Evaluation Report

Eurolyser Phenobarbital test kit (VT0211) on solo Analyser

Location

Location: Eurolyser Diagnostica GmbH
Operator: Bettina Schönauer
Date: February 2016 - March 2016

Specimens

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

Equipment

- Eurolyser solo Analyser: Ae5052, Ae5053, Ae5054, Ae3611
- Testkit LOT Pheno VT0211: 1602-1

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1. Introduction and Scope

Phenobarbital is administered to control seizures and epilepsy in dogs and cats. As a barbiturate it loosens up cramps. Depending on the dosage a sedative or hypnotic action of the drug can be observed. In cats phenobarbital is the first choice as antiepileptic treatment. In dogs it may be administered together with potassium-bromide, depending on individual reaction of the patient to the treatment. Blood phenobarbital concentration is recommended to be checked approximately 12-14 days after administration. Best sampling time is approximately two hours prior to the next scheduled drug treatment.

In the literature different therapeutical ranges are found.
Therefore each Laboratory has to establish its own reference ranges.
Section 8 of this evaluation recommends the therapeutical range in combination with the Eurolyser Phenobarbital assay.

Principle:
Quantitative turbidimetric test for the measurement of phenobarbital in serum and plasma.
Latex particles coated with phenobarbital are agglutinated when mixed with phenobarbital antibody solution. When a sample containing phenobarbital is used, the agglutination reaction is partially inhibited, slowing the process of agglutination. The agglutination causes an absorbance change (546nm), inversely dependent upon the phenobarbital contents of the patient sample.

2. Comparison Studies

The comparison study is based on the correlation between the results of the Eurolyser SOLO Phenobarbital assay and the Immulite 2000 Phenobarbital assay.

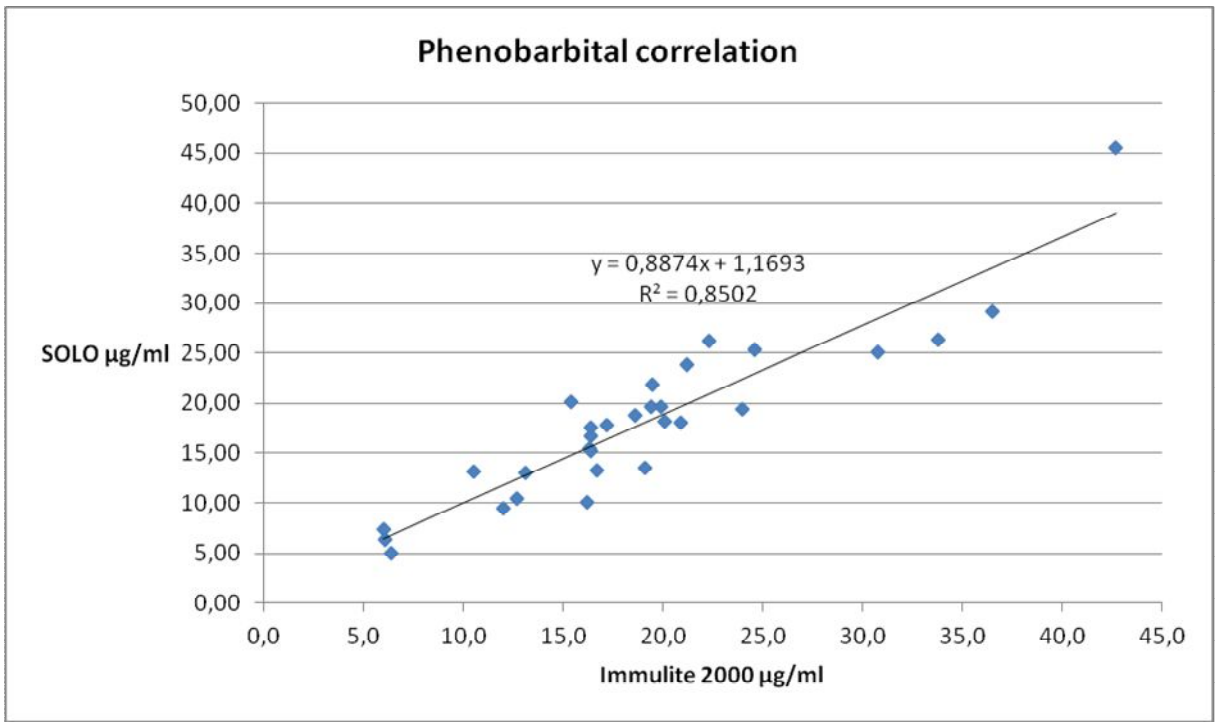
The Immulite 2000 Analyzer was calibrated with a multipoint calibration kit.
The SOLO Phenobarbital assay was calibrated against 6 levels of calibrators, including a 0 point calibrator. The linearity range is from 10 - 100 µg/ml. The calibration curve was established with a linear regression to fit the curve.

24 dog & 6 cat samples (lithium-heparin plasma as well as serum) have been tested.

The acceptance criterion for this comparison study is a coefficient of determination **$R^2 > 0.85$** obtained from the linear regression function between the Eurolyser Phenob. and Immulite.
Correlation factors: SLOPE 0.8 – 1.2 and OFFSET +/- 3.

Sample	Species	Immulite 2000 µg/ml	Sample Quality	mean SOLO µg/ml
Serum	Dog	22.3 µg/ml		26.24 µg/ml
Serum	Cat	6.0 µg/ml		7.33 µg/ml
Lithium-heparin plasma	Cat	30.8 µg/ml		25.11 µg/ml
Serum	Dog	20.9 µg/ml		18.10 µg/ml
Serum	Dog	24.6 µg/ml		25.36 µg/ml
Serum	Cat	20.1 µg/ml	hemolytic	18.25 µg/ml
Serum	Dog	33.8 µg/ml		26.37 µg/ml
Lithium-heparin plasma	Dog	19.5 µg/ml		21.94 µg/ml
Lithium-heparin plasma	Dog	12.0 µg/ml		9.46 µg/ml
Lithium-heparin plasma	Dog	18.6 µg/ml		18.82 µg/ml
Lithium-heparin plasma	Dog	19.9 µg/ml		19.67 µg/ml
Lithium-heparin plasma	Dog	12.7 µg/ml		10.48 µg/ml
Lithium-heparin plasma	Cat	21.2 µg/ml		23.79 µg/ml
Lithium-heparin plasma	Dog	16.3 µg/ml		15.49 µg/ml
Serum	Dog	16.7 µg/ml		13.32 µg/ml
Serum	Dog	19.4 µg/ml		19.70 µg/ml
Serum	Dog	6.4 µg/ml	hemolytic	5.00 µg/ml
Serum	Dog	16.4 µg/ml		17.53 µg/ml
Serum	Dog	13.1 µg/ml		12.97 µg/ml
Serum	Cat	19.1 µg/ml	hemolytic	13.48 µg/ml
Serum	Dog	24.0 µg/ml		19.46 µg/ml
Lithium-heparin plasma	Dog	17.2 µg/ml	hemolytic	17.82 µg/ml
Lithium-heparin plasma	Dog	6.1 µg/ml	hemolytic	6.43 µg/ml
Lithium-heparin plasma	Dog	15.4 µg/ml	lipemic	20.13 µg/ml
Serum	Dog	36.5 µg/ml		29.10 µg/ml
Serum	Cat	10.5 µg/ml		13.10 µg/ml
Serum	Dog	16.2 µg/ml		10.12 µg/ml
Serum	Dog	16.4 µg/ml		16.68 µg/ml
Serum	Dog	16.4 µg/ml		15.27 µg/ml
Serum	Dog	42.7 µg/ml	lipemic	45.50 µg/ml

Phenobarbital SAMPLE Correlation:



The result for the correlation between Eurolyser Phenob. and Immulite is the linear regression function y (SOLO) = 0.8874 x (Immulite-2000) + 1.1693 and an **R^2 = 0.8502**.

3. Imprecision “within-run”

The imprecision “within-run” of Eurolyser Phenobarbital has been obtained through twelve measurements of two different levels.

NO	CONTROL TDA18012	CONTROL TDA18013	Analyzer
1	22.40 µg/ml	42.90 µg/ml	SOLO Ae5052
2	18.30 µg/ml	45.50 µg/ml	SOLO Ae5053
3	17.60 µg/ml	46.30 µg/ml	SOLO Ae5054
4	16.90 µg/ml	49.80 µg/ml	SOLO Ae3611
5	21.30 µg/ml	42.40 µg/ml	SOLO Ae5052
6	18.60 µg/ml	45.40 µg/ml	SOLO Ae5053
7	19.40 µg/ml	43.80 µg/ml	SOLO Ae5054
8	22.00 µg/ml	48.20 µg/ml	SOLO Ae3611
9	23.10 µg/ml	47.60 µg/ml	SOLO Ae5052
10	19.20 µg/ml	42.90 µg/ml	SOLO Ae5053
11	21.10 µg/ml	40.30 µg/ml	SOLO Ae5054
12	19.60 µg/ml	46.50 µg/ml	SOLO Ae3611
mean	**20.00 µg/ml**	**45.10 µg/ml**	
Stabwn	**1.99**	**2.75**	
CV	**9.98%**	**6.10%**	

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

9.98% at 20.0 µg/ml
6.10% at 45.1 µg/ml

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

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

The following results can be used to evaluate the reproducibility.

Summary	01.03.2016	to	06.03.2016	Instrument
Level	CONTROL TDA18012		CONTROL TDA18013	
Unit	µg/ml		µg/ml	
Day 1-1/3	21.8 µg/ml		45.40 µg/ml	SOLO Ae5053
Day 1-2/3	17.5 µg/ml		38.60 µg/ml	SOLO Ae5054
Day 1-3/3	22.3 µg/ml		49.20 µg/ml	SOLO Ae3611
Day 2-1/3	16.8 µg/ml		46.40 µg/ml	SOLO Ae5052
Day 2-2/3	19.4 µg/ml		44.30 µg/ml	SOLO Ae5052
Day 2-3/3	19.6 µg/ml		42.90 µg/ml	SOLO Ae5053
Day 3-1/3	22.5 µg/ml		45.10 µg/ml	SOLO Ae5054
Day 3-2/3	18.0 µg/ml		43.20 µg/ml	SOLO Ae3611
Day 3-3/3	22.3 µg/ml		44.90 µg/ml	SOLO Ae5052
Day 4-1/3	17.8 µg/ml		47.20 µg/ml	SOLO Ae5053
Day 4-2/3	23.4 µg/ml		44.30 µg/ml	SOLO Ae5054
Day 4-3/3	17.4 µg/ml		43.20 µg/ml	SOLO Ae5052
Day 5-1/3	22.8 µg/ml		39.90 µg/ml	SOLO Ae5053
Day 5-2/3	21.3 µg/ml		44.80 µg/ml	SOLO Ae5054
Day 5-3/3	19.6 µg/ml		39.90 µg/ml	SOLO Ae3611
Mean	**20.2**		**44.0**	
Std	**2.3**		**2.8**	
CV	**11.4%**		**6.5%**	

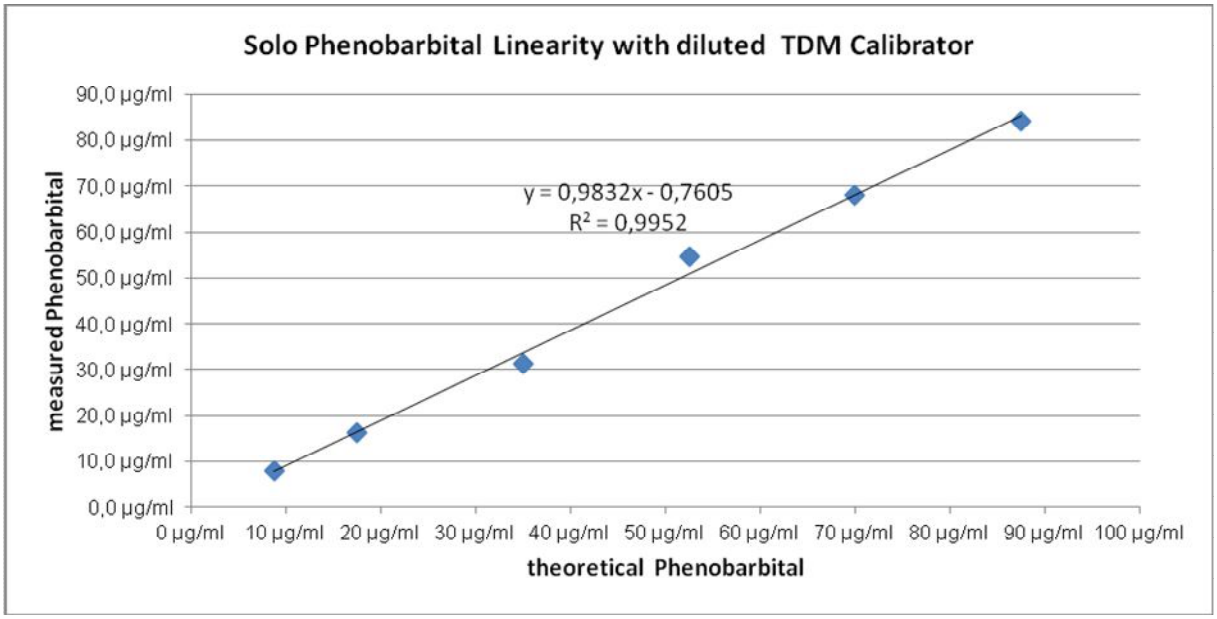
The results for the imprecision “day-to-day” of Eurolyser Phenobarbital are as follows at two different levels:

Low	(mean: 20.2 µg/ml; n = 15)**:**	**CV = 11.4%**
High	(mean: 44.0 µg/ml; n = 15)**:**	**CV = 6.5%**

5. Linearity Study

A high calibrator 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 10% between expected and recovered (obtained) values.

Target 90-110% above 10 µg/ml	Level 6 TDM	Ae 5052	Ae 5053	Ae 5054				
Dilution NaCL 0.9%	Theoretical	meas 1	meas 2	meas 3	mean	stabwn	CV	Recovery
100	87.5 µg/ml	88.3 µg/ml	84.9 µg/ml	78.9 µg/ml	84.0 µg/ml	4.76	5.66%	96%
80	70.0 µg/ml	68.5 µg/ml	72.2 µg/ml	63.3 µg/ml	68.0 µg/ml	4.47	6.58%	97%
60	52.5 µg/ml	54.2 µg/ml	53.1 µg/ml	56.9 µg/ml	54.7 µg/ml	1.96	3.57%	104%
40	35.0 µg/ml	33.2 µg/ml	29.9 µg/ml	31.0 µg/ml	31.4 µg/ml	1.68	5.36%	90%
20	17.5 µg/ml	14.8 µg/ml	15.9 µg/ml	18.1 µg/ml	16.3 µg/ml	1.68	10.33%	93%
10	8.8 µg/ml	7.4 µg/ml	6.6 µg/ml	9.6 µg/ml	7.9 µg/ml	1.56	19.91%	90%



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

y (measured) = 0.9832 x (theoretical) - 0.7605 **R^2 = 0.9952**

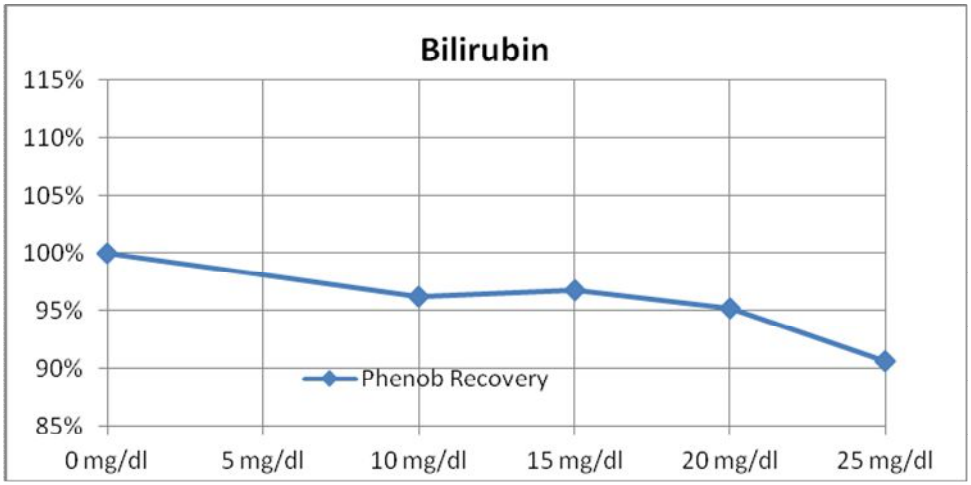
6. Interferences of the Eurolyser Phenobarbital Assay

A phenobarbital sample of around 31 µg/ml was spiked with 1000 mg/dl haemoglobin, 1000mg/dl triglycerides, 25mg/dl bilirubin and 800mg/dl intralipid. The samples were compared to control samples containing saline and water instead of the spiked material.

The spiked samples showed the following interferences:

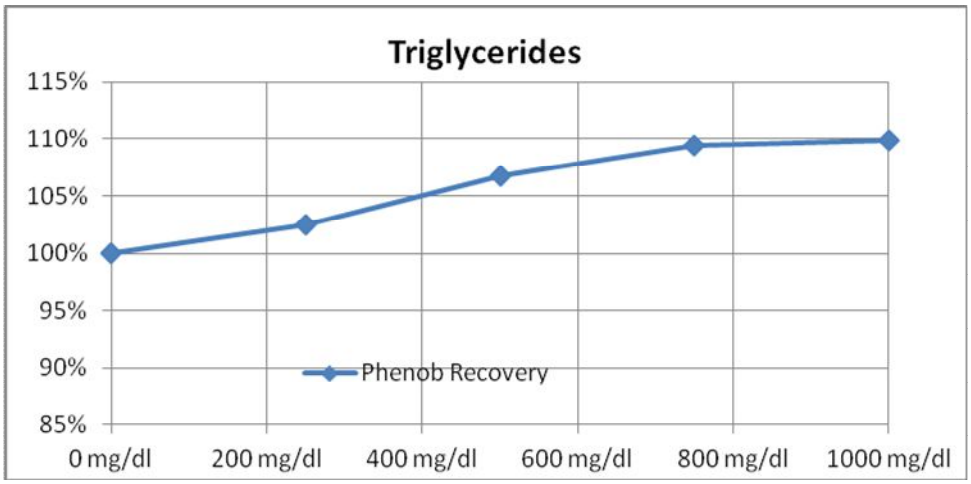
BILIRUBIN:

Bilirubin Concentration	Phenob. Recovery
0 mg/dl	100%
10 mg/dl	96.2%
15 mg/dl	96.8%
20 mg/dl	95.2%
25 mg/dl	90.7%



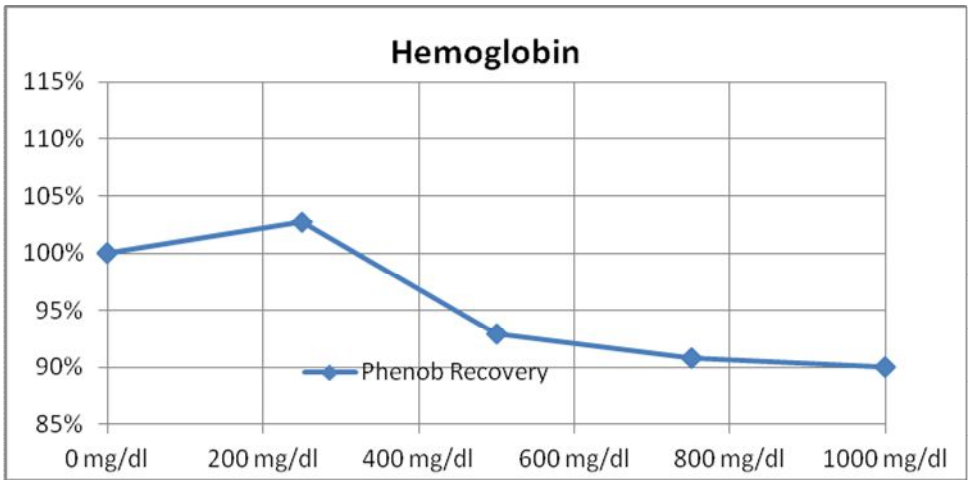
TRIGLYCERIDES:

Triglycerides Concentration	Phenob. Recovery
0 mg/dl	100%
250 mg/dl	102.5%
500 mg/dl	106.7%
750 mg/dl	109.4%
1000 mg/dl	109.9%



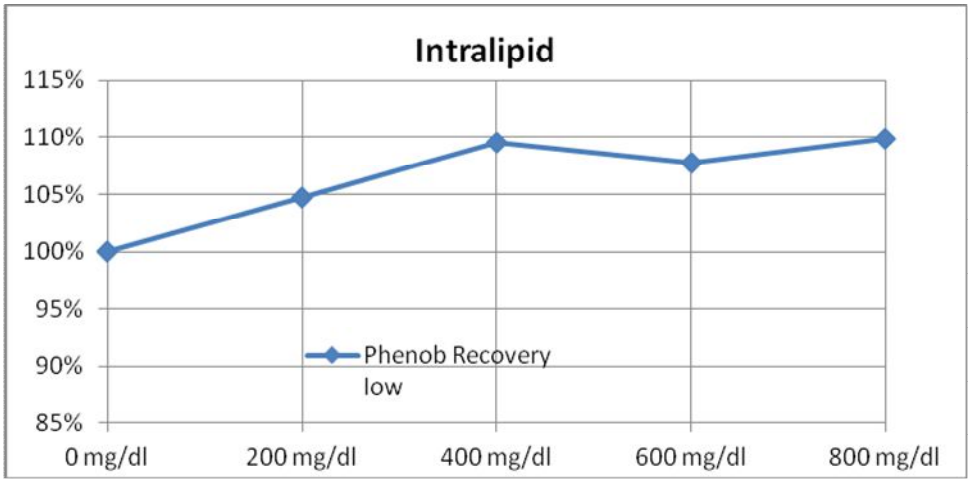
HAEMOGLOBIN (HGB):

HGB Concentration	Phenob. Recovery
0 mg/dl	100.0%
250 mg/dl	102.7%
500 mg/dl	93.0%
750 mg/dl	90.8%
1000 mg/dl	90.0%



INTRALIPID:

Intralipid Concentration	Phenob. Recovery low
0 mg/dl	100.0%
200 mg/dl	104.7%
400 mg/dl	109.6%
600 mg/dl	107.8%
800 mg/dl	109.9%



SUMMARY:

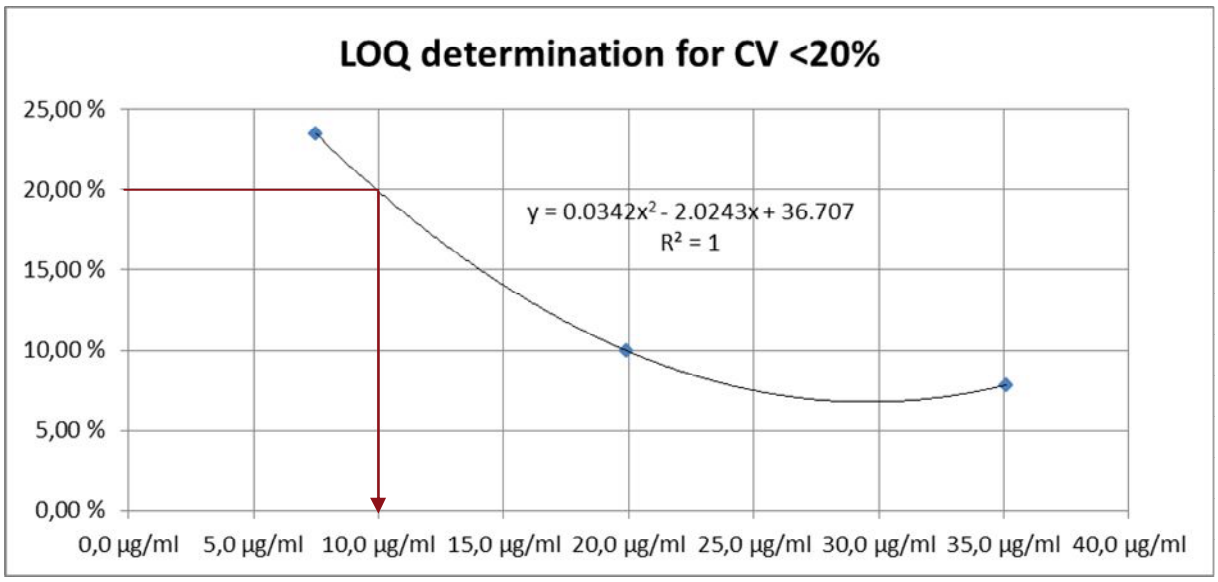
The interferences are as follows:

Bilirubin:	No interference up to	25 mg/dl
Triglycerides:	No interference up to	1000 mg/dl
Haemoglobin:	No interference up to	1000 mg/dl
Intralipid:	No interference up to	800 mg/dl

7. Limit of Quantitation (LOQ) of the Eurolyser-Phenobarbital Assay

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

NO	CONTROL TDA18011	CONTROL TDA18012	CONTROL TDA18013 diluted	Analyzer
1	6.6 µg/ml	22.40 µg/ml	32.90 µg/ml	SOLO Ae5052
2	8.3 µg/ml	18.30 µg/ml	35.50 µg/ml	SOLO Ae5053
3	6.7 µg/ml	17.60 µg/ml	36.30 µg/ml	SOLO Ae5054
4	9.2 µg/ml	16.90 µg/ml	39.80 µg/ml	SOLO Ae3611
5	9.7 µg/ml	21.30 µg/ml	32.40 µg/ml	SOLO Ae5052
6	7.8 µg/ml	18.60 µg/ml	35.40 µg/ml	SOLO Ae5053
7	8.8 µg/ml	19.40 µg/ml	33.80 µg/ml	SOLO Ae5054
8	8.5 µg/ml	22.00 µg/ml	38.20 µg/ml	SOLO Ae3611
9	7.2 µg/ml	23.10 µg/ml	37.60 µg/ml	SOLO Ae5052
10	8.9 µg/ml	19.20 µg/ml	32.90 µg/ml	SOLO Ae5053
11	3.7 µg/ml	21.10 µg/ml	30.30 µg/ml	SOLO Ae5054
12	5.2 µg/ml	19.60 µg/ml	36.50 µg/ml	SOLO Ae3611
mean	**7.6 µg/ml**	**20.0 µg/ml**	**35.1 µg/ml**	
Stabwn	**1.77**	**1.99**	**2.75**	
CV	**23.45%**	**9.98%**	**7.83%**	



The LOQ is set to 10 µg/ml.

8. Therapeutic Ranges

The therapeutic range is recommended based on the following references:

Dog: 15-35 µg/ml
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4913615/

Ravis WR, Pedersoli WM, Wike JS. Pharmacokinetics of phenobarbital in dogs given multiple doses. Am J Vet Res 1989;50:1343–1347. [PubMed]

Schwartz-Porsche D, Loscher W, Frey HH. Therapeutic efficacy of phenobarbital and primidone in canine epilepsy: a comparison. J Vet Pharmacol Therap 1985;8:113–119. [PubMed]

Cunningham JG, Haidukewych D, Jensen HA. Therapeutic serum concentrations of primidone and its metabolites, phenobarbital and phenylethylmalonamide in epileptic dogs. J Am Vet Med Assoc 1983;182:1091–1094. [PubMed]

Cat: 15-35 µg/ml
Seizures and narcolepsy. Thomas WB. In Dewey CW (ed): A Practical Guide to Canine and Feline Neurology—Ames: Iowa State University Press, 2003, pp 193-212.

Anticonvulsants and other neurologic therapies in small animals. Boothe DM. In Boothe DM (ed): Small Animal Clinical Pharmacology and Therapeutics, 1st ed— Philadelphia: WB Saunders, 2000, pp 431-456.

Antiepileptic drug therapy. Podell M. Clin Tech Small Anim Pract 13:185-192,

Horse: 15-45 µg/ml
Equine Neurology Edited by Martin Furr and Stephen Reed
Jarvis C. 1976. The evolutionary strategy of the equidae and the origins of rumen and cecal digestion. *Evolution* 30:757–74.

Cat	15-35 µg/ml
Dog	15-35 µg/ml
Horse	15-45 µg/ml
Foal	5-30 µg/ml

Each laboratory should establish its own therapeutic range!