

Evaluation Report

Eurolyser GLDH test kit (VT0200_VT0201) on solo Analyser

Location

Location: Eurolyser Diagnostica GmbH
Operators: Simone Wieser, Michael Gruber
Date: October 2015 – February 2016

Specimens

The specimens used for analysis were taken from multiple sites and were frozen horse, dog and cat lithium-heparin plasma samples.

Equipment

- Eurolyser solo Analyser: Ae5052, Ae5053, Ae5054, Ae3611
 - Test kit LOT GLDH VT020: 1601-1
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1. Introduction and scope

Glutamate dehydrogenase (GLDH) is a liver specific enzyme which is located in mitochondria of centrolobular hepatocytes. Due to this localization, GLDH dominantly increases in hypoxic states of the liver as well as in pronounced necrosis of hepatocytes. Increased release of GLDH from hepatocytes can further be observed in i.e. inflammatory, neoplastic or infectious liver pathology as well as in all diseases leading to liver cell degeneration.

When GLDH was established in veterinary diagnostics, the DGKC method was performed at a temperature of 25 °C.

Nowadays enzyme measurements – including the Eurolyser solo – are standardised to 37 °C and therefore the clinical reference values from the 1980s are no longer valid, although those reference ranges are still used in many laboratories worldwide.

Eurolyser therefore strongly recommends that each laboratory should establish its own reference ranges!

The reference ranges used for the Eurolyser test kits are based on data from established veterinary universities, using the same DGKC method and having established the reference ranges based on 37 °C measurement temperature.

Principle:

$2\text{-oxoglutarate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{GLDH}} \text{L-glutamate} + \text{NAD}^+ + \text{H}_2\text{O}$

Glutamate dehydrogenase catalyses the reduction of oxoglutaric acid while simultaneously NADH oxidatises to NAD.

The reaction speed of the absorption-increase is directly proportional to the GLDH activity, measured on the SOLO system at 340 nm.

2. Comparison studies

The comparison study is based on the correlation between the results of the Eurolyser SOLO GLDH assay and the ROCHE GLDH opt DGKC assay performed on a COBAS 701 Analyzer.

The COBAS 701 Analyzer was calibrated with a 2 point calibration against 0.9% NaCL and the CFAS Calibrator.

The SOLO GLDH assay was calibrated against 3 levels of calibrators and NaCL as the 0 point.

The linearity range is from 6 - 120 U/L

The calibration curve was established with a linear regression to fit the curve.

9 dog, 20 cat and 8 horse samples (lithium-heparin plasma), have been tested.

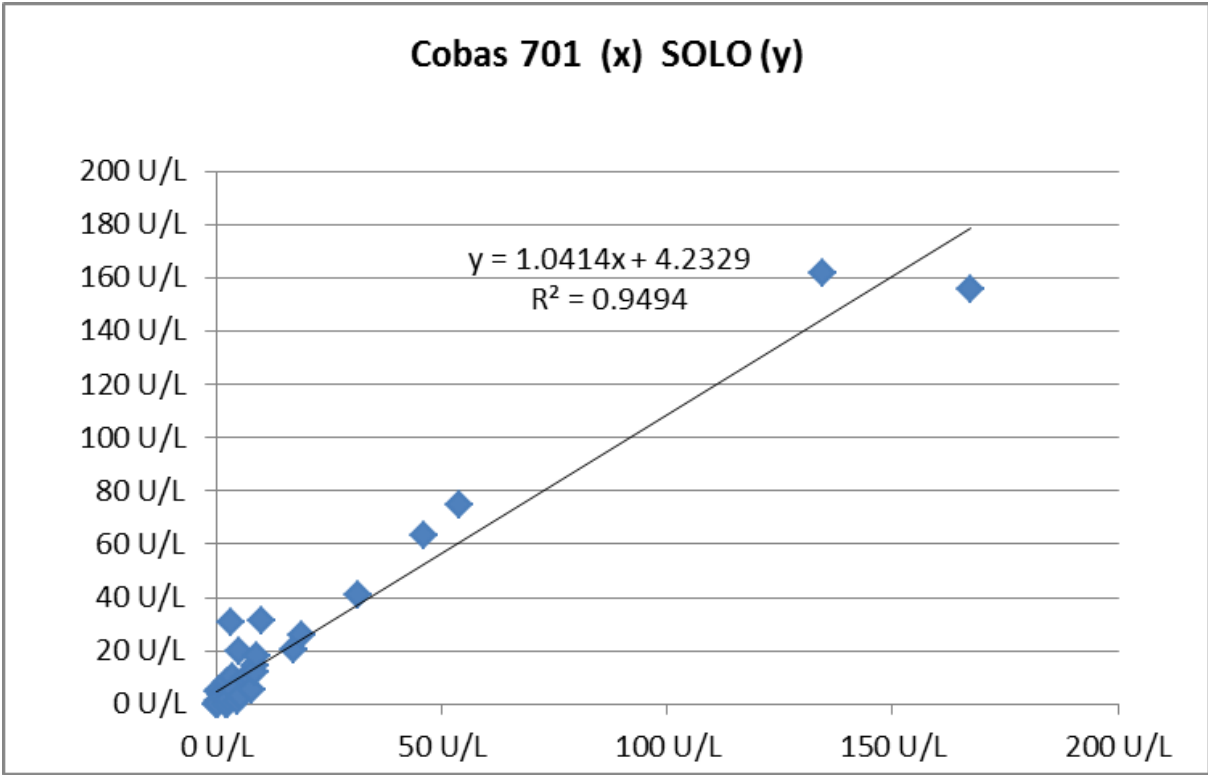
The acceptance criterion for this comparison study is a coefficient of determination $R^2 > 0.92$ obtained from the linear regression function between the Eurolyser GLDH and COBAS 701 GLDH.

Correlation factors: SLOPE 0.9 – 1.1 and OFFSET +/- 5.

Sample ID	Species	GLDH U/L COBAS 701	GLDH U/L SOLO	Comment
1601-S-18179	Cat	0.10 U/L	0.00 U/L	
1601-S-18180	Dog	0.10 U/L	0.00 U/L	
1601-S-07950	Cat	0.40 U/L	0.89 U/L	
1601-S-06759	Cat	0.50 U/L	4.59 U/L	
1601-S-09303	Cat	0.60 U/L	0.00 U/L	strongly haemolytic
1601-S-08613	Cat	0.70 U/L	5.30 U/L	
1601-S-09769	Cat	1.20 U/L	3.31 U/L	
1601-S-04437	Horse	1.40 U/L	2.74 U/L	
1601-S-09625	Horse	1.60 U/L	2.46 U/L	
1601-S-09774	Horse	2.10 U/L	7.58 U/L	
1601-S-18732	Dog	2.10 U/L	0.00 U/L	
1601-S-13501	Dog	2.30 U/L	4.45 U/L	
1601-S-09771	Horse	2.50 U/L	1.32 U/L	
1601-S-16751	Dog	2.50 U/L	0.00 U/L	
1601-S-18962	Cat	2.50 U/L	5.30 U/L	
1601-S-09992	Horse	2.60 U/L	8.30 U/L	
1601-S-09776	Horse	3.00 U/L	1.60 U/L	
1601-S-05523	Dog	3.30 U/L	30.38 U/L	strongly lipemic

Sample ID	Species	GLDH U/L COBAS 701	GLDH U/L SOLO	Comment
	Dog	3.50 U/L	10.29 U/L	
1601-S-08720	Cat	4.00 U/L	5.16 U/L	
1601-S-03959	Cat	4.90 U/L	1.17 U/L	
1601-S-10683	Cat	5.20 U/L	19.84 U/L	haemolytic and lipemic
1601-S-05481	Cat	5.60 U/L	2.60 U/L	
1601-S-04523	Cat	6.40 U/L	9.29 U/L	strongly hemolytic
1601-S-02401	Dog	7.10 U/L	10.72 U/L	
1601-S-18560	Horse	7.90 U/L	5.30 U/L	
1601-S-04764	Dog	8.60 U/L	14.14 U/L	
1601-S-08721	Cat	8.70 U/L	12.14 U/L	
1601-S-02871	Cat	9.10 U/L	18.13 U/L	strongly hemolytic
1601-S-15125	Cat	10.20 U/L	31.52 U/L	haemolytic and lipemic
1601-S-02599	Cat	17.30 U/L	20.55 U/L	
1601-S-16717	Dog	19.10 U/L	25.54 U/L	
1601-S-09927	Cat	31.60 U/L	41.21 U/L	
1601-S-04224	Cat	46.00 U/L	63.57 U/L	
1601-S-13740	Cat	54.00 U/L	74.55 U/L	
1601-S-00301	Cat	134.40 U/L	161.74 U/L	
1601-P-00239	Horse	167.50 U/L	155.61 U/L	

GLDH SAMPLE correlation:



The result for the correlation between Eurolyser GLDH and COBAS GLDH is the linear regression function y (Solo GLDH) = $1.0414 x$ (COBAS GLDH) + 4.2329 and a $R^2 = 0.9494$.

3. Imprecision “within-run”

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

NO	CONTROL N (13 U/L)	CONTROL P (24.3 U/L)	SAMPLE HIGH (74.5 U/L)	Analyzer
1	13.4 U/L	26.8 U/L	74.5 U/L	SOLO Ae5052
2	10.9 U/L	23.0 U/L	79.8 U/L	SOLO Ae5053
3	14.9 U/L	24.2 U/L	76.3 U/L	SOLO Ae5054
4	12.0 U/L	26.0 U/L	75.3 U/L	SOLO Ae3611
5	11.0 U/L	23.3 U/L	81.1 U/L	SOLO Ae5052
6	13.5 U/L	21.9 U/L	83.3 U/L	SOLO Ae5053
7	13.8 U/L	27.8 U/L	72.1 U/L	SOLO Ae5054
8	12.9 U/L	25.5 U/L	70.5 U/L	SOLO Ae3611
9	10.9 U/L	24.9 U/L	74.5 U/L	SOLO Ae5052
10	13.2 U/L	23.6 U/L	78.8 U/L	SOLO Ae5053
11	14.1 U/L	26.5 U/L	sample size limited	SOLO Ae5054
12	15.2 U/L	21.9 U/L	sample size limited	SOLO Ae3611
13	12.6 U/L	20.8 U/L	sample size limited	SOLO Ae5052
14	13.8 U/L	24.3 U/L	sample size limited	SOLO Ae5053
15	12.9 U/L	25.5 U/L	sample size limited	SOLO Ae5054
16	11.5 U/L	26.6 U/L	sample size limited	SOLO Ae3611
17	11.2 U/L	24.5 U/L	sample size limited	SOLO Ae5052
18	14.8 U/L	24.1 U/L	sample size limited	SOLO Ae5053
19	14.9 U/L	24.9 U/L	sample size limited	SOLO Ae5054
20	15.5 U/L	21.1 U/L	sample size limited	SOLO Ae3611
mean	13.1 U/L	24.4 U/L	76.6 U/L	
Stabwn	1.51	1.95	4.06	
CV	11.51%	8.02%	5.30%	

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

11.51 % at 13.1 U/L

8.02 % at 24.4 U/L

5.30 % at 76.6 U/L

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

The imprecision “day-to-day” of Eurolyser GLDH has been obtained through the measurement of two different controls on five consecutive days.

The following results can be used in order to evaluate the reproducibility.

Summary	18.01.2016	to	23.01.2016	Instrument
Level	Control low 13 U/L		Control high 24.3	
Unit	U/L		U/L	
Day 1-1/3	12.0		24.8	SOLO Ae5052
Day 1-2/3	15.2		20.9	SOLO Ae5053
Day 1-3/3	13.0		21.8	SOLO Ae5054
Day 2-1/3	11.2		22.3	SOLO Ae3611
Day 2-2/3	12.5		24.9	SOLO Ae5052
Day 2-3/3	14.5		20.8	SOLO Ae5053
Day 3-1/3	14.3		23.7	SOLO Ae5054
Day 3-2/3	12.7		22.1	SOLO Ae3611
Day 3-3/3	11.9		19.8	SOLO Ae5052
Day 4-1/3	14.4		18.9	SOLO Ae5053
Day 4-2/3	12.2		21.1	SOLO Ae5054
Day 4-3/3	12.0		24.5	SOLO Ae5052
Day 5-1/3	15.5		23.6	SOLO Ae5053
Day 5-2/3	15.6		22.9	SOLO Ae5054
Day 5-3/3	11.0		22.3	SOLO Ae3611
mean	13.2		22.3	
std	1.6		1.8	
%CV	11.9%		8.1%	

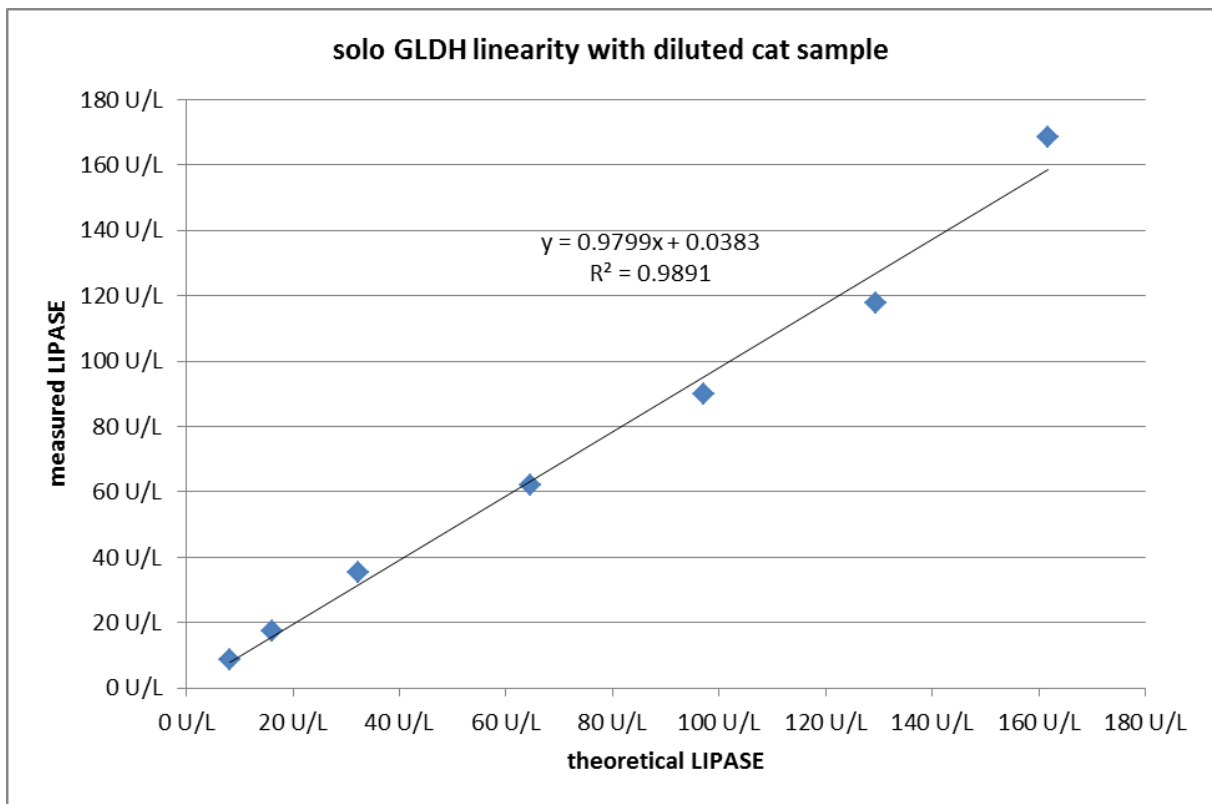
The results for the imprecision “day-to-day” of Eurolyser GLDH are as follows at two different levels:

Low	(mean: 13.2 U/L; n = 15):	CV = 11.9 %
High	(mean: 22.3 U/L; n = 15):	CV = 8.1 %

5. Linearity study

A sample near the upper linearity range was used to obtain linearity data. The acceptance criterion is a linear regression with a coefficient of determination $R^2 > 0.9$ and the recovery of **90 - 110 %** down to a dilution of 5 % between expected and recovered (obtained) values.

Target 90-110% above 8 U/L									
1601-S-00301	Sample 25	Ae 5052	Ae 5053	Ae 5054					
Dilution	NaCL	Theoretical	meas 1	meas 2	meas 3	mean	stabwn	cv	Recovery
0.9%									
100		161.7 U/L	159.0 U/L	170.7 U/L	175.4 U/L	168 U/L	8.45	5.02%	104%
80		129.4 U/L	119.0 U/L	111.0 U/L	123.8 U/L	118 U/L	6.47	5.48%	91%
60		97.0 U/L	94.3 U/L	90.2 U/L	85.3 U/L	90 U/L	4.51	5.01%	93%
40		64.7 U/L	58.2 U/L	61.3 U/L	67.2 U/L	62 U/L	4.57	7.35%	96%
20		32.3 U/L	32.0 U/L	36.3 U/L	37.5 U/L	35 U/L	2.89	8.20%	109%
10		16.2 U/L	15.2 U/L	17.6 U/L	19.0 U/L	17 U/L	1.92	11.13%	107%
5		8.1 U/L	7.5 U/L	10.0 U/L	8.0 U/L	9 U/L	1.32	15.56%	105%



The result for the correlation between theoretical and recovered (measured) values for Eurolyser GLDH is the linear regression function:

$$y \text{ (measured)} = 0.9799 x \text{ (theoretical)} + 0.0383$$

$$R^2 = 0.989$$

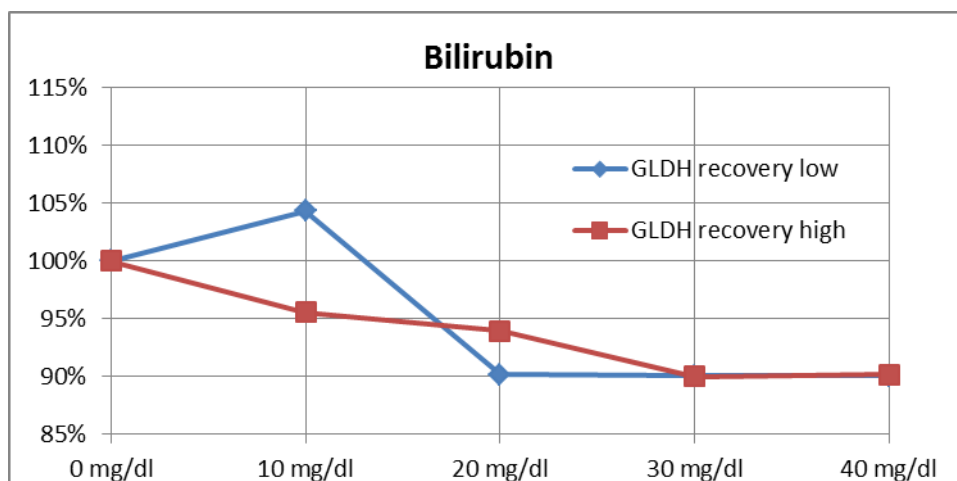
6. Interferences of the Eurolyser GLDH assay

GLDH samples of about 63 U/L and 20.55 U/L were spiked with 1000 mg/dl haemoglobin, 400mg/dl triglycerides, 40mg/dl bilirubin and 10mM ascorbic acid. The samples were compared to control samples containing saline and water instead of the spike material.

The spiked samples showed the following interferences:

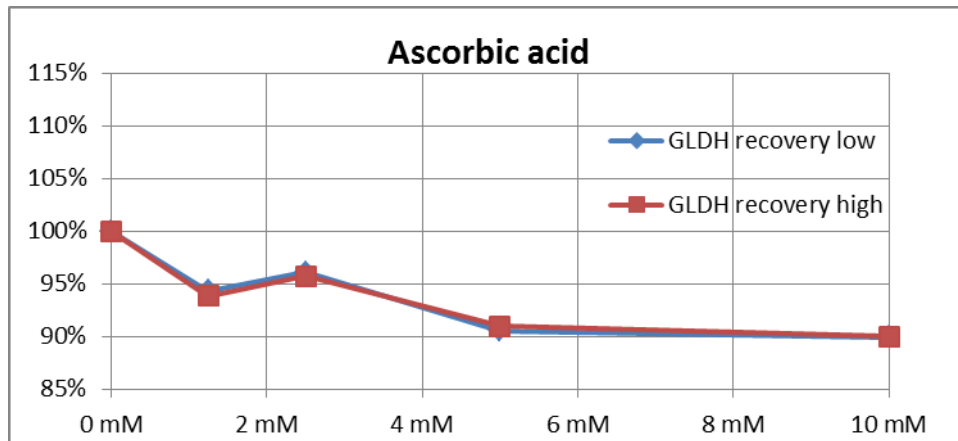
BILIRUBIN:

Bilirubin concentration	GLDH recovery low	GLDH recovery high
0 mg/dl	100%	100%
10 mg/dl	104,3%	95,6%
20 mg/dl	90,2%	94,0%
30 mg/dl	90,0%	90,0%
40 mg/dl	90,0%	90,2%



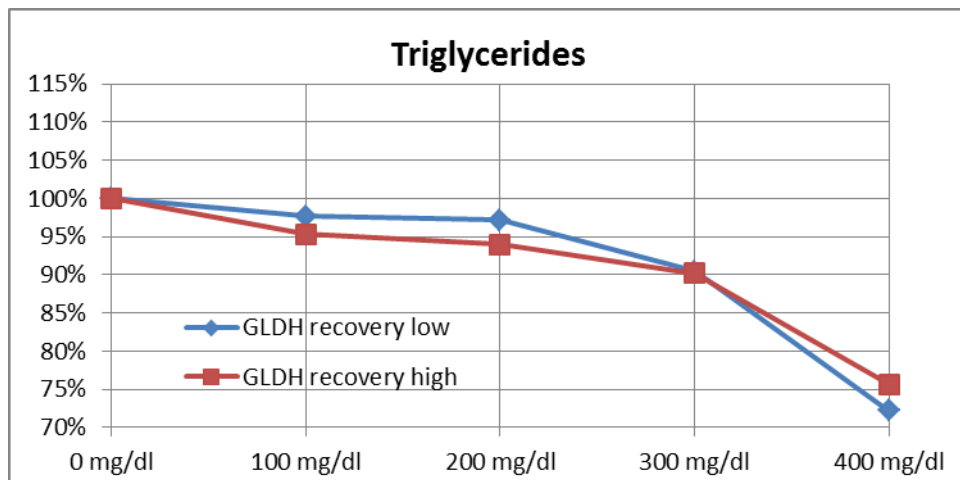
ASCORBIC ACID:

Ascorbic acid	GLDH recovery low	GLDH recovery high
0.0 mM	100%	100%
1.3 mM	94.3%	93.8%
2.5 mM	96.1%	95.7%
5.0 mM	90.5%	91.0%
10.0 mM	90.0%	90.0%



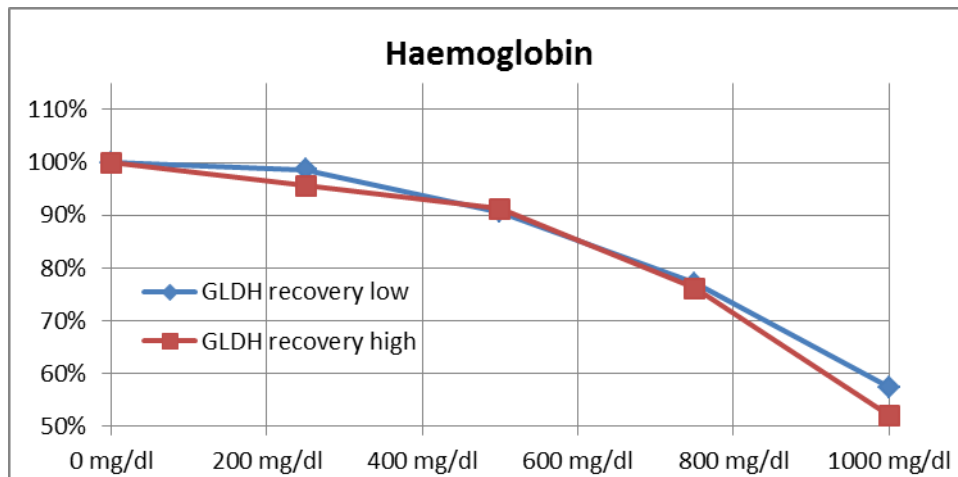
TRIGLYCERIDES:

Triglycerides concentration	GLDH recovery low	GLDH recovery high
0 mg/dl	100%	100%
100 mg/dl	97.7%	95.3%
200 mg/dl	97.2%	94.0%
300 mg/dl	90.5%	90.2%
400 mg/dl	72.2%	75.7%



HAEMOGLOBIN:

HGB concentration	GLDH recovery low	GLDH recovery high
0 mg/dl	100%	100%
250 mg/dl	98.6%	95.6%
500 mg/dl	90.7%	91.1%
750 mg/dl	77.1%	76.1%
1000 mg/dl	57.5%	52.2%



SUMMARY:

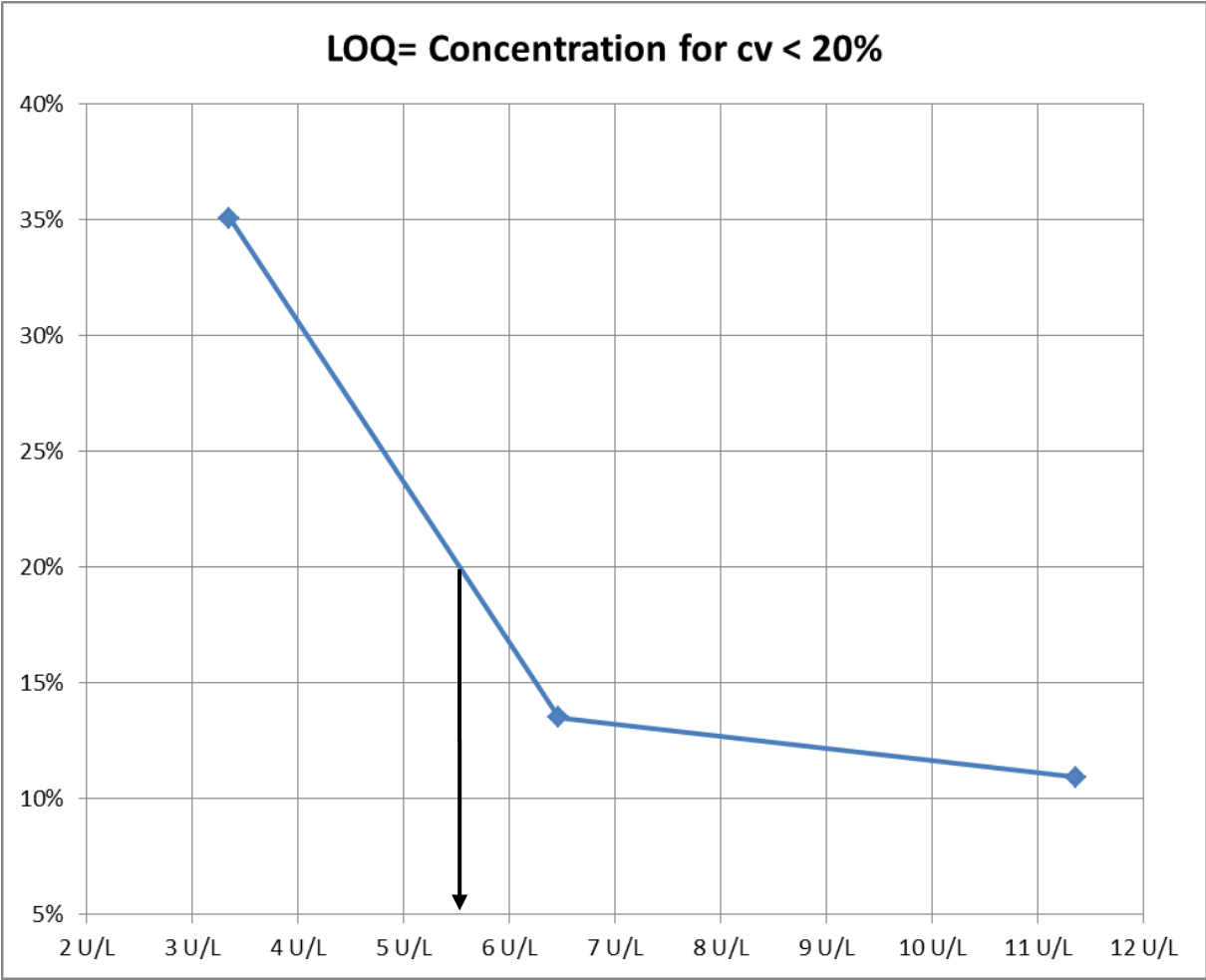
The interferences are as follows:

Bilirubin: No interference up to 40 mg/dl
Ascorbic acid: No interference up to 10 mM
Triglycerides: No interference up to 300 mg/dl (**avoid lipemic samples!**)
Haemoglobin: No interference up to 500 mg/dl (**avoid hemolytic samples!**)

7. Limit of quantitation (LOQ) of the Eurolyser GLDH assay

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

LOQ-determination for activity @ cv< 20%			
Date	15.01.2016		
Testkit:	GLDH		
Lot:	1601		
Exp:	Jan. 2017		
Wavelength	340		
Sample volume:	100		
Target:	13.00U/L	6.50U/L	3.25U/L
1	10.06U/L	7.22U/L	3.07U/L
2	11.45U/L	5.91U/L	3.01U/L
3	10.26U/L	6.73U/L	3.19U/L
4	11.09U/L	7.41U/L	4.17U/L
5	12.90U/L	5.26U/L	3.24U/L
6	13.02U/L	6.61U/L	3.82U/L
7	10.91U/L	6.98U/L	2.17U/L
8	13.98U/L	5.80U/L	6.26U/L
9	10.77U/L	6.40U/L	4.00U/L
10	11.33U/L	4.87U/L	3.08U/L
11	12.09U/L	8.51U/L	1.19U/L
12	11.35U/L	5.43U/L	2.67U/L
13	9.88U/L	6.02U/L	4.73U/L
14	10.09U/L	6.45U/L	1.44U/L
15	11.22U/L	5.96U/L	3.09U/L
16	9.30U/L	6.92U/L	4.43U/L
17	12.40U/L	6.89U/L	3.30U/L
18	12.30U/L	7.01U/L	3.34U/L
mw	11.36U/L	6.47U/L	3.34U/L
std	1.24U/L	0.87U/L	1.17U/L
cv	10.9%	13.5%	35.1%



The LOQ is set to 6 U/L

8. Normal values

The normal values have been set (recommended) based on reference labs using DGKC GLDH methods:

Cat	< 10 U/L
Cattle	< 15 U/L
Dog	< 13 U/L
Guinea pig	< 17 U/L
Horse	< 13 U/L
Pig	< 7 U/L
Rabbit	< 19 U/L

Each laboratory should establish its own reference range!