theranos	LDT Validation Report	Theranos Potassium Assay	Rev:
		CL RPT-14039	1
Description	Validation Report for Modified Siemens Assay of Potassium in Lithium Heparin Plasma		
Originator: Curtis Schneider		Date: 09/26/2013	

Validation of Modified Siemens Potassium Assay

Author(s):

Signature:	Date: 3/24/14
Name: Paul Patel, Ph.D.	Title: Team Lead, General Chemistry

Reviewer(s):

Signature:	Date:
Name:	Title:

Signature:	Date: 3/26/2014
Name: Daniel Young, Ph.D.	Title: Vice President

Approver(s):

Signature:	Date: 3/24/14
Name: Adam Rosendorff, M.D.	Title: Laboratory Director

9/19/15

Sunil S. Dhawan M.D.

Potassium Plasma Assay

I. Overview

Potassium is the major cation of the intracellular fluid. Disturbance of potassium homeostasis (intra- and extracellular) can cause serious health effects.

Decreases in extracellular potassium are characterized by muscle weakness, irritability, and eventual paralysis. Cardiac effects include tachycardia, other cardiac conduction abnormalities that are apparent by electrocardiographic examination, and eventual cardiac arrest. Hypokalemia is common in vomiting, diarrhea, alcoholism, and folic acid deficiency. Additionally, >90% of hypertensive patients with aldosteronism have hypokalemia.

Abnormally high extracellular potassium levels produce symptoms of mental confusion; weakness, numbness and tingling of the extremities; weakness of the respiratory muscles; flaccid paralysis of the extremities; slowed heart rate; and eventually peripheral vascular collapse and cardiac arrest. Hyperkalemia may be seen in end-stage renal failure, hemolysis, trauma, Addison's disease, metabolic acidosis, acute starvation, dehydration, and with rapid potassium infusion.

II. Method Principle

The sample is mixed with ISE buffer, thereby providing a constant pH and a constantionic strength solution. As the buffered sample is moved through the ion-selective electrode, changes in the electrical potential take place. These electrical potential changes are measured against the potential of a reference electrode to derive the correct analog value for that sample. This assay has been modified from its original test method by pre-diluting the sample (3.5 fold) prior to performing the test. The pre-dilution occurs on the Tecan liquid handling robot. While the Tecan has very high precision, its accuracy can drift over time. Therefore, controls are run with each set of samples to correct for any volumetric drift. Namely, two low QC levels and two high QC levels also pre-diluted in parallel with the samples to be analyzed. A linear fit is applied to the QC control data and is used to correct the results from the sample analysis.

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: $LoB < LoD \leq LoQ$.

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*

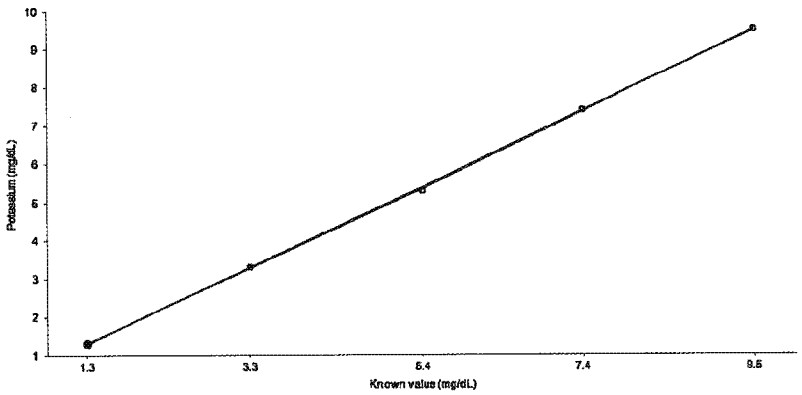
a. Analytical Measurement Range

i. Limits of Blank, Detection and Quantitation

The analytical range 1 – 10 mmol/L L for Potassium in plasma has been determined by Siemens. The precision and bias verified at 2.7 mmol/L was 2.9 % and 3.8% (n=80) respectively.

ii. Linearity

The Analytical Measurement Range (AMR) including linear measurement interval has been determined for Potassium in plasma. This method is linear from 1.3 – 9.5 mmol/L within the 10% allowable non-linearity in this interval.



Level	Mean	Linear fit	Nonlinear fit (2nd order polynomial)	Nonlinearity	Allowable nonlinearity
1	1.30	1.28	1.31	0.03	0.13
2	3.30	3.28	3.27	-0.01	0.33
3	5.30	5.38	5.35	-0.03	0.54
4	7.40	7.38	7.36	-0.01	0.74
5	9.50	9.48	9.51	0.03	0.95

Nonlinearity is less than allowable nonlinearity: 10%.
Performance requirement verified over the measuring interval.

b. Analytical Specificity

The analytical specificity for this assay was determined by testing the effect of hemoglobin (100 mg/dL), bilirubin (15 mg/dL) and triglycerides (438 mg/dL) on plasma samples spiked with the interferents and then compared with un-spiked controls. Potassium concentration at which the interference testing was performed was at 3.7 mM. Non-interference was defined as the mean result from testing of spiked samples within 10% of the mean of the un-spiked samples. Recoveries were within 94% to 96% (see table below).

Table 1. Interference Testing For Potassium

Analyte (mM)	% Recovery		
	Interferent		
	Bilirubin (15 mg/dL)	Hemoglobin (100 mg/dL)	Triglycerides (438 mg/dL)
Potassium (3.75)	94	96	95

No significant interference was observed.

c. Precision

The CV was determined at three levels using low, mid and high (level 3) QC controls (Liquid Assayed Multiquals) with 1 run per day over multiple days. The precision levels tested were at 2.6 mM (level 1), 4.1 mM (level 2) and 7.5 mM (level 3). The precision data for all levels are summarized below.

Level = 1

Number of observations	48
Number of runs	24
Number of runs excluded	1
Number of days	24
Runs per day	1
Replicates per run	2

CLSI guideline EP05-A2 section 10.4 recommends a minimum of 30 runs, with 2 replicates per run; or 20 runs, with 3 or more replicates per run.

Mean 2.6243460

	SD	95% CI	CV
Repeatability	0.0387618	0.0302663 to 0.0539236	1.5%
Between-day	0.0526675		2.0%
Within-laboratory	0.0653937	0.0526578 to 0.0863101	2.5%

Level = 2

Number of observations	48
Number of runs	24
Number of runs excluded	1
Number of days	24
Runs per day	1
Replicates per run	2

CLSI guideline EP05-A2 section 10.4 recommends a minimum of 30 runs, with 2 replicates per run; or 20 runs, with 3 or more replicates per run.

Mean 4.0772815

	SD	95% CI	CV
Repeatability	0.0735415	0.0574233 to 0.1023074	1.8%
Between-day	0.0291154		0.7%
Within-laboratory	0.0790953	0.0657108 to 0.0993777	1.9%

Level = 3

Number of observations	50
Number of runs	25
Number of days	25
Runs per day	1
Replicates per run	2

CLSI guideline EP05-A2 section 10.4 recommends a minimum of 30 runs, with 2 replicates per run; or 20 runs, with 3 or more replicates per run.

Mean 7.5086285

	SD	95% CI	CV
Repeatability	0.1100231	0.0862864 to 0.1518769	1.5%
Between-day	0.0299881		0.4%
Within-laboratory	0.1140367	0.0951964 to 0.1422442	1.5%

Where CV's are reported as zeros in the precision summary output this is 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, 50 unique patient samples were screened on the predicate method (Siemens, Advia) and on the Theranos method. One sample was excluded as outlier (mean absolute difference greater than 4). Using the predicate method twenty-six (26) values were within the reference range (3.6— 5.2 mM), twelve (12) were below the reference range, and eleven (11) 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 Potassium is 0.5mM. The table below shows the allowable bias and precision at 3 levels (values shown in parentheses) and the corresponding closest medical decision limits.

Table 2. Allowable Bias and Precision at the Medical Decision Levels

Medical Decision Levels (mM)	**3.0 (2.6)**	**5.8 (4.1)**	**7.5 (7.5)**
Precision (%)	**5.0**	**3.8**	**3.0**
Allowable Bias (%)	**14.2**	**8.4**	**3.7**

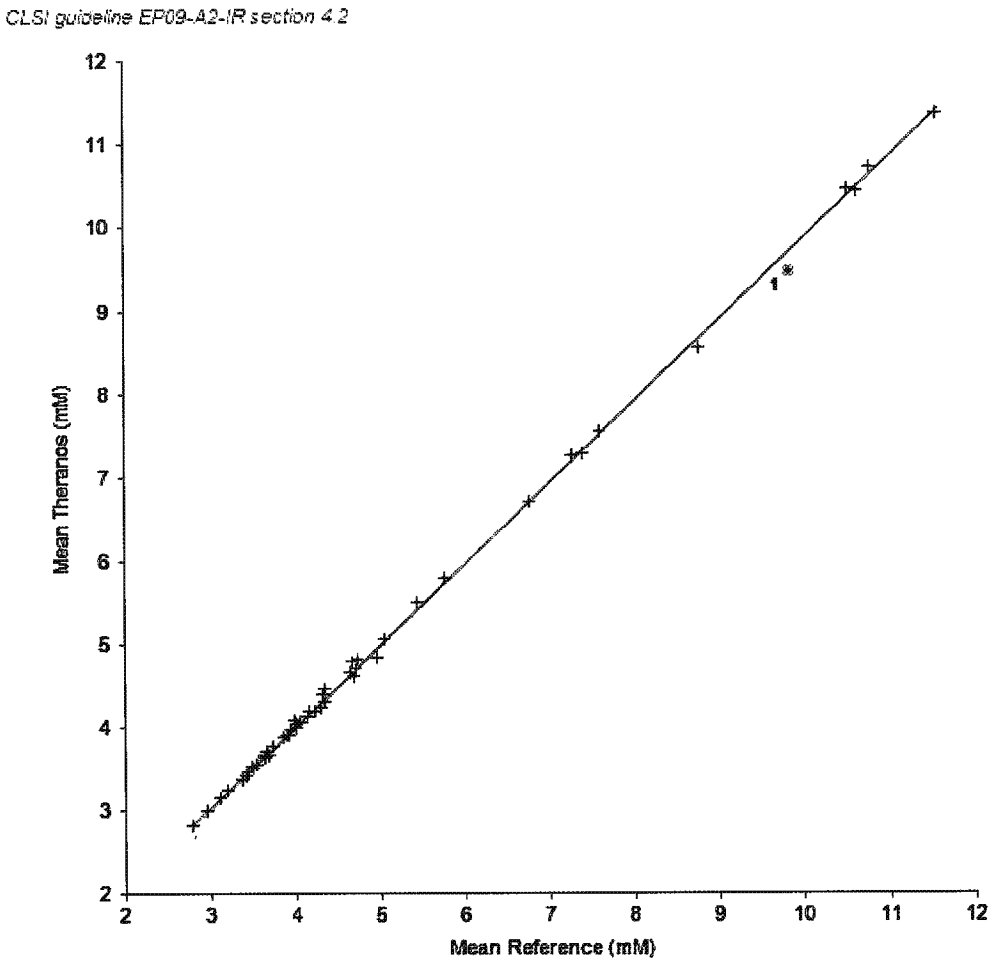


Figure 1. Graph showing Theranos method versus Predicate Method (Siemens Advia). Simple linear regression was used to establish a slope, intercept and an r2. The slope, intercept and clinical correlation were determined to be 0.98, 0.09 and 1.00 respectively.

Trueness

CLSI guideline EP09-A2-IR section 7

Level ID	Value	Bias	SE	95% CI	Allowable bias
L	3.00000000	0.020143920	0.010330650	-0.000638674 to 0.04092	0.154500000
M	5.80000000	-0.04093796	0.008293852	-0.057623045 to -0.0242	0.298700000
H	7.50000000	-0.07802339	0.011941510	-0.102046633 to -0.0540	0.386250000

Bias is less than allowable bias: 5.15%.

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 with venous samples using twenty four (24) new normal subjects. 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 lithium heparin venous verification 23 (95.5%) values fell within the new reference range and 1 (4.5%) values fell outside the new reference range. See graph below for venous samples verification.

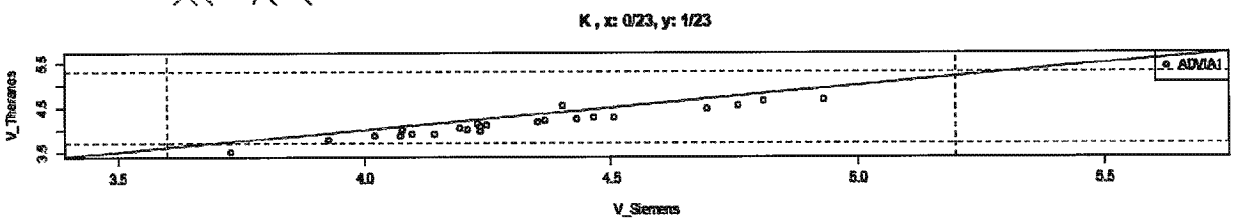


Figure 2. Graph showing venous sample reference range verification.

c. Verification of Reference Interval with Finger Stick Samples

Finger stick reference range was also verified with venous matched finger sticks (lithium heparin) samples from twenty three (23) new normal subjects. The finger stick samples were also collected in a Theranos blood collection devices (BCD) configured with Lithium heparin vessel only. For finger stick verification 22 (95.5%) values fell within the new reference range and 1 (4.5%) values fell outside the new reference range. See graphs below for finger stick samples verification.

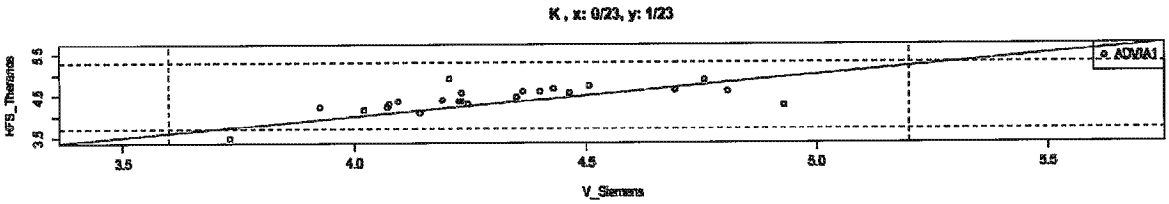


Figure 3. Finger stick sample reference range verification.

The Lithium heparin finger stick reference interval for Potassium was determined to be 3.6 – 5.2 mM.

VI. Stability

a. Reagents

On-board Reagent Stability

System	Stability
ADVIA 1200	30 days
ADVIA 1650/1800	30 days
ADVIA 2400	30 days

For all systems, unopened reagents are stable until the expiration date printed on the product label when stored at 5° - 25°C. Do not freeze reagents.

For complete details, refer to the Methods Introduction section of the system-specific Operator's Guide.

b. Sample

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

c. Calibrators

Potassium calibrator is stable at 2-8 °C for at least 6 months from date of manufacture.

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