theranos	LDT Validation Report	Theranos Creatinine Assay	Rev:
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Description	Validation Report for Modified Siemens Assay for Creatinine in Lithium Heparin Plasma		
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Validation of Modified Siemens Creatinine Assay

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Creatinine Plasma Assay

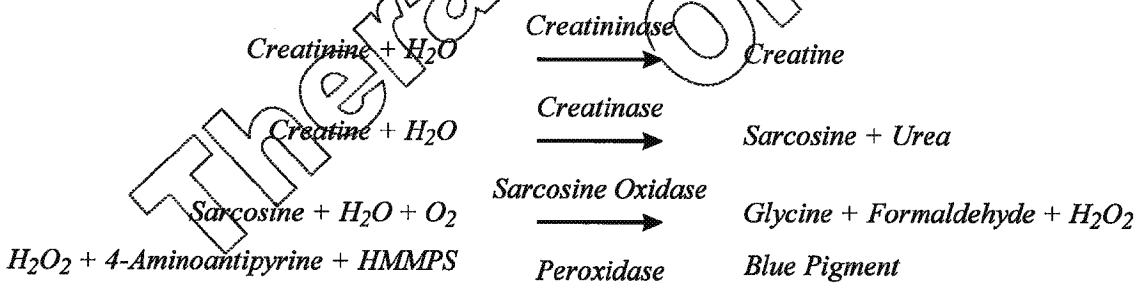
I. Overview

Creatinine is a breakdown product of creatine. Measurement of creatinine levels is used as one indicator of kidney function. Serum Creatinine is a waste product of the dehydration of Creatine. Most of the body Creatine is present in muscle tissue. It represents an important alternative energy source for the body. The primary source of energy for the body is the conversion of ATP to ADP by breaking a high-energy phosphate bond and releasing energy that can be used. Creatine is important as it is an efficient energy source used to covert ADP back to ATP.

II. Method Principle

Creatinine is converted to creatine by the action of creatininase. The creatine formed is hydrolyzed by creatinase to produce sarcosine, which is decomposed by sarcosine oxidase to form glycine, formaldehyde, and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed yields a blue pigment by quantitative oxidative condensation with N-(3-sulfopropyl)-3-methoxy-5-methylaniline (HMMPS) and 4-aminoantipyrine. The creatinine concentration is obtained by measuring the absorbance of the blue color at 596/694 nm, which is proportional to the creatinine concentration.

Reaction Equation



III. Definitions and Abbreviations

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

a. **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.

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

c. **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).

d. **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.

e. **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).

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

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

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

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

j. **Colorimetry:** A technique used to determine the concentration of colored compound(s) in solution.

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

l. **IUPAC:** International Union of Pure and Applied Chemistry

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

n. **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.

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

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

q. **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.

r. **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.

s. **Matrix:** All components of a material system, except the analyte. A specimen matrix is the biological milieu in which an analyte exists (e.g., plasma, serum, urine, or other body fluids).

t. **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).

u. **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).

v. **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.

w. **SOP:** Standard Operating Procedure.

x. **Spectrophotometry:** The quantitative measurement of the transmission (or reflection) properties of a material as a function of wavelength.

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

IV. Pre-clinical Validation

a. Analytical Measurement Range

i. Limits of Blank, Detection and Quantitation

The limits of blank, detection and quantitation were determined to be 0.000 mg/dL, 0.028 mg/dL and 0.028 mg/dL respectively.

Limit of blank

CLSI guideline EP17-A section 4.3.1

Level	Number of samples	N	Mean	SD
Blank	1	20	0.000	0.000

Alpha	5%
Parametric LoB	0.000

Limit of detection

CLSI guideline EP17-A section 4.3.2

Level	Number of samples	N	Pooled SD
Low	1	20	0.017

Beta	5%
Parametric LoD	0.028

Limit of quantitation

CLSI guideline EP17-A section 5.1

Level	Number of samples	N
Low	1	20

Bias	0.040
Pooled Imprecision	0.017
95% total error	0.073
Allowable error	0.10
LoQ	0.028

95% total error is less than allowable error: 30%.

LoQ has been established.

ii. Linearity

The Analytical Measurement Range (AMR) including linear measurement interval has been determined by Siemens. Refer to the **Analytical Range** section of the manufacturer product information insert for additional details.

b. Analytical Specificity

The analytical specificity for this assay was determined by observing the effects of bilirubin (10 mg/dL), hemoglobin (100 mg/dL), and triglycerides (400 mg/dL) on the recovery of creatinine (1.2 mg/dL) in spiked plasma samples. No significant interference (NSI) was determined if the mean analyte concentration of an interferent-spiked sample reported within 10% of the mean analyte concentration of an un-spiked sample. Recoveries of creatinine in the presence of bilirubin, hemoglobin, and triglycerides were 97%, 95.9%, and 99.6%, respectively (see table below).

Analyte:	% Recovery in the presence of each interferent:		
Creatinine (mg/dL)	Bilirubin (10 mg/dL)	Hemoglobin (100 mg/dL)	Triglycerides (400 mg/dL)
1.2	97*	95.9*	99.6*

* NSI observed at interferent level tested.

c. Precision

Level = L1

Number of observations	78
Number of runs	39
Number of runs excluded	1
Number of days	20
% of days with 1 run	5%
Runs per day	2
Replicates per run	2

CLSI guideline EP05-A2 section 10.4 recommends a minimum of 40 runs

Mean	0.9

	SD	95% CI	CV
Repeatability	0.0	0.0 to 0.0	3.2%
Between-run	0.0		0.0%
Between-day	0.1		15.5%
Within-laboratory	0.1	0.1 to 0.2	15.9%

Level = L2

Number of observations	80
Number of runs	40
Number of days	20
Runs per day	2
Replicates per run	2
Mean	2.2

	SD	95% CI	CV
Repeatability	0.0	0.0 to 0.0	1.4%
Between-run	0.0		0.0%
Between-day	0.1		4.7%
Within-laboratory	0.1	0.1 to 0.2	4.9%

Level = L3

Number of observations	78
Number of runs	39
Number of runs excluded	1
Number of days	20
% of days with 1 run	5%
Runs per day	2
Replicates per run	2

CLSI guideline EP05-A2 section 10.4 recommends a minimum of 40 runs.

Mean	6.6

	SD	95% CI	CV
Repeatability	0.1	0.1 to 0.1	1.6%
Between-run	0.0		0.0%
Between-day	0.1		1.0%
Within-laboratory	0.1	0.1 to 0.2	1.9%

The percent CV reported as zeros in the above precision summary are most likely a consequence of rounding values in StatisPro.

V. Clinical Validation

a. Method Comparison with Predicate (Accuracy/Comparability)

To test the accuracy of the assay on the Theranos System, 53 unique patient samples were screened on the predicate method (Siemens, Advia) and on the Theranos method. Using the predicate method thirty eight (38) values were within the reference range (Males: 0.5 – 1.2 mg/dL; Female: 0.4 – 1.1 mg/dL), one (1) was below the reference range, and three (3) were above the reference range. Based on the results of the data examination, either a simple linear regression or alternative procedures were used to estimate expected (average) bias and the confidence interval of expected bias at the desired medical decision level(s) as per CLSI guidance EP09-A2. StatisPro was used for bias calculations. These estimates were compared with internal criteria to judge the acceptability of the Theranos method. Each sample was run in duplicate on the predicate, and the average used for comparison to the Theranos method. Some samples were stored before analysis on both methods. If the confidence interval for the predicted bias includes the defined acceptable bias or if the acceptable bias is greater than the higher

limit of the confidence interval of the predicted bias, then the data do not show that the bias of the Theranos method is different from the acceptable bias or there is a high probability (97%) that the predicated bias is acceptable, respectively. The acceptable bias at each medical decision level was determined based on the total allowable error (TEa) minus the measured precision at the level closest to that decision level. Total allowable error (TEa) was taken from American Proficiency Institute (API) peer proficiency testing criteria or CLIA proficiency testing criteria for acceptable analytical performance, as printed in the Federal Register February 28, 1992;57(40):7002-186, when available. The TEa for Creatinine is 15%. The table below shows the allowable bias and precision at 2 levels (values shown in parentheses) and the corresponding closest medical decision limits.

Table 1. Allowable Bias and Precision at the Medical Decision Levels

Medical Decision Levels (mg/dL)	**0.6 (0.9)**	**1.6 (2.2)**	**6.0 (6.6)**
Precision (%)	**15.5**	**4.7**	**1.0**
Allowable Bias (%)	**-0.5**	**10.3**	**14.0**

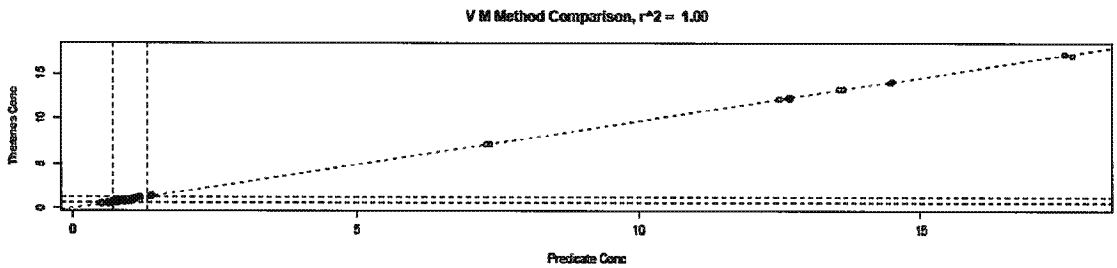


Figure 1. Graph showing Theranos method versus Predicate Method (Siemens Advia) Simple linear regression was used to establish a slope, intercept and an r2. The slope, intercept and clinical correlation were determined to be 0.97, 0.02 and 1.00 respectively.

Comparability

CLSI guideline EP09-A2-IR section 7

Level ID	Value	Difference	SE	95% CI	Allowable difference
	0.60	0.005	0.0091	-0.013 to 0.023	0.061
	1.60	-0.025	0.0085	-0.042 to -0.008	0.164
	6.00	-0.157	0.0098	-0.177 to -0.137	0.614

Difference is less than allowable bias: 10.24%.

The difference between the two methods is not greater than the allowable difference. The performance requirement is verified.

b. Transference and Verification of Reference Interval (Venous)

Reference ranges were modified by applying the regression equation to the lower and upper reference limits of existing reference interval to generate a new reference range. New reference ranges were verified using a minimum of twenty (20) new normal subjects

New reference ranges were verified using a total of ninety three (male 36 and female 57) new normal subjects with matched Lithium heparin venous and finger sticks samples. For a reference range to pass verification, 95% of values should fall within the upper and lower reference limits and 5% or fewer values fall outside of the upper and lower reference limits. For venous verification with the male population group 36 (100%) values fell within the new reference range and 0 (0%) values fell outside the new reference range. For the female population group 57 (100%) values fell within the new reference range and 0 (0%) values fell outside the new reference range See graphs below for male and female venous samples verification.

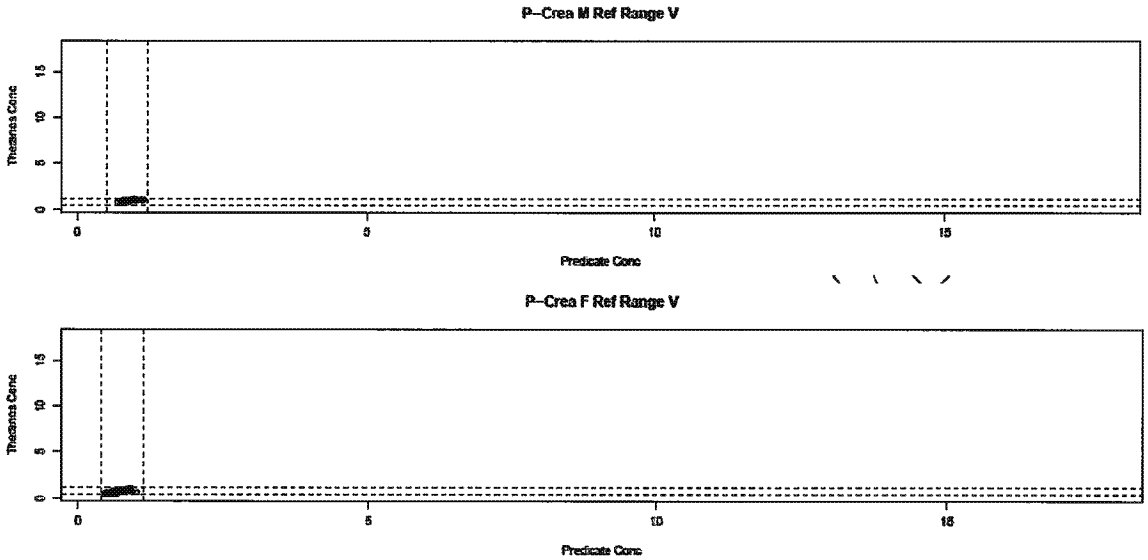
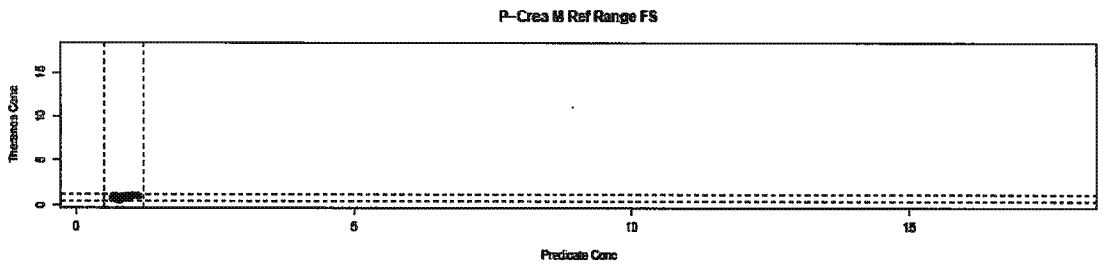


Figure 2. Graph showing male & female venous sample reference range verification.

c. Verification of Reference Interval with Finger Stick Samples

New reference ranges were also verified with venous matched finger sticks (Lithium heparin) from a total of eighty five (39 males & 56 females) new normal subjects. The finger stick samples were collected in a Theranos blood collection device (BCD) configured with separate Lithium heparin and EDTA vessels. For finger stick verification 38 values (97.4%) for the male group and 56 values (100%) for female group fell within the new reference range and 1 (2.6%) values for the male group fell outside the new reference range. See graphs below for finger stick samples verification.



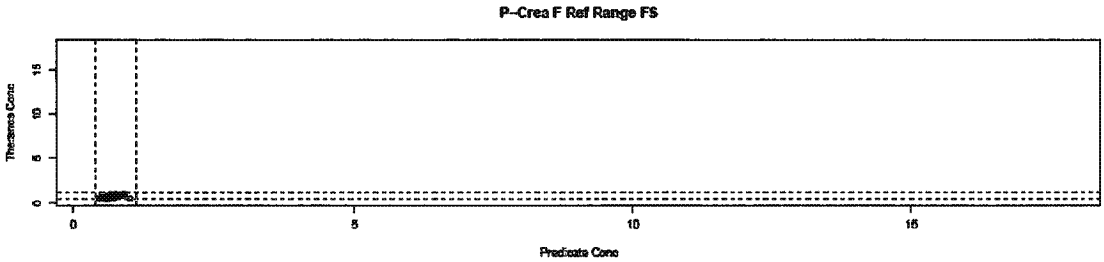


Figure 3. Graph showing male & female Finger stick sample reference range verification.

Theranos.Analyte	Anti-coagulant	Existing Reference Range (mg/dL)		New Reference Range (mg/dL)	
		Reference Range (low)	Reference Range (High)	Transfered RR (low)	Transfered RR (high)
Creatinine (Male)	Heparin	0.5	1.2	0.5	1.2
Creatinine (Female)	Heparin	0.4	1.1	0.4	1.1

The new reference range for finger stick Creatinine were determined to be 0.5 – 1.2 mg/dL (male) & 0.4 – 1.1 mg/dL (female).

VI. Stability

d. Reagents

On-board Reagent Stability

System	Reagent On Board Stability
ADVIA 1200 Systems	60 days
ADVIA 1650/1800 Systems	60 days
ADVIA 2400 Systems	60 days

Unopened creatinine reagents are stable at 2-8 °C until the expiration date printed on the product label. Do not freeze reagents.

a. Sample

Plasma samples for Creatinine analysis are stable for 2 weeks at 2-8 °C, or at least 90 days at -20 °C.

b. Calibrators

Siemens Chemistry Calibrators should be stored at 2-8 °C, protected from light, and are stable until the expiration date on the vial label. Opened calibrators are stable for 48 hours, except for total and direct, which are stable for 8 hours.

REVISION HISTORY

Revision Level	Effective Date	Initiator	ECO Number
A	11/06/2013	A. Rosendorff	CL ECO-00117

Section Number	Description and Justification of Changes
All	Initial Release