

## Evaluation Report

### **Eurolyser Phenobarbital test kit (VT0211) for solo and CUBE-VET analysers**

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Report created on 31<sup>st</sup> August 2020  
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#### **Specimens**

The specimens for sample correlation were taken from a reference lab/veterinary university from dogs and cats and were fresh serum or li-hep plasma. Samples were aliquoted and tested with the reference method (Siemens Phenobarbital test measured on an Immulite 2000 xPi). For all other tests the dedicated Phenobarbital controls have been used.

Sample volume: 20 µl

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#### **Equipment**

- Eurolyser CUBE-VET analyser: Cg20350, Cg20351, Cg20353, Ce19732  
- Eurolyser solo analyser: Ae5050, Ae5052, Ae5053, Bc14783  
- Test kits: LOT 20200226

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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 cramps. Depending on the dosage, a sedative or hypnotic action of the drug can be observed. In cats, phenobarbital is the first choice as anti-epileptic 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. The best sampling time is about two hours prior to the next scheduled drug treatment.

### **Principle:**

Homogeneous immunoturbidimetric test.

## 2. Comparison Study

Eurolyser vs Reference method (Siemens Phenobarbital)

The comparison study is based on the correlation between the results of the Eurolyser Phenobarbital test and the Siemens Phenobarbital test measured on an Immulite 2000 xPi.

47 canine samples and 28 feline samples have been analysed on solo and CUBE-VET analysers. 2 replicates of each sample have been measured.

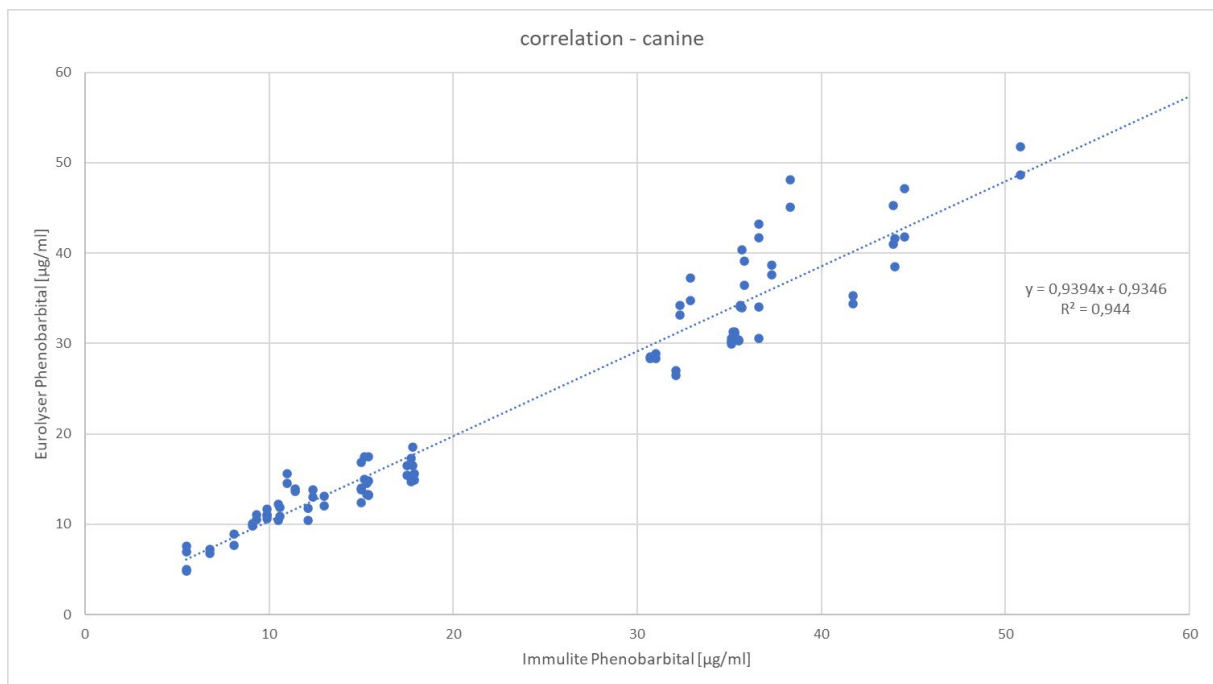
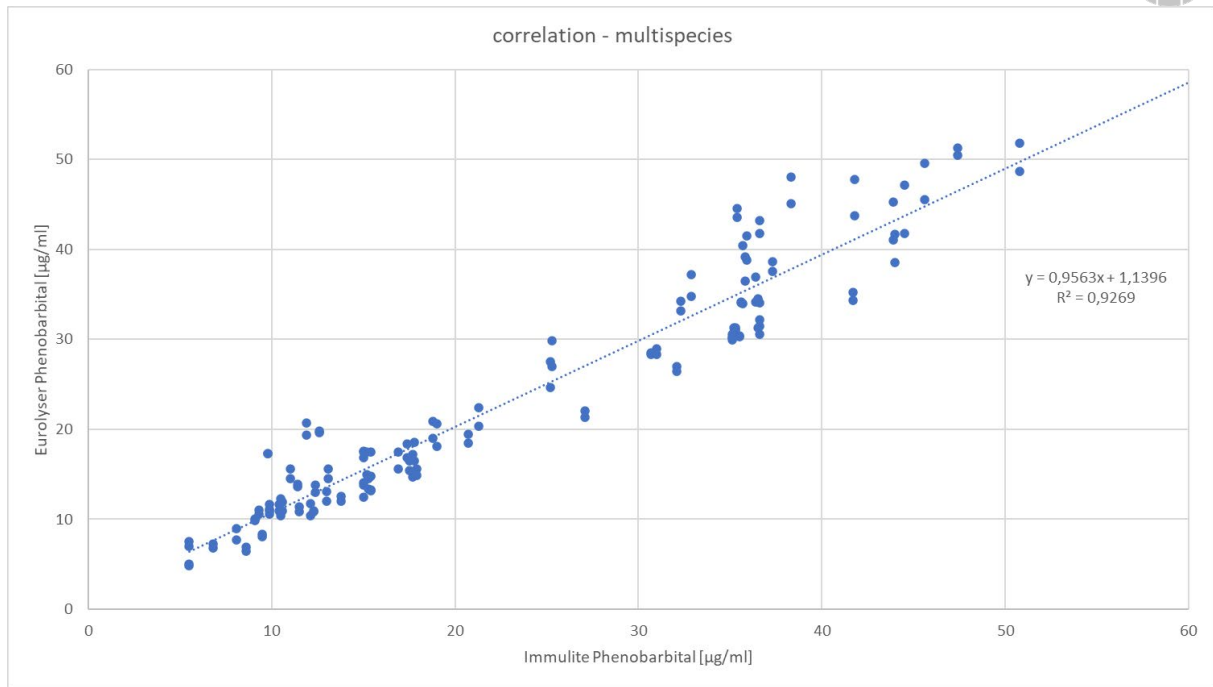
The acceptance criterion for this comparison study is a coefficient of determination  $R^2 > 0.90$  obtained from linear regression between the Eurolyser Phenobarbital and the Siemens (Immulite) Phenobarbital. Further, slope has to be within 0.8 and 1.2, and an intercept between -2 and 2 is acceptable.

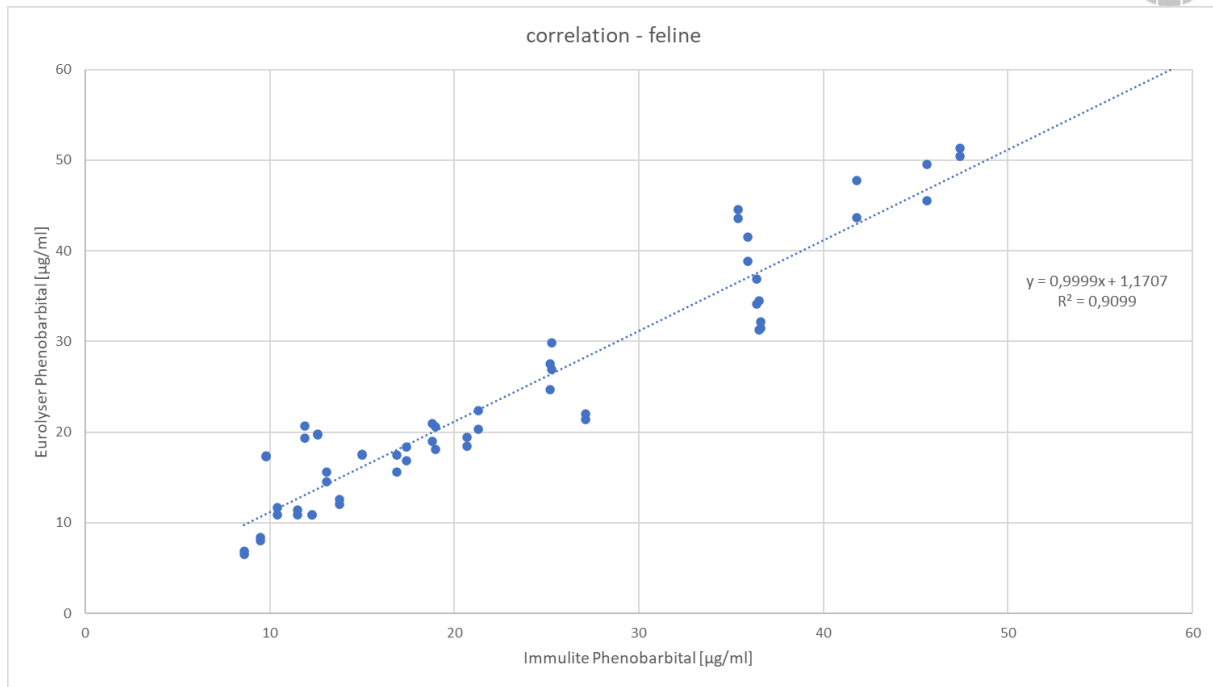
Correlation canine:

| Sample N° | Immulite (µg/ml) | Eurolyser #1 (µg/ml) | Eurolyser #2 (µg/ml) |
|-----------|------------------|----------------------|----------------------|
| 1         | 41.7             | 34.4                 | 35.3                 |
| 2         | 35.2             | 30.6                 | 31.3                 |
| 3         | 35.6             | 34.2                 | 34.0                 |
| 4         | 35.8             | 36.4                 | 39.1                 |
| 5         | 35.5             | 30.3                 | 30.4                 |
| 6         | 37.3             | 37.6                 | 38.6                 |
| 7         | 43.9             | 41.3                 | 45.2                 |
| 8         | 12.4             | 13.0                 | 13.8                 |
| 9         | 12.1             | 11.8                 | 10.4                 |
| 10        | 13.0             | 12.0                 | 13.1                 |
| 11        | 11.4             | 13.7                 | 13.9                 |
| 12        | 11.0             | 14.5                 | 15.6                 |
| 13        | 10.6             | 11.9                 | 10.9                 |
| 14        | 10.5             | 10.4                 | 12.2                 |
| 15        | 9.9              | 10.6                 | 10.9                 |
| 16        | 9.9              | 11.1                 | 11.7                 |
| 17        | 9.3              | 10.5                 | 11.0                 |
| 18        | 9.1              | 10.1                 | 9.8                  |
| 19        | 8.1              | 8.9                  | 7.7                  |
| 20        | 6.8              | 6.8                  | 7.2                  |
| 21        | 5.5              | 7.5                  | 7.2                  |
| 22        | 5.5              | 5.0                  | 4.8                  |
| 23        | 44.5             | 47.2                 | 41.8                 |
| 24        | 38.3             | 45.1                 | 48.1                 |
| 25        | 36.6             | 43.2                 | 41.7                 |
| 26        | 32.9             | 34.8                 | 37.2                 |
| 27        | 32.3             | 33.1                 | 34.2                 |
| 28        | 32.1             | 27.0                 | 26.4                 |
| 29        | 31.0             | 28.9                 | 28.3                 |
| 30        | 30.7             | 28.3                 | 28.5                 |
| 31        | 35.1             | 30.6                 | 30.2                 |
| 32        | 35.1             | 30.2                 | 29.9                 |
| 33        | 35.3             | 31.0                 | 31.3                 |
| 34        | 36.6             | 34.0                 | 30.5                 |
| 35        | 35.7             | 40.4                 | 33.9                 |
| 36        | 44.0             | 41.6                 | 38.5                 |
| 37        | 50.8             | 51.8                 | 48.6                 |
| 38        | 17.8             | 18.6                 | 16.5                 |
| 39        | 15.0             | 16.8                 | 13.8                 |
| 40        | 15.0             | 12.4                 | 14.0                 |
| 41        | 15.2             | 17.5                 | 15.0                 |
| 42        | 15.3             | 13.3                 | 14.6                 |
| 43        | 15.4             | 13.3                 | 17.5                 |
| 44        | 15.4             | 14.8                 | 13,2                 |
| 45        | 17.5             | 16.5                 | 15.4                 |
| 46        | 17.7             | 17.2                 | 14.7                 |
| 47        | 17.9             | 14.9                 | 15.6                 |

**Correlation feline:**

| Sample N° | Immulite (µg/ml) | Eurolyser #1 (µg/ml) | Eurolyser #2 (µg/ml) |
|-----------|------------------|----------------------|----------------------|
| 48        | 15.0             | 17.5                 | 17.5                 |
| 49        | 13.1             | 14.6                 | 15.6                 |
| 50        | 12.6             | 19.7                 | 19.8                 |
| 51        | 10.4             | 11.7                 | 10.9                 |
| 52        | 11.9             | 19.3                 | 20.7                 |
| 53        | 9.8              | 17.3                 | 17.3                 |
| 54        | 47.4             | 50.5                 | 51.3                 |
| 55        | 35.4             | 43.5                 | 44.6                 |
| 56        | 36.5             | 31.2                 | 34.5                 |
| 57        | 36.6             | 31.4                 | 32.2                 |
| 58        | 27.1             | 21.4                 | 22.0                 |
| 59        | 25.3             | 29.9                 | 26.9                 |
| 60        | 25.2             | 27.6                 | 24.7                 |
| 61        | 21.3             | 22.4                 | 20.3                 |
| 62        | 20.7             | 18.5                 | 19.4                 |
| 63        | 19.0             | 20.6                 | 18.1                 |
| 64        | 18.8             | 19.0                 | 20.9                 |
| 65        | 17.4             | 18.4                 | 16.8                 |
| 66        | 16.9             | 17.5                 | 15.6                 |
| 67        | 35.9             | 41.5                 | 38.8                 |
| 68        | 36.4             | 36.9                 | 34.1                 |
| 69        | 41.8             | 47.8                 | 43.7                 |
| 70        | 45.6             | 45.6                 | 49.6                 |
| 71        | 13.8             | 12.6                 | 12.0                 |
| 72        | 12.3             | 10.9                 | 10.9                 |
| 73        | 11.5             | 11.4                 | 10.9                 |
| 74        | 9.5              | 8.0                  | 8.4                  |
| 75        | 8.6              | 6.5                  | 6.9                  |





Sample correlation:

The result for the correlation between the Eurolyser Phenobarbital test and the Siemens (Immulite) Phenobarbital test is the linear regression function:

multispecies:

$$y \text{ (Eurolyser Phenob)} = 0.9563x \text{ (Immulite Phenob)} + 1.1396; \mathbf{R^2 = 0.9269}$$

canine:

$$y \text{ (Eurolyser Phenob)} = 0.9394x \text{ (Immulite Phenob)} + 0.9346; \mathbf{R^2 = 0.944}$$

feline:

$$y \text{ (Eurolyser Phenob)} = 0.9999x \text{ (Immulite Phenob)} + 1.1707; \mathbf{R^2 = 0.9099}$$

### 3. Reference Ranges

The following reference ranges are suggested.

Nonetheless, it is recommended that each laboratory establishes its own reference ranges.

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

#### 4. Reproducibility (within-run precision)

Two controls have been tested 20 times each and the CV values were calculated (tested with solo and CUBE-VET analysers):

| Sample #       | Control 1 (µg/ml) | Control 2 (µg/ml) |
|----------------|-------------------|-------------------|
| 1              | 10.5              | 30.2              |
| 2              | 9.3               | 24.4              |
| 3              | 9.4               | 26.4              |
| 4              | 8.2               | 26.5              |
| 5              | 7.7               | 24.6              |
| 6              | 9.8               | 29.5              |
| 7              | 10.1              | 27.3              |
| 8              | 9.1               | 27.5              |
| 9              | 8.9               | 25.1              |
| 10             | 8.2               | 25.1              |
| 11             | 9.5               | 30.0              |
| 12             | 9.9               | 26.6              |
| 13             | 8.3               | 27.7              |
| 14             | 8.3               | 22.9              |
| 15             | 7.4               | 26.0              |
| 16             | 7.9               | 27.4              |
| 17             | 9.1               | 29.5              |
| 18             | 9.5               | 27.1              |
| 19             | 9.1               | 24.1              |
| 20             | 8.3               | 26.8              |
| <b>average</b> | <b>8.9</b>        | <b>26.7</b>       |
| <b>stdev</b>   | <b>0.9</b>        | <b>2.0</b>        |
| <b>CV</b>      | <b>9.63%</b>      | <b>7.62%</b>      |

The CV values for the tested controls are 9.63% and 7.62%.

#### 5. Stability Test

A real time stability test was performed. Reagent stability was recorded over 4 months, during this time cuvettes have been stored at 4 °C.

3 control levels have been used.

The recovery of the control low, mid and high has to be within 10% of the target value.

Recovery:

| day | Control low |            | Control mid |            | Control high |            |
|-----|-------------|------------|-------------|------------|--------------|------------|
|     | µg/ml       | % recovery | µg/ml       | % recovery | µg/ml        | % recovery |
| 1   | 8.9         | 100.0      | 38.9        | 100.0      | 79.8         | 100.0      |
| 32  | 8.4         | 94.3       | 38.4        | 98.8       | 74.6         | 93.6       |
| 64  | 8.6         | 96.6       | 39.1        | 100.6      | 79.8         | 100.1      |
| 94  | 8.6         | 96.6       | 38.6        | 99.3       | 82.9         | 103.9      |
| 117 | 9.2         | 103.9      | 40.9        | 105.1      | 74.0         | 92.8       |

The reagent shows good stability in case of storage at 4 °C over 4 months, therefore, a 9 months expiry date can be assumed and is implemented.

## 6. Linearity Study

Do not use diluted samples for measurement.

## 7. Limit of Quantitation (LOQ)

LOQ is determined as the lowest sample run that displays a CV value < 20%.

|               | Control dilution |
|---------------|------------------|
| average       | 4.65             |
| stdev         | 0.81             |
| <b>CV (%)</b> | <b>17.41%</b>    |

Based on these results the LOQ is set to 5 µg/ml.

## 8. Interferences

The test system has been analysed for various interferences. Criterion was the recovery within 10% of initial values. The following substances show no interferences up to:

|                         |            |
|-------------------------|------------|
| Haemoglobin             | 525 mg/dl  |
| Albumin                 | 12 g/dl    |
| Bilirubin, conjugated   | 36 mg/dl   |
| Bilirubin, unconjugated | 36 mg/dl   |
| Cholesterol             | 620 mg/dl  |
| Rheumatoid factor       | 1080 IU/ml |
| Triglycerides           | 835 mg/dl  |
| Uric acid               | 30 mg/dl   |

## 9. Summary

The Eurolyser Phenobarbital test kit designed for solo and CUBE-VET analysers has a good correlation to the Siemens Phenobarbital test measured on an Immulite 2000 xPi. The reproducibility and stability of the test are very good.