

Evaluation Report

Eurolyser FRUCTOSAMINE “gold standard” test kit (VT0190) on solo Analyser

Locations

Location 1: Eurolyser Diagnostica GmbH
Operator: Michael Gruber
Date: November-Dezember 2014

V18.02.2015

Specimens

The specimens used for analysis were taken from multiple sites and were frozen dog and cat serum and EDTA plasma samples.

Equipment

- Eurolyser solo Analyser: Bc14581Bc14577, Bc14578, Bc14579, Bc14580
- Testkit FRUCTOSAMINE VT0110: R+D 1214-1



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1. Introduction and Scope

Eurolysers “Gold standard Fructosamine” assay is an enzymatic assay which is much more specific for glycated serum protein (Fructosamine) than the old-traditional NBT based fructosamine assay which is significantly interfered by endogenous reducing substances. The traditional method (NBT) was found to be not specific for Fructosamine. NBT reacts with various endogenous substances of reducing activity such as thiol groups, NADH, and ascorbate. In fact, studies showed that only about half of the reducing activity (Fructosamine) was due to specific non enzymatic glycation of proteins, and the remaining unspecific activity varied from serum to serum.

Therefore the Novel Eurolyser “Gold standard Fructosamine” is the assay of the first choice offering improved specificity and reliability as it does not suffer from non specific interferences.

Principle:

Eurolysers Fructosamine (Glycated Serum Protein) gold standard Assay uses proteinase K to digest GSP into low molecular weight glycated protein fragments (GPF), and uses Eurolysers specific fructosaminase, a microorganism originated amadoriase to catalyze the oxidative degradation of Amadori product GPF to yield PF or amino acids, glucosone and H₂O₂. The H₂O₂ released is measured by a colorimetric Trinder end-point reaction. The absorbance at 546 nm is proportional to the concentration of glycated serum proteins=Fructosamine.

2. Comparison Studies

The comparison study is based on the correlation between the results of the Eurolyser SOLO Fructosamine gold standard and the ABX Pentra 400 Fructosamine assay Ref A11A01679.

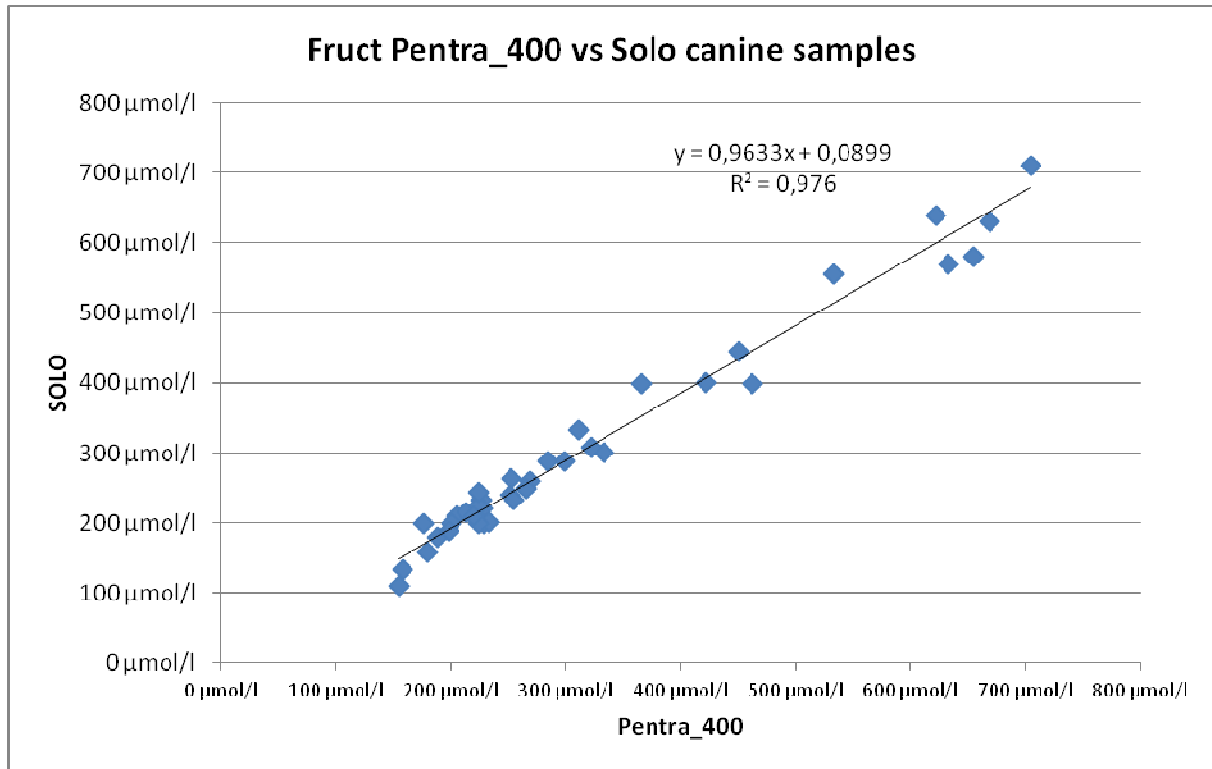
The ABX Pentra 400 was calibrated with a 2 point calibration against 0.9% NaCl and the ABX Pentra Fructo Cal, Ref. A11A01680

The SOLO Fructosamine gold standard assay was calibrated against the Diayzme-USA GSP calibrator DZ112B-Cal.

40 dog and 40 cat patient samples (EDTA plasma and serum), have been tested.

The acceptance criteria for this comparison study is a coefficient of determination $R^2 > 0.95$ obtained from linear regression between the Eurolyser and the ABX Pentra400 Fructosamine.

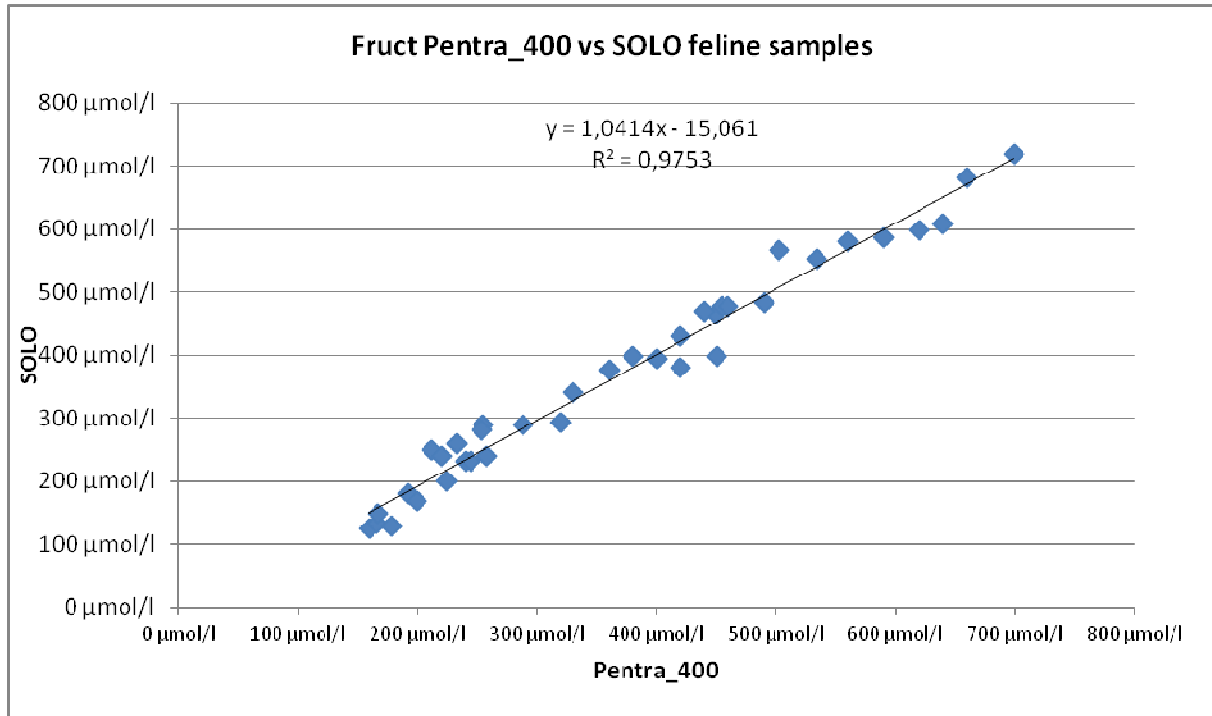
normal range			
dog	Material	fruct Pentra 400	Fruct SOLO
678537 IVL-2	EDTA plasma	155 µmol/l	110 µmol/l
677681 IVL-3	Serum	158 µmol/l	133 µmol/l
676690 IVL-4	Serum	462 µmol/l	399 µmol/l
675817 IVL-5	EDTA plasma	655 µmol/l	580 µmol/l
IVL 1	EDTA plasma	189 µmol/l	180 µmol/l
IVL 2	Serum	205 µmol/l	210 µmol/l
IVL 3	Serum	253 µmol/l	263 µmol/l
IVL 4	Serum	214 µmol/l	216 µmol/l
IVL 5	Serum	311 µmol/l	333 µmol/l
IVL 6	EDTA plasma	252 µmol/l	241 µmol/l
IVL 7	Serum	284 µmol/l	289 µmol/l
IVL 8	EDTA plasma	227 µmol/l	233 µmol/l
IVL 9	Serum	269 µmol/l	260 µmol/l
IVL 10	Serum	228 µmol/l	220 µmol/l
VU1	EDTA plasma	533 µmol/l	555 µmol/l
VU2	EDTA plasma	422 µmol/l	400 µmol/l
VU3	EDTA plasma	201 µmol/l	199 µmol/l
VU4	Serum	255 µmol/l	233 µmol/l
VU5	Serum	229 µmol/l	200 µmol/l
VU6	EDTA plasma	233 µmol/l	201 µmol/l
VU7	EDTA plasma	225 µmol/l	224 µmol/l
VU8	Serum	266 µmol/l	250 µmol/l
VU9	Serum	704 µmol/l	710 µmol/l
VU10	Serum	333 µmol/l	300 µmol/l
VU11	Serum	322 µmol/l	308 µmol/l
VU12	EDTA plasma	199 µmol/l	188 µmol/l
VU13	EDTA plasma	177 µmol/l	199 µmol/l
VU14	Serum	450 µmol/l	445 µmol/l
VU15	Serum	622 µmol/l	639 µmol/l
VU16	Serum	225 µmol/l	200 µmol/l
VU17	EDTA plasma	220 µmol/l	210 µmol/l
VU18	EDTA plasma	299 µmol/l	288 µmol/l
VU19	Serum	366 µmol/l	399 µmol/l
VU20	Serum	225 µmol/l	244 µmol/l
VU21	EDTA plasma	669 µmol/l	630 µmol/l
VU22-2	Serum	633 µmol/l	570 µmol/l
VU23-2	EDTA plasma	180 µmol/l	159 µmol/l



CANINE SAMPLE Correlation:

The result for the correlation between Eurolyser/SOLO FRUCT and PENTRA400 is the linear regression function y (Solo Fruct) = $0.9633 x$ (Pentra400 Fruct) + $0,0899$ and a $R^2 = 0.976$.

normal range			
cat		fruct Pentra 400	Fruct Solo
IVD 676432-1	EDTA plasma	159 µmol/l	125 µmol/l
IVD678537-2	Serum	166 µmol/l	133 µmol/l
IVD 680642-3	Serum	178 µmol/l	129 µmol/l
IVD 682747-4	EDTA plasma	192 µmol/l	180 µmol/l
IVD 684852-5	EDTA plasma	199 µmol/l	169 µmol/l
IVD 686957	Serum	255 µmol/l	289 µmol/l
IVD 689062	Serum	212 µmol/l	249 µmol/l
IVD 691167	Serum	220 µmol/l	239 µmol/l
IVD 693272	Serum	224 µmol/l	200 µmol/l
IVD 695377-7	EDTA plasma	240 µmol/l	233 µmol/l
IVD 697482	Serum	233 µmol/l	260 µmol/l
IVD 699587	EDTA plasma	254 µmol/l	282 µmol/l
IVD 701692	Serum	503 µmol/l	566 µmol/l
IVD 703797	Serum	258 µmol/l	239 µmol/l
IVD 705902	EDTA plasma	320 µmol/l	293 µmol/l
IVD 708007	EDTA plasma	449 µmol/l	466 µmol/l
IVD 710112	EDTA plasma	455 µmol/l	477 µmol/l
IVD 712217	Serum	490 µmol/l	483 µmol/l
IVD 714322	Serum	534 µmol/l	553 µmol/l
IVD 716427	EDTA plasma	560 µmol/l	580 µmol/l
IVD 718532	EDTA plasma	590 µmol/l	587 µmol/l
IVD 720637	Serum	620 µmol/l	599 µmol/l
IVD 722742	Serum	640 µmol/l	608 µmol/l
IVD 724847	Serum	660 µmol/l	681 µmol/l
IVD 726952	Serum	330 µmol/l	340 µmol/l
VU1	EDTA plasma	288 µmol/l	289 µmol/l
VU2	EDTA plasma	244 µmol/l	233 µmol/l
VU3	Serum	440 µmol/l	470 µmol/l
VU4	Serum	699 µmol/l	720 µmol/l
VU5	Serum	400 µmol/l	395 µmol/l
VU6	EDTA plasma	460 µmol/l	477 µmol/l
VU7	Serum	420 µmol/l	430 µmol/l
VU8	Serum	360 µmol/l	377 µmol/l
VU9	Serum	380 µmol/l	399 µmol/l
VU10	Serum	450 µmol/l	399 µmol/l
VU11-1	Serum	167 µmol/l	149 µmol/l
VU12-2	EDTA plasma	420 µmol/l	380 µmol/l



FELINE SAMPLE Correlation:

The result for the correlation between Eurolyser/SOLO Fruct and ABX Pentra400 Fruct is the linear regression function y (Solo Fruct) = 1.0414 x (ABXPentra_400 Fruct) – 15,061 and a **$R^2 = 0.9753$** .

3. Imprecision “within-run”

a. Precision/Reproducibility

In house precision

The precision of the Eurolyser FRUCT “gold standard” Test was evaluated according to Clinical and Laboratory Standards Institute (CLSI) EP5-A guideline. In the study, two controls and two levels of serum specimens containing 215, 720, 225 and 480 $\mu\text{mol/L}$ GSP, respectively, were tested 4 runs per day over 20 working days at Eurolysers OEM partner “Diazyme Laboratories”. The two levels of serum samples were purchased from ProMedDx, LLC and came with an IRB certification.

SMART (=SOLO) Analyzer

Control Level 1: 215 $\mu\text{mol/L}$ GSP

Day#	Run 1		Run 2		Daily Mean	Mean Run 1	Mean Run 2
	Result 1	Result 2	Result 1	Result 2			
1	222	228	223	227	225.0	225.0	225.0
2	202	207	216	219	211.0	204.5	217.5
3	207	225	221	206	214.8	216.0	213.5
4	206	206	221	223	214.0	206.0	222.0
5	193	197	225	219	206.5	195.0	222.0
6	221	219	215	211	216.5	220.0	213.0
7	212	214	209	222	214.3	213.0	215.5
8	219	221	209	194	210.8	220.0	201.5
9	208	212	220	219	214.8	210.0	219.5
10	214	218	219	218	217.3	216.0	218.5
11	217	224	217	216	218.5	220.5	216.5
12	211	210	212	207	210.0	210.5	209.5
13	208	195	195	227	206.3	201.5	211.0
14	207	216	216	204	210.8	211.5	210.0
15	197	212	210	210	207.3	204.5	210.0
16	214	208	208	212	210.5	211.0	210.0
17	236	219	223	218	224.0	227.5	220.5
18	206	208	209	209	208.0	207.0	209.0
19	200	195	193	211	199.8	197.5	202.0
20	222	206	248	218	223.5	214.0	233.0

Control Level 2: 720 $\mu\text{mol/L}$ GSP

Day#	Run 1		Run 2		Daily Mean	Mean Run 1	Mean Run 2
	Result 1	Result 2	Result 1	Result 2			
1	761	733	744	754	748.0	747.0	749.0
2	712	710	724	712	714.5	711.0	718.0
3	727	723	712	724	721.5	725.0	718.0
4	686	712	672	720	697.5	699.0	696.0
5	698	728	725	727	719.5	713.0	726.0
6	724	731	695	730	720.0	727.5	712.5
7	759	720	711	689	719.8	739.5	700.0
8	701	719	715	671	701.5	710.0	693.0
9	765	705	711	727	727.0	735.0	719.0
10	707	720	739	738	726.0	713.5	738.5
11	728	706	697	729	715.0	717.0	713.0
12	701	714	720	732	718.8	707.5	726.0
13	728	711	717	721	719.3	719.5	719.0
14	721	695	704	702	705.5	708.0	703.0
15	660	676	716	669	680.3	668.0	692.5
16	728	703	671	707	702.3	715.5	689.0
17	696	716	756	725	702.3	715.5	689.0
18	677	693	706	719	698.8	685.0	712.5
19	720	843	730	763	764.0	781.5	746.5
20	778	744	751	763	764.0	761.0	767.0

Serum Level 1: 225 µmol/L

Day#	Run 1		Run 2		Daily Mean	Mean Day#	Mean Date
	Result 1	Result 2	Result 1	Result 2			
1	225	235	235	235	232.5	230.0	235.0
2	225	232	223	230	227.5	228.5	226.5
3	224	221	233	230	227.0	222.5	231.5
4	196	223	244	236	224.5	209.5	239.5
5	222	233	216	197	217.0	227.5	206.5
6	230	211	216	228	221.3	220.5	222.0
7	222	223	218	234	224.3	222.5	226.0
8	216	227	214	237	223.5	221.5	225.5
9	226	199	240	222	221.8	212.5	231.0
10	234	225	220	235	228.5	229.5	227.5
11	222	211	227	239	224.8	216.5	233.0
12	228	229	221	240	229.5	228.5	230.5
13	221	243	221	240	231.3	232.0	230.5
14	226	222	228	235	227.8	224.0	231.5
15	210	215	222	233	220.0	212.5	227.5
16	228	236	233	216	228.3	232.0	224.5
17	235	242	229	228	233.5	238.5	228.5
18	219	230	218	225	223.0	224.5	221.5
19	203	223	225	217	217.0	213.0	221.0
20	235	212	227	230	227.5	223.5	231.5

Serum Level 2: 480 µmol/L GSP; ID #: 10936154

Day#	Run 1		Run 2		Daily Mean	Mean Run 1	Mean Run 2
	Result 1	Result 2	Result 1	Result 2			
1	478	459	482	477	474.0	468.5	479.5
2	487	440	471	457	463.8	463.5	464.0
3	505	476	479	499	489.8	490.5	489.0
4	470	472	489	491	480.5	471.0	490.0
5	456	478	485	479	474.5	467.0	482.0
6	490	519	518	503	507.5	504.5	510.5
7	457	445	481	477	465.0	451.0	479.0
8	445	488	464	486	470.8	466.5	475.0
9	487	471	497	465	480.0	479.0	481.0
10	491	479	472	505	486.0	485.0	488.5
11	488	508	485	475	489.0	498.0	480.0
12	471	502	491	457	480.3	486.5	474.0
13	495	495	486	474	487.5	495.0	480.0
14	499	446	472	475	473.0	472.5	473.5
15	466	491	475	482	478.5	478.5	478.5
16	482	458	472	492	476.0	470.0	482.0
17	466	456	473	458	463.3	461.0	465.5
18	467	482	500	470	479.8	474.5	485.0
19	467	471	489	483	477.5	469.0	486.0
20	471	497	467	493	482.0	484.0	480.0

The results on SOLO 8SMART) were calculated using the software precision statistic template and summarized in the following table:

Within Run Precision (S_r)

	Control Level 1: 215 µmol/L GSP	Control Level 2: 720 µmol/L GSP	Serum Level 1: 225 µmol/L GSP	Serum Level 2: 480 µmol/L GSP
Number of Data Points	80	80	80	80
Mean	213.26	719.21	225.51	478.96
SD	7.70	22.92	9.75	16.09
CV%	3.6 %	3.2 %	4.3 %	3.4 %

Within-Laboratory Precision (S_T)

	Control Level 1: 215 µmol/L GSP	Control Level 2: 720 µmol/L GSP	Serum Level 1: 225 µmol/L GSP	Serum Level 2: 480 µmol/L GSP
Number of Data Points	80	80	80	80
Mean	213.26	719.21	225.51	478.96
SD	9.91	28.22	10.14	16.48
CV%	4.6 %	3.9 %	4.5 %	3.4 %

$$S_r = [((1) + (2))/4I]^{1/2}$$

Where I = number of days

$$S_T = [(2B^2 + A^2 + S_r^2)/2]^{1/2}$$

Where A = [(3)/2I]^{1/2}

B= Standard deviation of "Daily Means"

Conclusion: For two levels of FRUCT (GSP) controls and two levels of serum specimen, 20-day reproducibility data showed that the within-run imprecision was from 3.2 % to 4.3 % and the within-laboratory imprecision was from 3.4 % to 4.6 %.

4. Linearity Study

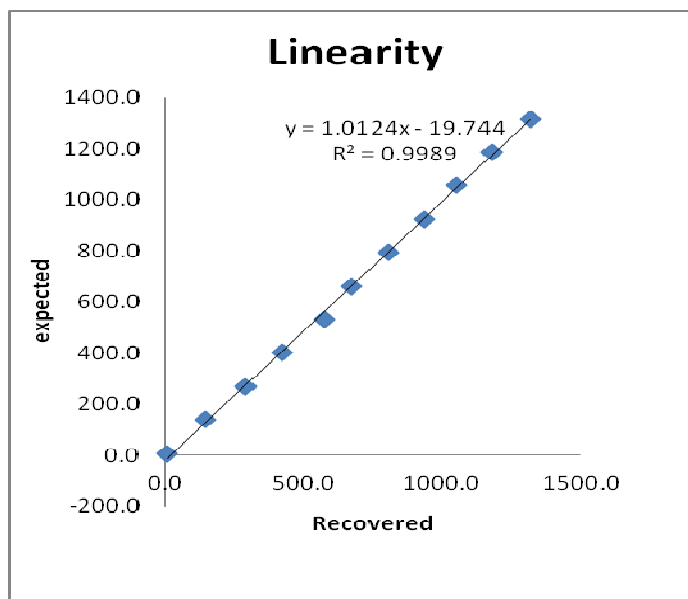
Eleven levels of linearity set were prepared by diluting a sample containing 1316 $\mu\text{mol/L}$ GSP with a sample containing 8.0 $\mu\text{mol/L}$ according to Clinical and Laboratory Standards Institute (CLSI) EP6-A as follows:

Level 10: 0 μl of 8.0 $\mu\text{mol/L}$ Fruct + 200 μl of 1316 $\mu\text{mol/L}$ Fruct
 Level 9: 20 μl of 8.0 $\mu\text{mol/L}$ Fruct + 180 μl of 1316 $\mu\text{mol/L}$ Fruct
 Level 8: 40 μl of 8.0 $\mu\text{mol/L}$ Fruct + 160 μl of 1316 $\mu\text{mol/L}$ Fruct
 Level 7: 60 μl of 8.0 $\mu\text{mol/L}$ Fruct + 140 μl of 1316 $\mu\text{mol/L}$ Fruct
 Level 6: 80 μl of 8.0 $\mu\text{mol/L}$ Fruct + 120 μl of 1316 $\mu\text{mol/L}$ Fruct
 Level 5: 100 μl of 8.0 $\mu\text{mol/L}$ Fruct + 100 μl of 1316 $\mu\text{mol/L}$ Fruct
 Level 4: 120 μl of 8.0 $\mu\text{mol/L}$ Fruct + 80 μl of 1316 $\mu\text{mol/L}$ Fruct
 Level 3: 140 μl of 8.0 $\mu\text{mol/L}$ Fruct + 60 μl of 1316 $\mu\text{mol/L}$ Fruct
 Level 2: 160 μl of 8.0 $\mu\text{mol/L}$ Fruct + 40 μl of 1316 $\mu\text{mol/L}$ Fruct
 Level 1: 180 μl of 8.0 $\mu\text{mol/L}$ Fruct + 20 μl of 1316 $\mu\text{mol/L}$ Fruct
 Level 0: 200 μl of 8.0 $\mu\text{mol/L}$ Fruct + 0 μl of 1316 $\mu\text{mol/L}$ Fruct

The linearity samples were tested with the SOLO/Smart Fructosamine Gold standard Test Kit in triplicate. The linearity analysis was performed using EP Evaluator Version 8 Software. The results are summarized below.

Levels	Recovery			Mean Recovery ($\mu\text{mol/L}$)	Expected Recovery ($\mu\text{mol/L}$)	%Error
	Rep 1	Rep 2	Rep 3			
Level 10	1334	1302	1314	1316.7	1316.7	0.0
Level 9	1157	1191	1189	1179.0	1185.8	-0.6
Level 8	1062	1032	1063	1052.3	1054.9	-0.2
Level 7	938	944	923	935.0	924.1	1.2
Level 6	812	790	812	804.7	793.2	1.4
Level 5	675	671	681	675.7	662.4	2.0
Level 4	581	581	570	577.3	531.5	8.6
Level 3	435	410	430	425.0	400.6	6.1
Level 2	288	296	290	291.3	369.8	8.0
Level 1	148	158	133	146.3	138.9	5.4
Level 0	6	3	15	8.0	8.0	0.0

The expected is plotted against mean recovered. The plot is shown below:



The data are also processed in the EP evaluator release 8. The results are shown below:

EP Evaluator

Research and Development – Diazyme Laboratories

GSP

Instrument: SMART

Linearity

	Assigned	N	Est	Mean	Residual	Linearity
11	8	3	8.4	8.0	-0.4	Pass
10	138.9	3	144.2	146.3	2.1	Pass
9	269.8	3	280.1	291.3	11.2	Pass
8	400.6	3	415.9	425.0	9.1	Pass
7	531.5	3	551.8	577.3	25.5	Pass
6	662.4	3	687.7	675.7	-12.1	Pass
5	793.2	3	823.5	804.7	-18.8	Pass
4	924.1	3	959.4	935.0	-24.4	Pass
3	1054.9	3	1095.2	1052.3	-42.9	Pass
2	1185.8	3	1231.1	1179.0	-52.1	Pass
1	1316.7	3	1367.0	1316.7	-50.3	Pass

See User's Specifications for Pass/Fail criteria

Linearity Summary

Overall	
Slope	1.038
Intercept	0.0
Obs. Err.	4.4%
N	11
LINEAR within Allowable Systematic Error of 4.5%	

Experimental Results

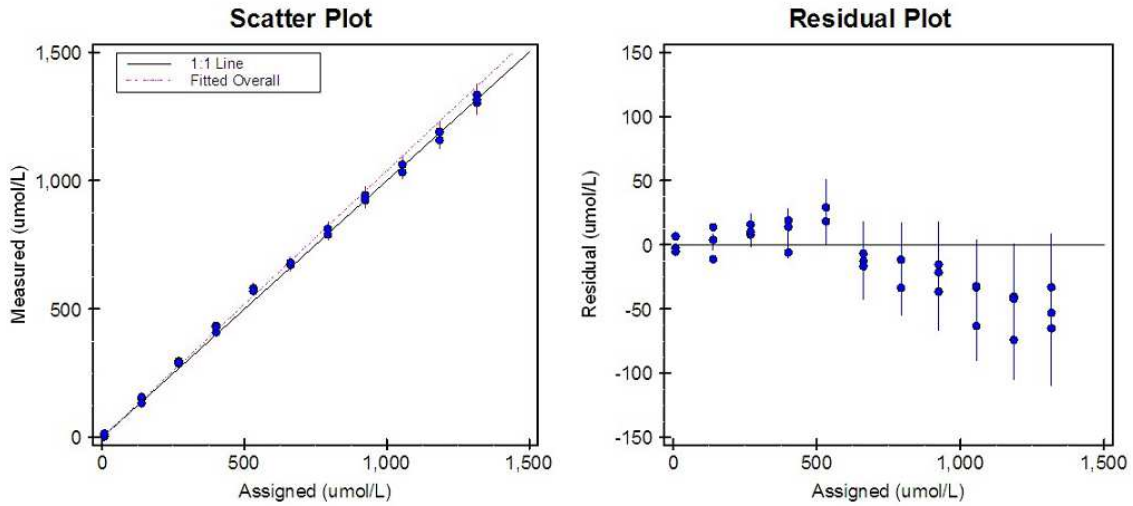
11	6	3	15
10	148	158	133
9	288	296	290
8	435	410	430
7	581	581	570
6	675	671	681
5	812	790	812
4	938	944	923
3	1062	1032	1063
2	1157	1191	1189
1	1334	1302	1314

X: Excluded from calculations

User's Specifications

Allowable Total Error:	10.0%
Systematic Error Budget:	45%
Allowable Systematic Error:	4.5%

Linearity



Conclusion: The bias between expected and recovered Fruct is less than 10% for the eleven levels of linearity set ranging from 8 µmol/L to 1316 µmol/L GSP. The linearity data of GSP was analyzed with the EP evaluator software with allowable systematic error (Sea) of 4.5 % and results showed that the assay is linear from 8.0 to 1316 µmol/L Fruct with a correlation coefficient of (R^2) of 0.99.

For VET-sample testing the Linearity Ranges up to at least 2600 µmol/l by using the 20µl Sample type option.

As shown in the later section, Limit of Quantitation (LOQ) of the assay is determined to be 61 µmol/L. Therefore we claim the linearity range of 61.0 to 2600 µmol/L.

5. Interference Study

Acceptance criteria: $\leq 10\%$ deviation from unspiked samples

To determine the level of interference from the substances normally present in serum, the Fructosamine gold standard Test Kit were used to test two serum samples with “low” and “high” Fruct concentration spiked with various concentrations of substances following Clinical and Laboratory Standards Institute EP7-A “Interference Testing in Clinical Chemistry”: dose-response guidelines.

To ensure a suitable degree of precision, each sample spiked with interference substances was tested in triplicates. For any substance, five levels will be tested: $C1 = L^*$, $C2 = (3L+H)/4$, $C3 = (L+H)/2$, $C4 = (L+3H)/4$ and $C5 = H^*$

L^* : The concentration of low range of interference substance;

H^* : The concentration of high range of interference substance

The interference substances examined and their concentrations tested are listed in the following table:

Interference Substances	C1	C2	C3	C4	C5
Ascorbic acid	0 mg/dL	1.25 mg/dL	2.5 mg/dL	3.75 mg/dL	5.0 mg/dL
Bilirubin	0 mg/dL	2.5 mg/dL	5.0 mg/dL	7.5 mg/dL	10 mg/dL
Bilirubin Conjugated	0 mg/dL	1.25 mg/dL	2.5 mg/dL	3.75 mg/dL	5 mg/dL
Glucose	0 mg/dL	600 mg/dL	1200 mg/dL	1800 mg/dL	2400 mg/dL
Triglycerides	0 mg/dL	500 mg/dL	1000 mg/dL	1500 mg/dL	2000 mg/dL
Uric Acid	0 mg/dL	8.75 mg/dL	17.5 mg/dL	26.25 mg/dL	35 mg/dL
Hemoglobin	0 mg/dL	50 mg/dL	100 mg/dL	150 mg/dL	200 mg/dL

The stock solutions for interference testing are prepared as following:

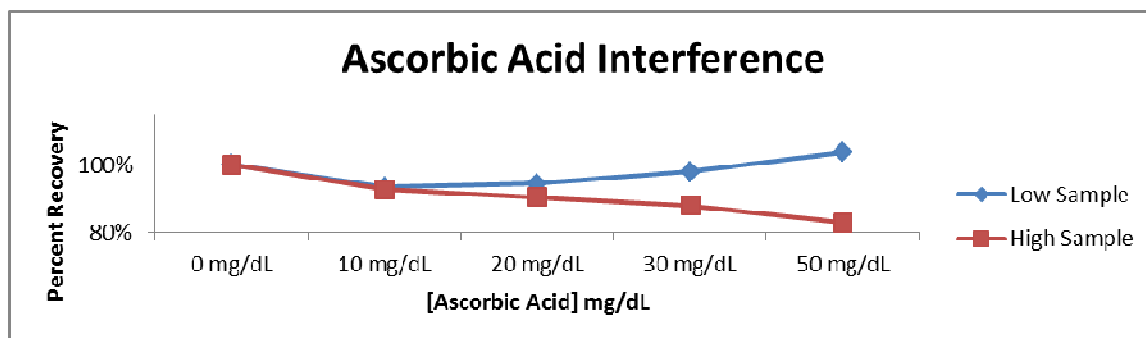
Interference Substances	Stock solution	Preparation Method
Ascorbic acid	5 mg/mL	Dissolve 5 mg in 1 mL of cold water deoxygenated by nitrogen bubbling; Keep the stock and serum solution on ice before testing
Bilirubin	10 mg/mL	Dissolve 10 mg bilirubin unconjugated in 1 mL 0.1N NaOH
Bilirubin Conjugated	5 mg/mL	Dissolve 5 mg bilirubin unconjugated in 1 mL water
Glucose	1200 mg/mL	Dissolve 1200mg Glucose in 1 mL of water
Triglycerides	1000 mg/mL	Neat solution
Uric Acid	35 mg/mL	Dissolve 35mg Uric Acid in 1 mL of water
Hemoglobin	200 mg/mL	Dissolve 200mg Hemoglobin in 1 mL of water

Spiking 1mL of serum samples containing approximately 219µmol/L and 550µmol/L Fruct with the stock solutions prepared above.

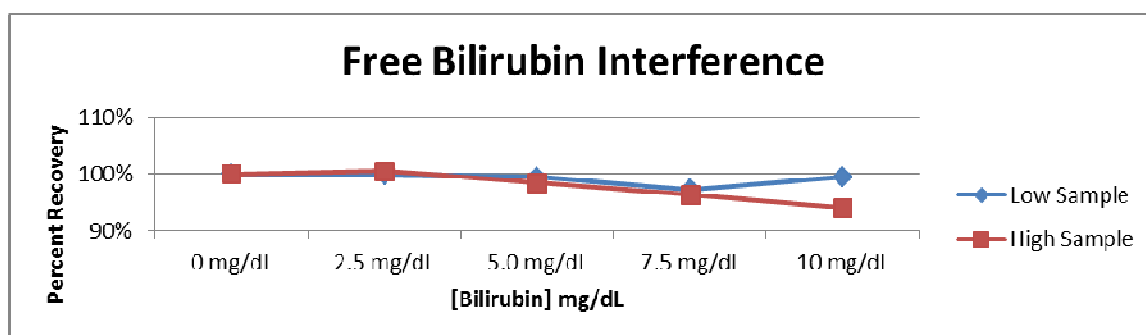
Interference Substances	C1	C2	C3	C4	C5
Ascorbic acid	0 mg/dL	1.25 mg/dL	2.5 mg/dL	3.75 mg/dL	5 mg/dL
Stock	0 µL	2.5 µL	5.0 µL	7.5 µL	10 µL
H ₂ O	10 µL	7.5 µL	5.0 µL	2.5 µL	0 µL
Bilirubin	0 mg/dL	2.5 mg/dL	5.0 mg/dL	7.5 mg/dL	10 mg/dL
Stock	0 µL	2.5 µL	5.0 µL	7.5 µL	10 µL
0.1N NaOH	10 µL	7.5 µL	5.0 µL	2.5 µL	0 µL
Bilirubin-conj.	0 mg/dL	1.25 mg/dL	2.5 mg/dL	3.75 mg/dL	5 mg/dL
Stock	0 µL	2.5 µL	5.0 µL	7.5 µL	10 µL
H ₂ O	10 µL	7.5 µL	5.0 µL	2.5 µL	0 µL
Glucose	0 mg/dL	600 mg/dL	1200 mg/dL	1800 mg/dL	2400 mg/dL
Stock	0 µL	5 µL	10 µL	15 µL	20 µL
H ₂ O	20 µL	15 µL	10 µL	5 µL	0 µL
Triglycerides	0 mg/dL	500 mg/dL	1000 mg/dL	1500 mg/dL	2000 mg/dL
Stock	0 µL	5 µL	10 µL	15 µL	20 µL
	20 µL	15 µL	10 µL	5 µL	0 µL
Uric Acid	0 mg/dL	8.75 mg/dL	17.5 mg/dL	26.25 mg/dL	35 mg/dL
Stock	0 µL	2.5 µL	5.0 µL	7.5 µL	10 µL
H ₂ O	10 µL	7.5 µL	5.0 µL	2.5 µL	0 µL
Hemoglobin	0 mg/dL	50 mg/dL	100 mg/dL	150 mg/dL	200 mg/dL
Stock	0 µL	2.5 µL	5.0 µL	7.5 µL	10 µL
H ₂ O	10 µL	7.5 µL	5.0 µL	2.5 µL	0 µL

Results:

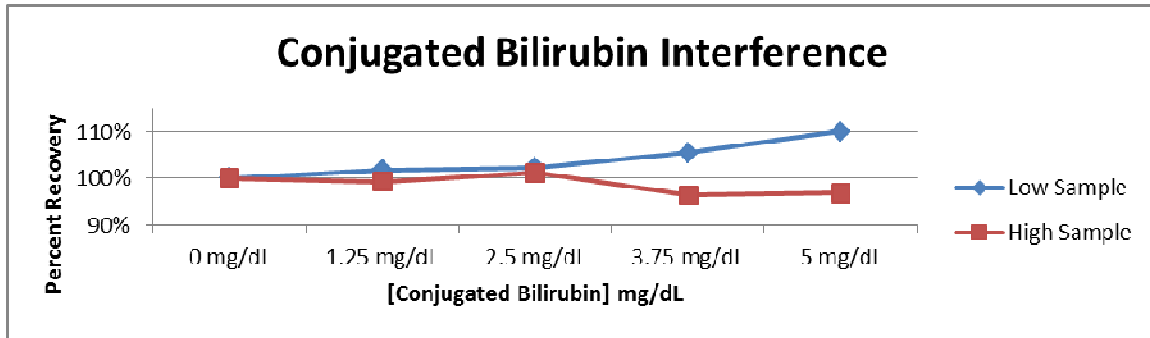
1. Ascorbic acid					
	0 mg/dL	10 mg/dL	20 mg/dL	30 mg/dL	50 mg/dL
Sample 1	219	199	200	206	218
212 µmol/L	206	197	200	210	205
	210	197	200	207	236
Mean	212	198	200	207	220
Recovery%	100%	93%	95%	98%	104%
Sample 2	527	501	474	452	435
524 µmol/L	517	432	469	469	431
	528	524	479	463	439
Mean	524	485.7	474	461.3	435
Recovery%	100%	93%	91%	88%	83%



2. Bilirubin					
	0 mg/dL	2.5 mg/dL	5.0 mg/dL	7.5 mg/dL	10 mg/dL
Sample 1	239	238	236	232	231
240 µmol/L	237	237	237	235	248
	242	241	240	230	234
mean	239	239	238	232	238
Recovery %	100%	100%	99%	97%	99%
Sample 2	554	550	540	526	524
550 µmol/L	565	556	539	530	512
	527	549	542	531	512
Mean	549	552	540	529	516
Recovery %	100%	100%	98%	96%	94%

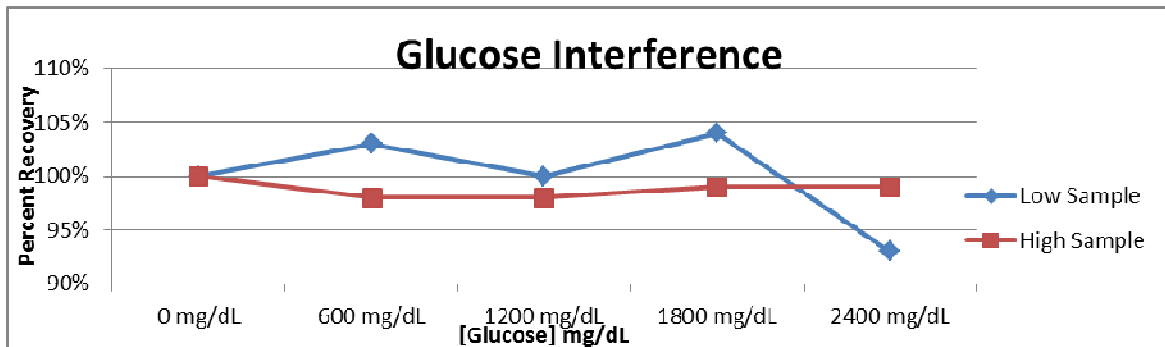


3. Bilirubin Conjugated					
	0 mg/dL	1.25 mg/dL	2.5 mg/dL	3.75 mg/dL	5 mg/dL
Sample 1	239	242	244	254	262
240 µmol/L	237	245	246	249	263
	242	243	244	253	264
Mean	239	243	245	252	263
Recovery%	100%	102%	102%	105%	110%
Sample 2	554	550	548	532	548
550 µmol/L	565	534	560	528	521
	527	549	555	525	524
Mean	549	544	554	528	531
Recovery%	100%	99%	101%	96%	97%



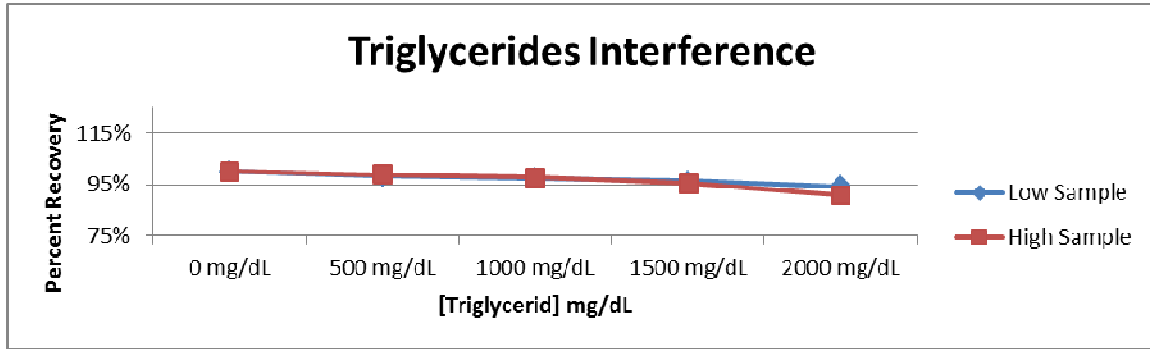
4. Glucose

	0 mg/dL	600 mg/dL	1200 mg/dL	1800 mg/dL	2400 mg/dL
Sample 1	219	208	215	222	194
212 µmol/L	206	232	202	207	211
	210	214	219	232	184
Mean	211.7	218	212	220.3	196.4
Recovery%	100%	103%	100%	104%	93%
Sample 2	527	486	486	522	526
524 µmol/L	517	542	530	534	544
	528	512	519	498	487
Mean	524	513.3	511.7	518	519
Recovery%	100%	98%	98%	99%	99%

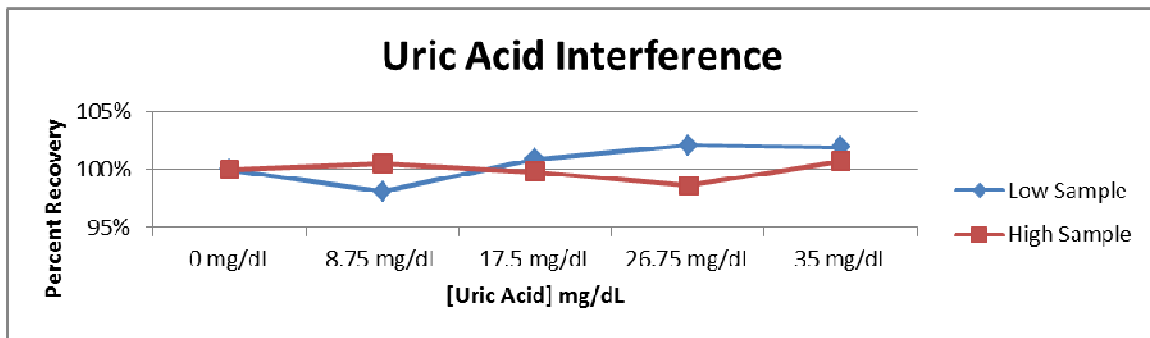


5. Triglycerides

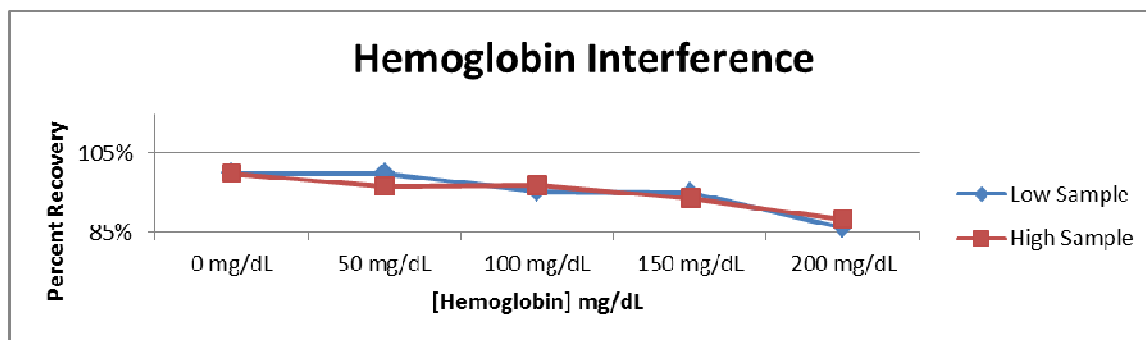
	0 mg/dL	500 mg/dL	1000 mg/dL	1500 mg/dL	2000 mg/dL
Sample 1	287	280	272	271	267
280 µmol/L	272	275	275	274	271
	290	278	280	273	262
Mean	283	278	276	273	267
Recovery%	100%	98%	97%	96%	94%
Sample 2	644	635	628	610	608
640 µmol/L	646	638	625	614	612
	643	634	634	618	598
Mean	644	636	629	614	584
Recovery%	100%	99%	98%	95%	91%



6. Uric Acid					
	0 mg/dL	8.75 mg/dL	17.5 mg/dL	26.75 mg/dL	35 mg/dL
Sample 1	187	195	196	204	191
190 µmol/L	190	190	191	193	193
	195	176	190	187	199
Mean	191	187	192	195	194
Recovery%	100%	98%	101%	102%	102%
Sample 2	482	477	482	480	499
490 µmol/L	488	513	510	485	496
	500	487	475	484	486
Mean	490	492	489	483	494
Recovery%	100%	101%	100%	99%	101%



7. Hemoglobin					
	0 mg/dL	50 mg/dL	100 mg/dL	150 mg/dL	200 mg/dL
Sample 1	186	195	186	187	170
219 µmol/L	196	189	179	184	160
	195	193	185	177	167
Mean	192	192	183	183	166
Recovery%	100%	100%	95%	95%	86%
Sample 2	523	503	492	482	444
550 µmol/L	517	488	498	486	454
	500	498	503	473	462
Mean	513	496	498	480	453
Recovery%	100%	97%	97%	94%	88%



Conclusion: The common serum interfering substances ascorbic acid, bilirubin, glucose, triglyceride, uric acid and hemoglobin showed no significant interference ($\leq 10\%$) up to the concentrations summarized below.

Interference	Concentration
Ascorbic Acid	20 mg/dL
Bilirubin	7.5 mg/dL
Bilirubin Conjugated	5 mg/dL
Triglyceride	2000 mg/dL
Glucose	2400 mg/dL
Uric Acid	35 mg/dL
Hemoglobin	100 mg/dL

6. Limit of Quantitation (LOQ) of the FRUCT “gold standard”

To calculate the LOQ of the Test Kit, five serum samples from commercial source were diluted with True blank to targeted concentrations and tested with the Test Kit reagent on (SOLO) SMART analyzers. Each diluted serum sample was tested on five separate runs with eight replicates per run. Evaluated Protocol software was used to estimate the LOQ.

Run	Replicate	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
1	1	54	71	99	222	256
	2	34	74	96	214	246
	3	33	99	97	221	233
	4	36	65	92	213	234
	5	40	69	96	237	245
	6	38	72	91	190	276
	7	44	68	102	219	276
	8	44	65	111	200	245
2	1	57	68	102	218	234
	2	36	59	97	202	220
	3	51	73	86	240	276
	4	32	66	97	224	245
	5	42	54	84	218	267
	6	36	63	85	211	260
	7	41	55	94	200	250
	8	55	67	91	211	235
3	1	46	74	109	206	267
	2	54	69	106	196	267
	3	55	78	99	195	232
	4	47	61	102	191	231
	5	58	69	95	208	250
	6	29	64	92	183	222
	7	35	67	90	208	250
	8	30	62	83	209	274
4	1	26	60	80	209	274
	2	23	68	84	195	271
	3	33	56	77	223	274
	4	23	64	92	195	245
	5	32	64	132	175	274
	6	19	71	116	190	250
	7	28	69	122	181	241
	8	34	60	78	194	240
5	1	37	81	92	187	231
	2	33	54	89	211	261
	3	41	57	82	198	254

	4	34	57	82	192	254
	5	33	88	107	185	268
	6	45	59	106	185	248
	7	64	88	93	170	258
	8	44	63	91	188	221
	Mean	39	67	95	203	251
	SD	11	10	12	16	17
	CV%	27%	14%	12%	8%	7%

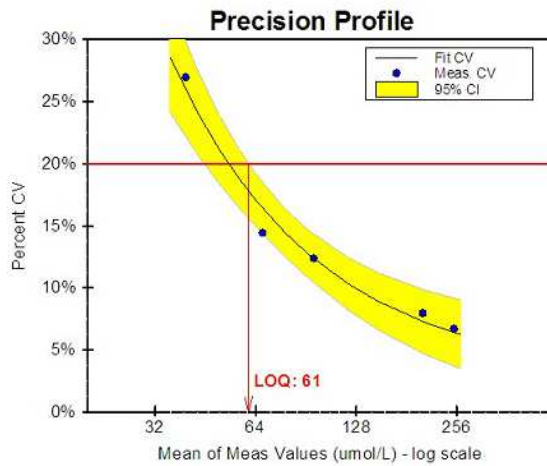
EP Evaluator

Research and Development – Diazyme Laboratories

GSP

Instrument: Smart

Sensitivity-Limit of Quantitation



Analytical Claim

GSP was analyzed by Smart to determine the LOQ (lowest concentration for which CV is less than a target of 20%).

Specimens with mean measured concentration ranging from 39.4 to 251.4 umol/L were assayed. A curve was fit to estimate the relationship between Mean and CV. Based on the fitted model, the LOQ is 61 umol/L. This is the point where the upper 95% confidence interval for the curve has a CV of 20%.

Sample	Target Conc.	N	Mean	SD	Meas. CV (%)	Fitted CV (%)	95% CI for Fitted	
							Low	High
sample1	40.0	40	39.4	10.6	27.0	26.0	22.2	29.8
Estimated LOQ		--	61	--	--	17.8	15.6	20.0
sample2	67.0	40	67.0	9.7	14.4	16.4	14.4	18.5
sample3	95.5	40	95.5	11.8	12.4	12.4	10.4	14.3
sample4	202.8	40	202.8	16.1	8.0	7.3	4.7	9.9
sample5	251.4	40	251.4	16.9	6.7	6.4	3.7	9.2

x: Excluded because either Mean or SD was zero.

Conclusion: The LOQ is 61 μmol/L based on data analysis.

7. Normal Values

As the Correlation under 2. COMPARISON STUDY fits perfectly to the ABX Pentra400 reference, the Normal Values have been set same as on the ABX Pentra400 for dog and cat samples.

Nevertheless each Laboratory should establish its own reference range.