theranos	LDT Validation Report	Theranos Ferritin Immunoassay	Rev:
		CL RPT-14038	A
Description	Validation Report for Ferritin (FRT) Immunoassay on the ADVIA 1800		
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1 ASSAY BACKGROUND

Ferritin is a 450 KDa protein complex consisting of 24 subunits, that form a shell, and is the primary intracellular iron storage molecule in eukaryotes. When fully bound by iron, it is estimated that over 20% of Ferritin protein consists of iron by weight. Denatured ferritin is called hemosiderin and ferritin without bound iron is known as apoferritin. Free iron is toxic to cells as it leads to the production of free radicals via the Fenton reaction. Ferritin binds iron and keeps it from producing free radicals. Measurements of ferritin may aid in the diagnosis of diseases affecting iron metabolism, such as hemochromotosis (iron overload) and iron deficiency anemia. Recent literature suggests that ferritin may decline before increases in TIBC and transferrin saturation, providing an earlier indication of iron-deficiency. The combination of MCV and Ferritin may provide an extremely accurate method for discriminating beta-thallassemia (low MCV, normal ferritin) from iron deficiency anemia (low MCV, low ferritin).

2 REGULATION AND GUIDANCE

The qualification/validation of the ELISA assays on the Theranos device will be in accordance with C.F.R. Ch IV, § 493.1253 "Standard: Establishment and verification of performance specifications" and outlined in CLSI guideline C28A3.

3 PRINCIPLE OF THE TEST

In the ADVIA Chemistry Ferritin (FRT) assay, sample is diluted and reacted with a buffer that contains latex particles coated with antibody specific for ferritin. The formation of the antibody-antigen complex during the reaction results in an increase in turbidity, the extent of which is measured as the amount of light absorbed at 658 nm. By constructing a standard curve from the absorbance of standards, ferritin concentration of a sample can be determined

Plasma samples were diluted 3 fold in saline prior to analysis.

4 CALIBRATION

4.1 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.

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

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

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

4.2 Calibrators were diluted 3 fold and verified on the ADVIA system

4.2.1 This dilution factor is within the acceptable limits of the ADVIA internal calibration test.

4.3 For the purposes of this Validation Plan, calibration was carried out with every new lot of reagents.

4.3.1 Each level was tested in replicates of 3 and the average was used to create a standard curve for testing.

4.3.2 The calibration was verified using quality controls.

5 QUALITY CONTROL

5.1 Two to four level quality control samples, as appropriate to the assay, were analyzed with each calibration and before each test during the validation.

5.1.1 High = 29.1 ng/mL

5.1.2 Mid = 164 ng/mL

5.1.3 Low = 273 ng/mL

5.2 The QC levels are not included when generating the calibration curve.

6 PRECISION

6.1 Precision was evaluated according to CLSI standard EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods.

6.1.1 A total of 20 runs were performed over 10 days with 2 runs per day and 2 replicates per run for a total of 40 data points. The following tables indicate the between-run, between-day and within-laboratory precision at 3 levels (64.6 mg/dL, 127.3 mg/dL and 182 mg/dL):

Table I: Precision at 3 decision levels

Precision

CLSI guideline EP05-A2 section 10.8

Level = L1

Number of observations	40
Number of runs	20
Number of days	10
Runs per day	2
Replicates per run	2

CLSI guideline EP05-A2 section 10.4 recommends a minimum of 40 runs, with 2 replicates per run.

Mean	29.09

	SD	95% CI	CV	Allowable Total SD
Repeatability	0.63	0.48 to 0.90	2.2%	-
Between-run	0.33		1.1%	-
Between-day	0.46		1.6%	-
Within-laboratory	0.84	0.67 to 1.15	2.9%	7.27

Imprecision is less than allowable total imprecision: 25%.

Level = L2

Number of observations	40
Number of runs	20
Number of days	10
Runs per day	2
Replicates per run	2

CLSI guideline EP05-A2 section 10.4 recommends a minimum of 40 runs, with 2 replicates per run.

Mean	163.51

	SD	95% CI	CV	Allowable Total SD
Repeatability	0.72	0.55 to 1.05	0.4%	-
Between-run	0.35		0.2%	-
Between-day	1.21		0.7%	-
Within-laboratory	1.46	1.07 to 2.29	0.9%	40.88

Imprecision is less than allowable total imprecision: 25%.

Level = L3

Number of observations	40
Number of runs	20
Number of days	10
Runs per day	2
Replicates per run	2

CLSI guideline EP05-A2 section 10.4 recommends a minimum of 40 runs, with 2 replicates per run.

Mean	273.28

	SD	95% CI	CV	Allowable Total SD
Repeatability	1.06	0.81 to 1.53	0.4%	-
Between-run	3.84		1.4%	-
Between-day	4.95		1.8%	-
Within-laboratory	6.35	4.63 to 10.12	2.3%	68.32

Imprecision is less than allowable total imprecision: 25%.

6.2 Acceptance criteria:

Total allowable error (TAE %) of 25%, was selected as the acceptance criteria for this assay following CLIA proficiency guidelines as printed in the Federal Register February 28, 1992;57(40):7002-186, as well as published acceptance criteria published in the American Proficiency Institute Peer Data for 2013 CHEMISTRY / IMMUNOLOGY / IMMUNOHEM -1ST EVENT. Allowable bias was calculated as the residual error budget after precision values (CV %) were subtracted from TAE (%). Values in brackets indicate the closest/corresponding API levels obtained from 2013 proficiency data.

Bias was calculated by comparing the average Level 1, Level 2 and Level 3 QC values obtained with the Theranos values, versus the average values assigned to each Level by the predicate method (n=6 measurements for each level).
Table II

	Level 1	Level 2	Level 3
CV%	2.9	0.9	2.3
Bias%	0.1	0.1	0.6
TAE%	25	25	25
Allowable Bias (%)	22.1	24.1	22.7

For each level allowable bias exceeds observed bias therefore the TAE criteria are satisfied.

7 BLOOD COLLECTION DEVICE (BCD) COMPARISON

7.1 Because the ADVIA system allows users to extend the reportable range, a pre-dilution of sample is valid, and the volume of sample obtained from a fingerstick in sufficient for testing on the system. To verify the comparability of fingerstick blood to venous blood, 20 unique patients donated 2 venous tubes of blood and 2-4 fingerstick samples in EDTA. Each sample of venous blood was tested and the 2 results were used as replicate tests. Fingerstick samples were pooled and tested in replicates of 2. All samples should be within the reference range, and were also subject to the reference range criteria.

7.2 Calculated concentrations are based on the mean of 2 replicate tests.

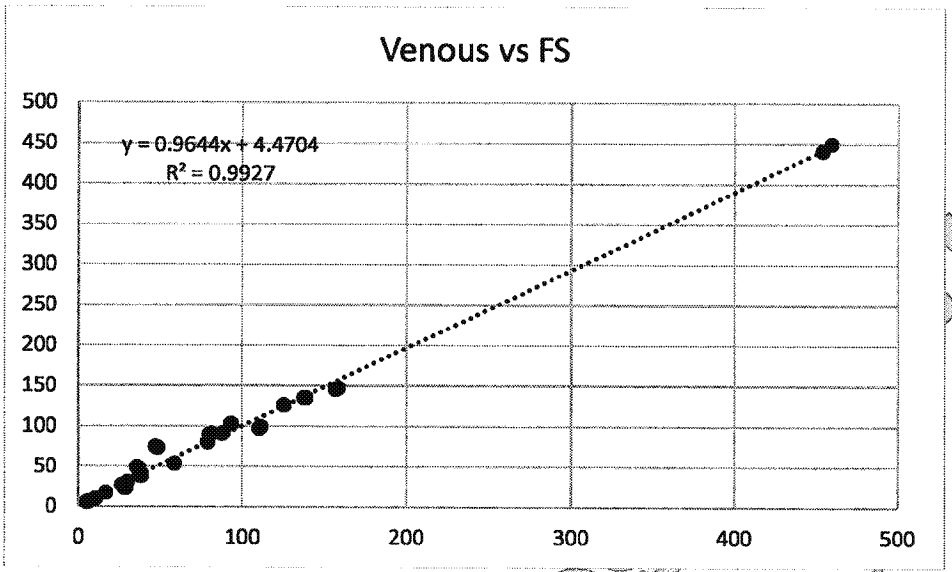
7.3 Acceptance Criteria:

7.3.1 All samples must have %CV within 20%

7.3.2 Scatter plots should have a slope of 1 +/- 0.15 and R^2 greater than 0.9

7.3.3 Regression equation for BCD versus matched venous samples is shown below and passes acceptance criteria.

Figure 2 BCD versus matched venous sample comparison



REFERENCE RANGE VERIFICATION

7.4 20 unique venous and matched BCD samples were collected and assayed in duplicate using the predicate and Theranos methods, and the average value was calculated. All samples were collected in EDTA, therefore to verify the published Siemens reference range that was established using Lithium-Heparin plasma, both venous and BCD values were transformed to normalized values according to the regression equation established in Section 7. Values obtained from venous blood that fell out of the Siemens published reference range, as well as the corresponding values from matched BCD samples, were excluded from analysis. Of the 20 values obtained, 18 were included for analysis, and 17 (94-95%) fell within the Siemens published reference range (5-148 ng/mL (F) and 28-365 ng/mL (M).

7.5 The RR is therefore verified (CLSI guidance C28-A3c).

8 REFERENCES

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

8.2 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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8.4 R (version 2.13.1). The R Foundation for Statistical Computing, 07/08/2011.

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

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

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

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

8.9 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.

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

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

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

9 Revision History

REVISION HISTORY			
Revision Level	**Effective Date**	**Initiator**	**CL ECO Number**
A	10/23/2013	Adam Rosendorff	CL ECO-00115
Section Number	**Description and Justification of Changes**		
All	Initial Release		