more samples are to be tested, repeat from steps 13.4

13.5.5 Allow protocol to run through to completion.

13.6 Press "view results" then "remove cartridge"

13.6.1.1 Remove the used cartridge and dispose of it into the appropriate biohazardous waste container immediately.

13.6.1.1.1 Avoid tossing or dropping cartridges as this may produce aerosols.

13.6.1.2 A new cartridge can be inserted from this point. If this is the case, insert the new cartridge and press "press to close" then repeat this protocol from (8.5.4)

13.6.1.3 If there is no new cartridge to be loaded, press "press to close" to finish the test.

14 DATA ACQUISITION

14.1 Once the test(s) have completed and cartridges have been discarded in the appropriate manner, raw data is accessed using the Theranos Alchemist system.

14.2 A user ID and passcode are required to log onto the system and retrieve data.

14.2.1 Select the appropriate experiment details to access the raw data and export into excel

14.2.1.1 If necessary find the appropriate "chemist" from the drop down list

14.2.2 Select the appropriate "Assay ID", "Experiment Name", "Condition Name", and "Sub-Condition Name" to view the "Cartridge Data"

14.2.2.1 Select barcodes to be exported to excel using the check boxes. In most cases "select all" is appropriate.

14.2.2.2 Select "Export Selected" and follow prompts for data summarization, file name, and location.

14.2.3 Save the data as an excel file and open it from the "downloads" list in the internet browser.

15 DATA ANALYSIS

15.1 Calculations and Statistical Analysis

15.1.1 Necessary device corrections will be performed by the Theranos Operating System.

15.1.2 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.

15.2 All data calculations will be performed in Excel. *(There is a template that will make calculations easier for now.)*

15.2.1 Calculate the intra- and inter-cartridge means and CVs.

15.2.2 Using the Dexter analysis provided for the cartridge reagent lot, calculate the TSH level based on the inter-cartridge RLU mean. *(This should self-populate if the template is used.)*

15.3 Reporting Results

15.3.1 Acceptance Criteria: If any replicates do not meet these criteria, the patient sample should be retested.

15.3.1.1 Intra-cartridge CVs should be lower than 20% (25% at LLOQ)

15.3.1.2 RLU values less than 100 are considered dark counts should be excluded/repeated.

15.3.1.3 System errors

15.4 Reportable Range: 5-150 ng/mL

15.5 Reference Range: 30-100 ng/mL

15.6 Critical Values:

15.6.1 Deficiency: Lower than 10 ng/mL

15.6.2 Insufficiency: 10-30 ng/mL

15.6.3 Toxicity: Higher than 100 ng/mL

15.7 Interferences and Cross-reactants

15.7.1 Bilirubin and Triglycerides affect assay precision, thus icteric and lipemic samples should not be tested.

15.7.1.1 40 mg/dL bilirubin does not give acceptable recoveries or CVs

16 TECHNICAL SUPPORT

16.1 Tecan Machine: William Westrick, Michelle Johnson

16.2 Edison Reader: Jared O'Leary, Samartha Anekal

16.3 Supplies: Darren Crandall, Aurelie Souppe

16.4 Software: Tina Lin

17 REFERENCES

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

17.2 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.

17.3 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.

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

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

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

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

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

17.9 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.

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

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

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

18 REVISION HISTORY

REVISION HISTORY

Revision Level	Effective Date	Initiator	ECO Number
A	10/23/2013	M. Johnson	CL ECO-00116

Section Number	Description and Justification of Changes
ALL	Initial Release