Summary of Plasma-Serum Bias Observed at Spectra and Bias Reported in Literature

Executive Summary

No difference between plasma and serum tests was observed for calcium, and the variance was smaller than those reported in the literature cited.

- CLIA allowable total error = 1.0 mg/dL
- Fraser "desirable bias" = 0.8%
- Target concentrations : 8.5, 9.5, 10.2 mg/dL
- Target biases: 0.068, 0.076, 0.082 mg/dL, respectively (very small relative to CLIA allowable error)
- Analytical SD approx. 0.14 mg/dL @ target (Very difficult to detect target bias with analytical SD)
- **ESRD Bias (PST-SST) = +0.128 mg/dL @ patient mean of 9.09 mg/dL, from a sample size of 101 ESRD patients**

Introduction

Spectra Laboratories is evaluating conversion from blood serum samples (collected in "Serum Separator Tubes" or SST) to blood plasma samples (collected in "Plasma Separator Tubes" or PST) because [a] plasma samples do not require waiting for the sample to clot (calcium is consumed in the clotting process), and [b] because of potential differences between ESRD patient samples compared to those from the general population samples, where the former at times, either do not clot completely before sampling and/or clot while on the analyzer (because the patient was partially anticoagulated, sample amount was inadequate, or perhaps due to unaccounted uremic effect). Of note, the CLIA allowable error for inter-assay differences in calcium is 1.0 mg/dL or ~10.5% (at 9.5 mg/dL). The CLIA limit is the minimum standard for performance that is adhered to by instrument manufacturers, and sets the level of current practice in most laboratories.

Methods

Studies were conducted at Spectra Laboratory to determine the differences (biases) between plasma and serum samples collected in separator tubes from ESRD patients. At least 100 or more pairs of patient plasma and serum samples were targeted to be compared. The biases observed in Spectra's studies were then compared to biases reported in the literature to check for consistency. This report summarizes Spectra's findings for phosphorus.

Results

The conditions of the literature reports are summarized below.

- Doumas BT, Hause LL, Simuncak DM, Breltenfeld D. Differences between Values for Plasma and Serum in Tests Performed in the Ektachem 700 XR Analyzer, and Evaluation of "Plasma Separator Tubes (PST)." Clin Chem 35:151-153(1989). This study involved comparison samples from 25 normal adults, and compared plasma from SST tubes to "serum." The means of the patient results were within the normal ranges. Testing was done on a Kodak Ektachem 700 XR.
- Miles RR, Roberts RF, Putnam AR, Roberts WL. Comparison of Serum and Heparinized Plasma Samples for Measurement of Chemistry Analytes. Clin Chem 50:1704-1706 (2004). This study involved comparison samples from 20 normal adults, and compared lithium heparin plasma to serum (no separating gel). Means of patient results were within the normal ranges. Testing was done on a Roche Modular P analyzer and a Vitros 950 (a later model of Ektachem).
- Wei Y-h, Zhang C-b, Yang X-w, Ji M-d. The Feasibility of Using Lithium-Heparin Plasma from a Gel Separator Tube as a Substitute for Serum in Clinical Biochemical Tests. Lab Medicine 41: 215-219 (2010). This study involved comparison samples from 40 "donors," and compared plasma to serum (SST) and PST to SST. Testing was done on an Olympus AU2700 (same as instrument at Spectra). Unfortunately, the authors summarized results from most tests as showing equivalency rather than providing bias estimates.

The Spectra experiments had a large sample of ESRD patients, whereas most of the published studies from other institutions involved smaller numbers of samples from relatively normal subjects. Results of testing are reported below and biases are given as PST - SST (plasma - serum).

Calcium: A bias of +0.1 mg/dL was observed at Spectra with 101 patient samples. Biases reported in the literature cited range from 0.1 to -0.2 mg/dL. Small amounts of calcium are consumed in the clotting process, potentially decreasing the measured serum calcium concentration. Fraser's desirable bias is 0.8%. Table 1 summarizes the results relative to the bias reported in the literature. The Appendix contains the actual data summary from the lab.

Conclusion

There was minimal difference observed for serum vs. plasma calcium, and the results for ESRD within Spectra laboratories showed minimal variance, consistent with those reported in the literature cited.

Implications

Most instruments are not capable of distinguishing calcium differences of the order of 0.1 mg/dL, due to inherent imprecision, without testing samples in large numbers of replicates. Calcium is customarily reported to the nearest 0.1 mg/dL, further minimizing the practical impact of the observed difference. For purposes of the ESRD QIP, a 0.1 mg/dL difference is small and anticipated to add minimal risk for a higher incidence of measured hypercalcemia in a dialysis provider's patient population, particularly when a 3-month rolling average is utilized to determine hypercalcemia.

Table 1. Spectra plasma vs. serum bias (PST-SST) compared to bias reported in literature.

Test Name	Spectra n	Spectra Bias[1]	Spectra % Bias[2]	Mean Absolute Bias 8 ESRD Labs[3]	Doumas Bias[4]	Miles Modular Bias[5]	Miles Vitros Bias[5]	Wei, et. al Plasma-SST[6]	Wei, et. al PST-SST[7]
Calcium	101	0.1	1.4%	0.09	0.1	-0.1	-0.2	equivalent	equivalent

Note: "Fraser" desirable bias reference: "Desirable Specifications for Total Error, Imprecision, and Bias, derived from intra- and inter-individual biologic variation." In: Ricos C, Alvarez V, Cava F, Garcia-Lario JV, Hernandez A, Jimenez CV, Minchinela J, Perich C, Simon M. "Current databases on biologic variation: pros, cons and progress." Scand J Clin Lab Invest 1999;59:491-500. This database was most recently updated in 2010, from Westgard QC website, http://www.westgardqc.com/biodatabase1.htm#SCC, accessed October 21, 2010.

[1] Simple bias observed at Spectra West in experiments conducted in 2010 and 2011
[2] Percent simple bias observed at Spectra West in experiment conducted in 2010 or 2011
[3] Mean absolute bias from ESRD laboratory comparison experiment
[4] Bias reported (PST - serum) by Doumas, et. al, Clin Chem 35:151-153 (1989)
[5] Bias reported (plasma - serum) by Miles, et. al, Clin Chem 50:1704-1706 (2004); bias reported by the authors in percent was converted to reporting units
[6] Bias reported (plasma - SST) by Wei, et. al, Lab Medicine 41: 215-219 (2010)
[7] Bias reported (PST plasma - SST) by Wei, et. al, Lab Medicine 41: 215-219 (2010)

APPENDIX:

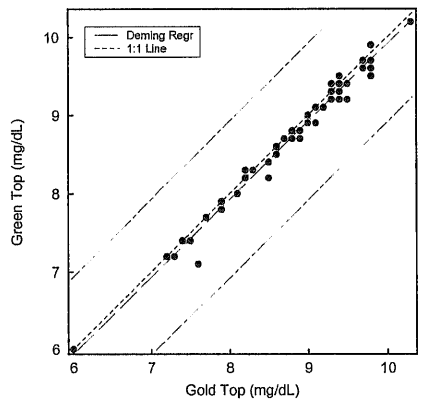
Actual results summary of the tests:

Test Name	date of analysis	n	slope	intercept	r	bias	bias %	mean ± SD SST	mean ± SD PST	result ranges SST	result ranges PST	analytical range
Calcium	2/20/10	101	0.999	0.139	0.981	0.128	1.4%	9.09 ± 0.80	9.21 ± 0.80	7.1 - 11.2	7.5 - 11.3	4.0-18.0

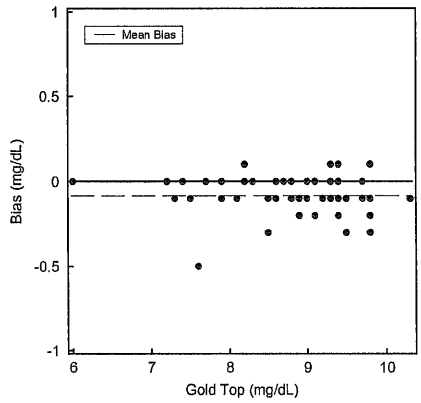
Alternate Method Comparison

X Method: Gold Top **Y Method: Green Top**

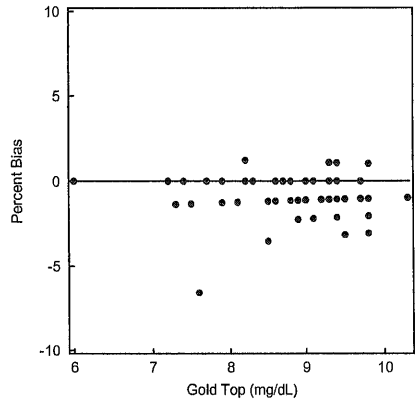
Scatter Plot



Bias



Percent Bias



Regression Analysis

	Deming	Regular
Slope:	0.995 (0.958 to 1.032)	0.986 (0.949 to 1.022)
Intercept:	-0.04 (-0.36 to 0.29)	0.04 (-0.28 to 0.37)
Std Err Est:	0.11	0.11

95% Confidence Intervals are shown in parentheses

Supporting Statistics

Corr Coef (R):	0.9909	T Probability:	<0.001
Bias:	-0.08	Degrees Freedom:	54
XMean ± SD:	8.84 ± 0.82	SubRange Bounds:	None
YMean ± SD:	8.76 ± 0.82	Points (Plotted/Total):	56/56
Std Dev Diffs:	0.11	Outliers:	None
Paired T Test:	5.67	Scatter Plot Bounds:	Allowable Error

Experiment Description

	X Method	Y Method
ExptDate:	30 Dec 2003	30 Dec 2003
Rep SD:	1	1
Result Ranges:	6.0 to 10.3	6.0 to 10.2
Units:	mg/dL	mg/dL
Analyst:	Marty Blair	Marty Blair
Comment:		

Accepted by: _____ _____

Signature Date

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Alternate Method Comparison

X Method: Gold Top **Y Method: Green Top**

Experimental Results

Specimen	X	Y	Bias	Specimen	X	Y	Bias	Specimen	X	Y	Bias
S00001	8.7	8.7	0.0	S00020	8.3	8.3	0.0	S00039	9.4	9.2	-0.2
S00002	9	9	0	S00021	9.4	9.3	-0.1	S00040	8.8	8.7	-0.1
S00003	8.6	8.5	-0.1	S00022	8.5	8.4	-0.1	S00041	8.9	8.8	-0.1
S00004	7.9	7.9	0.0	S00023	7.4	7.4	0.0	S00042	9.3	9.3	0.0
S00005	8.9	8.8	-0.1	S00024	9.5	9.4	-0.1	S00043	9.8	9.6	-0.2
S00006	9	8.9	-0.1	S00025	8.8	8.7	-0.1	S00044	8.5	8.2	-0.3
S00007	7.2	7.2	0.0	S00026	9.3	9.2	-0.1	S00045	8.9	8.7	-0.2
S00008	7.7	7.7	0.0	S00027	9.3	9.4	0.1	S00046	8.8	8.7	-0.1
S00009	9.4	9.5	0.1	S00028	8.2	8.3	0.1	S00047	9.7	9.7	0.0
S00010	9.8	9.9	0.1	S00029	8.8	8.8	0.0	S00048	9.4	9.2	-0.2
S00011	8.5	8.4	-0.1	S00030	9.2	9.1	-0.1	S00049	9.8	9.5	-0.3
S00012	8.1	8	-0.1	S00031	7.9	7.8	-0.1	S00050	9.7	9.6	-0.1
S00013	6	6	0	S00032	9.7	9.7	0.0	S00051	7.3	7.2	-0.1
S00014	7.6	7.1	-0.5	S00033	8.6	8.6	0.0	S00052	9.5	9.4	-0.1
S00015	8.2	8.2	0.0	S00034	10.3	10.2	-0.1	S00053	9.8	9.7	-0.1
S00016	9.5	9.2	-0.3	S00035	9.8	9.6	-0.2	S00054	9.4	9.4	0.0
S00017	9.1	8.9	-0.2	S00036	8.7	8.7	0.0	S00055	8.9	8.8	-0.1
S00018	9	9	0	S00037	7.5	7.4	-0.1	S00056	9.1	9.1	0.0
S00019	9.3	9.2	-0.1	S00038	9.4	9.2	-0.2				

Values with an "X" were excluded from the calculations. Outliers "O" were also excluded.

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