

Evaluation Report

Eurolyser Lipase Test Kit (VT0190) on Solo Analyser

Location

Location: Eurolyser Diagnostica GmbH
Operator: Michael Gruber
Date: March - June 2014

Specimens

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

Equipment

- Eurolyser solo analyser: Bc14577, Bc14578, Bc14579, Bc14580, Bc14581
- Test kit LOT lipase VT0190: 0414-1



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1. Introduction and scope

Eurolyser solo uses the lipase enzymatic method DGGR, which is well known as a highly sensitive and specific method for detection of pancreatitis in animals.

It is a highly effective biomarker and has shown a >95% concordance by comparison with tissue and species specific quantitative immunoassay.
(*vet clin path* 41:E10-11.2012)

Further studies published:

Agreement of the Serum SpecfPL TM and 1,2-O-Dilauryl-RacGlycero-3-Glutaric Acid-(6' -Methylresorufin) Ester Lipase Assay for the Determination of Serum Lipase in Cats with Suspicion of Pancreatitis S. Oppliger, S. Hartnack, B. Riond, C.E.Reusch, and P.H. Kook

Agreement of Serum Spec cPL with the 1,2-o-Dilauryl-Rac-Glycero Glutaric Acid-(6' -methylresorufin) Ester (DGGR) Lipase Assay and with Pancreatic Ultrasonography in Dogs with Suspected Pancreatitis P.H. Kook, N. Kohler, S. Hartnack, B. Riond, and C.E. Reusch

Principle:

In the presence of colipase and bile acids lipase splits the synthetic substrate (1.2-o-dilauryl-rac-glycero-3-glutaric acid -(6-methylresorufin) ester) to glycerol and methylresorufin-ester, which is spontaneously degraded to glutaric acid and methylresorufin. The combination of colipase and bile acid makes the reaction specific for pancreatic lipase without interference of esterases and lipolytic enzymes.

The measured absorbance is proportional to the lipase activity in the sample.

2. Comparison Studies

The comparison study is based on the correlation between the results of the Eurolyser solo lipase assay and the ROCHE LIPC lipase colorimetric assay performed on a COBAS 6000 analyser.

The COBAS 6000 analyser was calibrated with a 2 point calibration against 0.9% NaCL and the CFAS calibrator. Its linearity range is from 3 - 300 U/L.

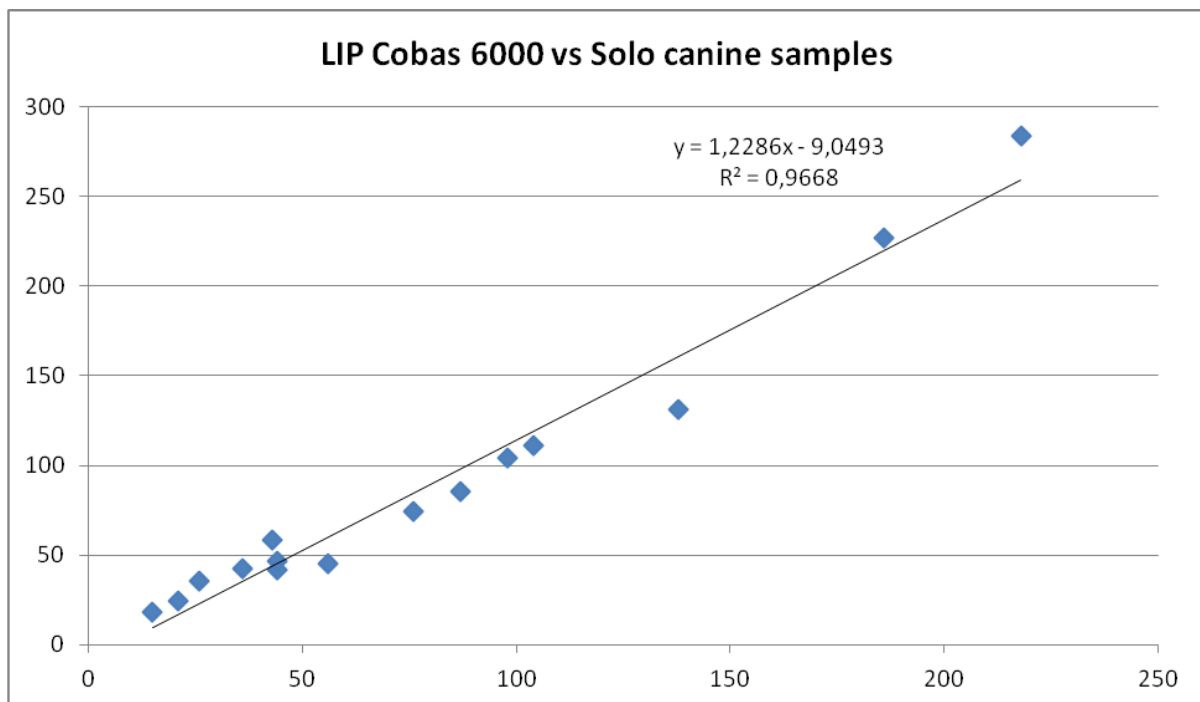
The solo lipase assay was calibrated against 2 levels of calibrators (88 and 40 U/L) and NaCL as the 0 point.

To set a higher calibration point the sample amount of the 88 U/L calibrator was doubled and so a 4 point calibration curve was established with a polynome to fit the curve.

20 dog and 20 cat patient samples (lithium-heparin plasma and serum), have been tested.

The acceptance criteria for this comparison study is a coefficient of determination $R^2 > 0.9$ obtained from linear regression between the Eurolyser solo lipase and COBAS 6000 lipase.

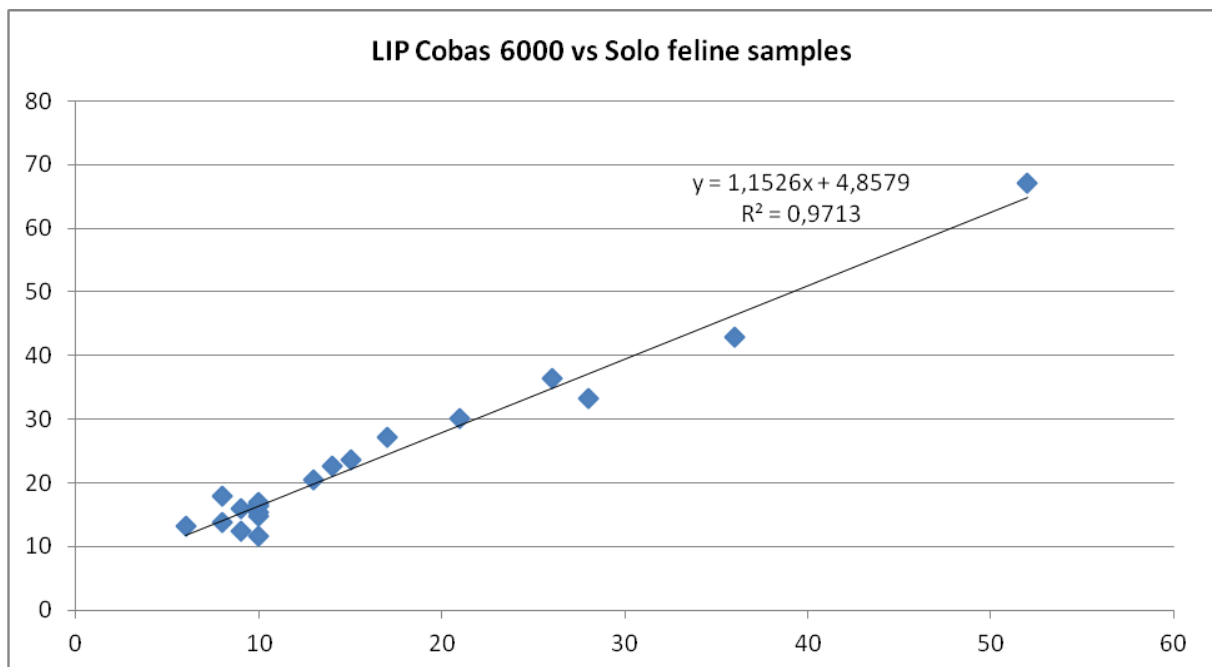
Normal range	Canine	0 - 125 U/L	0 - 125 U/L	Instrument
Dog	Material	LIP Cobas 6000	LIP Solo	
131402111	S	15	18.1	SOLO BC14577
491402131	S	21	24.5	SOLO BC14578
461402141	P	26	35.7	SOLO BC14579
951402101	P	44	41.5	SOLO BC14580
431402141	P	36	42.4	SOLO BC14581
64102071	P	56	45.6	SOLO BC14577
140218-1	S	44	46.4	SOLO BC14578
651402071	S	43	58.3	SOLO BC14579
691402131	S	76	74.2	SOLO BC14580
91402141	S	87	85.4	SOLO BC14581
411402071	P	98	104	SOLO BC14577
451402171	P	104	110.9	SOLO BC14578
371402121	S	138	131.2	SOLO BC14579
51402191	S	186	227	SOLO BC14580
251402041	S	218	283.6	SOLO BC14581
671402171	S	275	>300	SOLO BC14577
91402111	P	1168	>300	SOLO BC14578
401402041	P	257	>300	SOLO BC14579
581402131	P	289	>300	SOLO BC14580
381402111	P	>3027	>300	SOLO BC14581



Canine sample correlation:

The result for the correlation between Eurolyser lipase and COBAS LIPC is the linear regression function y (Solo LIPASE) = 1.2286 x (COBAS LIPC) – 9.0493 and a $R^2 = 0.9668$.

Normal range	Feline	0 - 30 U/L	0 - 35 U/L	Instrument
Cat	Material	LIP Cobas 6000	LIP Solo	
421402131	P	10	11.7	SOLO BC14577
391402031	P	9	12.4	SOLO BC14578
581402191	P	6	13.1	SOLO BC14579
741401311	P	8	13.7	SOLO BC14580
351402111	P	10	14.7	SOLO BC14581
71401221	P	10	15.3	SOLO BC14577
311402101	P	9	15.9	SOLO BC14578
981401171	P	10	16.4	SOLO BC14579
341402191	P	10	16.5	SOLO BC14580
461401241	p	10	17	SOLO BC14581
191402031	P	8	17.9	SOLO BC14577
201402121	p	13	20.5	SOLO BC14578
271402041	p	14	22.6	SOLO BC14579
261402121	p	15	23.6	SOLO BC14580
161402031	p	17	27.1	SOLO BC14581
1081401171	p	21	30.2	SOLO BC14577
521402181	p	28	33.3	SOLO BC14578
1111401211	P	26	36.4	SOLO BC14579
301402131	P	36	42.9	SOLO BC14580
231402061	p	52	67.1	SOLO BC14581



Feline sample correlation:

The result for the correlation between Eurolyser lipase and COBAS LIPC is the linear regression function y (Solo LIPASE) = 1.1526 x (COBAS LIPC) + 4.8579 and a $R^2 = 0.9713$.

3. Imprecision “within-run”

The imprecision “within-run” of Eurolyser lipase has been obtained through 20 measurements of 3 different levels.

NO	Cat (30 U/L)	Dog (138 U/L)	Dog (218 U/L)	Instrument
1	30 U/L	138 U/L	220 U/L	SOLO BC14577
2	28 U/L	141 U/L	230 U/L	SOLO BC14578
3	32 U/L	139 U/L	220 U/L	SOLO BC14579
4	29 U/L	142 U/L	221 U/L	SOLO BC14580
5	31 U/L	134 U/L	229 U/L	SOLO BC14581
6	27 U/L	138 U/L	228 U/L	SOLO BC14577
7	33 U/L	142 U/L	223 U/L	SOLO BC14578
8	30 U/L	141 U/L	206 U/L	SOLO BC14579
9	31 U/L	139 U/L	204 U/L	SOLO BC14580
10	33 U/L	138 U/L	220 U/L	SOLO BC14581
11	29 U/L	127 U/L	221 U/L	SOLO BC14577
12	27 U/L	135 U/L	218 U/L	SOLO BC14578
13	27 U/L	138 U/L	219 U/L	SOLO BC14579
14	33 U/L	128 U/L	220 U/L	SOLO BC14580
15	30 U/L	141 U/L	220 U/L	SOLO BC14581
16	28 U/L	133 U/L	221 U/L	SOLO BC14577
17	28 U/L	138 U/L	228 U/L	SOLO BC14578
18	29 U/L	137 U/L	225 U/L	SOLO BC14579
19	29 U/L	138 U/L	218 U/L	SOLO BC14580
20	34 U/L	138 U/L	217 U/L	SOLO BC14581
mean	30 U/L	137 U/L	220 U/L	
std dev	2.20	4.12	6.54	
CV	7.35%	3.00%	2.97%	

As degree of the imprecision „within-run“, the percentage of the coefficient of variation is:

7.35 % at 30 U/L
 3.00 % at 137 U/L
 2.97 % at 220 U/L

4. Imprecision “day-to-day” / Reproducibility

The imprecision “day-to-day” of Eurolyser LIPASE has been obtained through the measurement of 2 different controls on 5 consecutive days. The following results can be used in order to evaluate the reproducibility.

Summary	05.05.2014	to	10.05.2014	Instrument
Level	Control low 46.4		Control high 88.2	
Unit	U/L		U/L	
Day 1 - 1/3	45		82	SOLO BC14580
Day 1 - 2/3	41		91	SOLO BC14580
Day 1 - 3/3	48		88	SOLO BC14580
Day 2 - 1/3	49		86	SOLO BC14580
Day 2 - 2/3	44		83	SOLO BC14580
Day 2 - 3/3	46		88	SOLO BC14580
Day 3 - 1/3	43		91	SOLO BC14580
Day 3 - 2/3	46		92	SOLO BC14580
Day 3 - 3/3	44		94	SOLO BC14580
Day 4 - 1/3	53		83	SOLO BC14580
Day 4 - 2/3	48		81	SOLO BC14580
Day 4 - 3/3	46		88	SOLO BC14580
Day 5 - 1/3	52		88	SOLO BC14580
Day 5 - 2/3	43		86	SOLO BC14580
Day 5 - 3/3	43		89	SOLO BC14580
Mean	46		87	
std dev	3.4		3.8	
%CV	7.4%		4.4%	

The results for the imprecision “day-to-day” of Eurolyser lipase are as follows at three different levels:

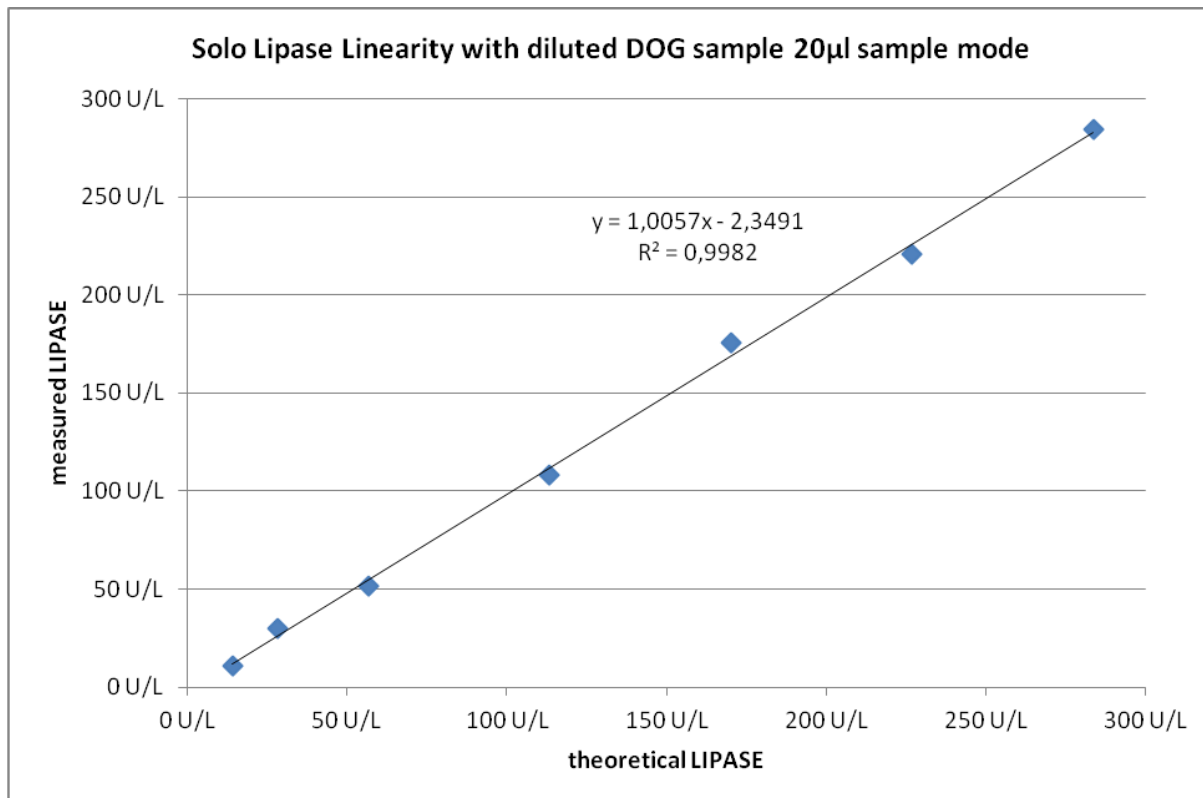
Low	(mean: 46 U/L; n = 15):	CV = 7.4 %
High	(mean: 87 U/L; n = 15):	CV = 4.4 %

5. Linearity Study

A sample near the upper linearity range was used to obtain linearity data. The acceptance criteria is a linear regression with a coefficient of determination $R^2 > 0.9$ and the Recovery of **90 - 110 %** down to a dilution of 10 % between expected and recovered (obtained) values.

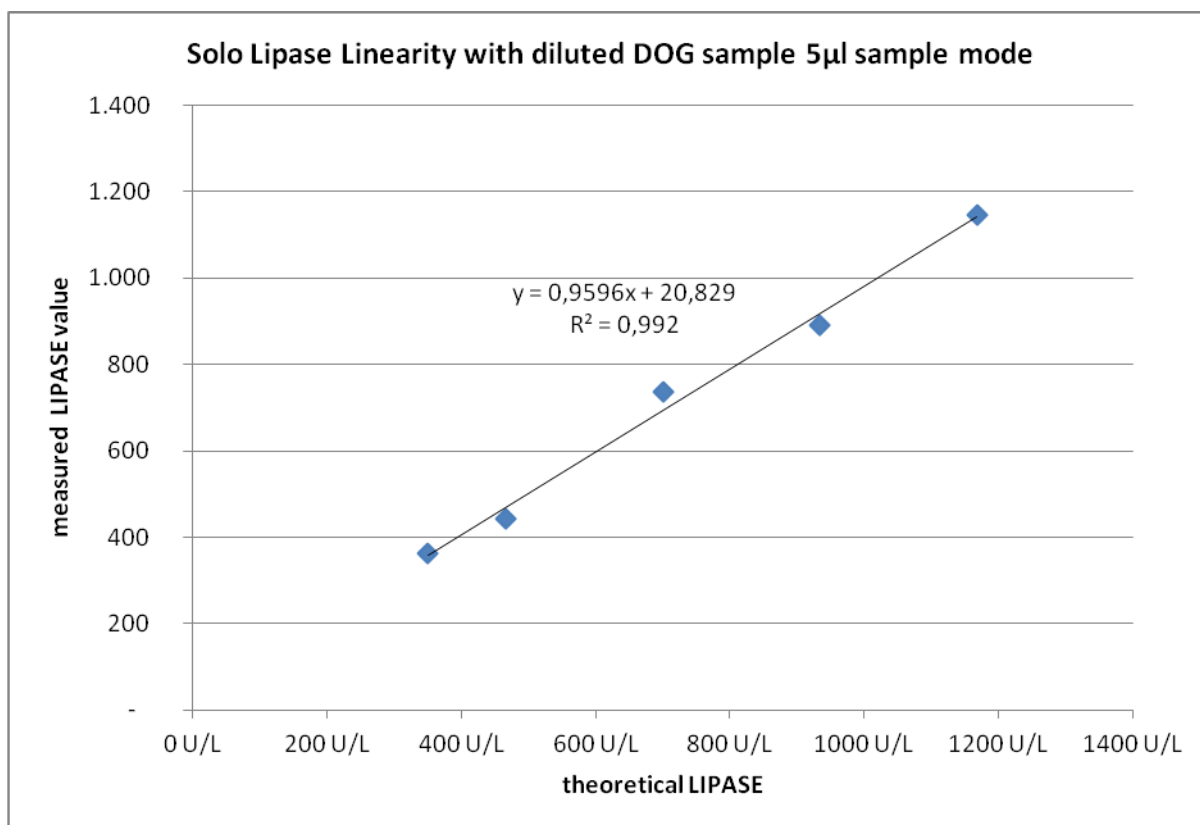
The study was made for the routine Mode (20 μ l) as well as for the dilution mode (5 μ l)

Target 90-110% above 28 U/L	Routine mode with 20 μ l sample volume							
251402041	Sample 13	SOLO BC14577	SOLO BC14578	SOLO BC14579	U/L			
Concentration (diluted with NaCl)	Theoretical	meas 1	meas 2	meas 3	mean	Std deviation	cv	Recovery
100 %	284 U/L	278	291	284	284	6.51	2.29%	100%
80 %	227 U/L	212	233	218	221	10.82	4.89%	97%
60 %	170 U/L	168	177	182	176	7.09	4.04%	103%
40 %	113 U/L	109	111	104	108	3.61	3.34%	95%
20 %	57 U/L	55	49	51	52	3.06	5.91%	91%
10 %	28 U/L	31	27	33	30	3.06	10.07%	107%
5 %	14 U/L	12	9	12	11	1.73	15.75%	78%



The result for the correlation between theoretical and recovered (measured) values for Eurolyser lipase (20 μ l mode) is the linear regression function y (measured) = 1.0057 x (theoretical) – 2.3491 and an $R^2 = 0.9982$.

Target 90 - 110% above 350 U/L	Dilution Mode with 5µl Sample Volume							
91402111	Sample 13	SOLO BC14577	SOLO BC14578	SOLO BC14579				
Concentration (diluted with NaCL)	Theoretical	meas 1	meas 2	meas 3	mean	std dev	cv	Recovery
100 %	1168 U/L	1200	1150	1090	1.147	55.08	4.80%	98%
80 %	934 U/L	848	933	888	890	42.52	4.78%	95%
60 %	701 U/L	777	733	699	736	39.11	5.31%	105%
40 %	467 U/L	410	445	477	444	33.51	7.55%	95%
30 %	350 U/L	310	388	388	362	45.03	12.44%	103%
10 %	117 U/L	<300	<300	<300	<300			
5 %	58 U/L	<300	<300	<300	<300			



The result for the correlation between theoretical and recovered (measured) values for Eurolyser lipase (5µl dilution mode) is the linear regression function y (measured) = 0.9596 x (theoretical) + 20.829 and an $R^2 = 0.9596$.

6. Interferences of the Eurolyser LIPASE Assay

A canine lipase sample of about 110.9 U/L (sample no. 451402171) was spiked with 500 mg/dl haemoglobin, 1000mg/dl triglycerides and 60mg/dl conjugated bilirubin. The samples were compared to control samples containing saline and water instead of the spike material. The spiked sample did not demonstrate a significant interference – defined as more than 10 % difference between test sample and control sample.

Sample No 451402171 (27mg/l)	Control sample	Test sample	Recovery	Instrument
Bilirubin (60mg/dl)	110	99	90 %	SOLO BC14579
Triglycerides (1000 mg/dl)	108	98	90 %	SOLO BC14579
Haemoglobin (500mg/dl)	112	123	109%	SOLO BC14580

7. Limit of Quantitation (LOQ) of the Eurolyser Lipase-VET

LOQ (Limit of quantitation) is determined as the lowest sample run that displayed **CV% < 20%**

SEQ	24.5 U/L sample 491402131	Sample 491402131 diluted to 50% with NAACL	Sample 36 diluted to 33.3%	
1	24 U/L	12 U/L	9 U/L	SOLO BC14577
2	28 U/L	11 U/L	7 U/L	SOLO BC14578
3	28 U/L	14 U/L	7 U/L	SOLO BC14579
4	22 U/L	13 U/L	8 U/L	SOLO BC14580
5	27 U/L	12 U/L	9 U/L	SOLO BC14581
6	26 U/L	9 U/L	8 U/L	SOLO BC14579
7	23 U/L	11 U/L	7 U/L	SOLO BC14579
8	25 U/L	8 U/L	7 U/L	SOLO BC14579
9	25 U/L	13 U/L	11 U/L	SOLO BC14579
10	27 U/L	12 U/L	7 U/L	SOLO BC14577
11	24 U/L	11 U/L	9 U/L	SOLO BC14578
12	26 U/L	8 U/L	8 U/L	SOLO BC14579
13	27 U/L	9 U/L	6 U/L	SOLO BC14580
14	26 U/L	11 U/L	6 U/L	SOLO BC14581
15	23 U/L	10 U/L	7 U/L	SOLO BC14582
16	23 U/L	11 U/L	6 U/L	SOLO BC14583
17	24 U/L	12 U/L	8 U/L	SOLO BC14584
18	23 U/L	12 U/L	8 U/L	SOLO BC14585
19	27 U/L	11 U/L	8 U/L	SOLO BC14586
20	28 U/L	13 U/L	4 U/L	SOLO BC14587
mean	25 U/L	11 U/L	8 U/L	
std deviation	1.95	1.66	1.47	
cv	7.70%	14.92%	19.59%	

The LOQ is set to 8 U/L

8. Normal Values

As the correlation (see **2. Comparison Study**) fits perfectly to the COBAS 6000 reference, the normal values have been set same as on the COBAS 6000 for dog samples.

On cat samples the normal values have been set according to the slope and offset of the correlation with COBAS 6000.

The normal range for dogs is set as: 0-125 U/L

The normal range for cats is set as: 0-35 U/L

Nevertheless, it is recommended that each laboratory establishes its own reference range.