Evaluation of the EUROLYSER-SOLO Total T4 assay

for veterinary samples

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Scope of the study

Goal of this study was the validation of the new veterinary Total T4 assay assigned for analysis with the Eurolyser-SOLO analyzer. For this, the system was evaluated for:

- Precision at different concentrations
- Linearity over the measuring range
- Method comparison to the present gold standard

Materials and methods

Samples

Serum samples from dogs and cats with and without thyroid disorders were collected at two different sites in Germany. The animals need not to be fasting, and no special preparations are necessary. Blood samples taken by venipuncture were collected in plain tubes without anti-coagulants. Samples were stored at room temperature for at least 10 minutes to allow a complete cell aggregation. Afterwards, serum was separated by centrifugation. Serum samples were subjected to direct analysis or frozen at -20°C until they were subjected to analysis. Before testing samples were allowed to come to room temperature and mix by gentle swirling or inversion.

EUROLYSER-SOLO analyzer system

The EUROLYSER-SOLO analyzer (single method automated reading technology) is a fully automated instrument allowing a simple quantitative determination of analytes in a point-of-care format. The EUROLYSER-SOLO analyzer is designed for measurement of veterinarian samples.

The system comprises from the analyzer itself and the respective reagents. The reagents are provided in form of “Single-use units” avoiding the usual open stability issue of wet chemistry reagents. The smart Total T4 kit includes cuvettes and ERS caps containing the reagent and in addition RF-ID cards containing all important assay information like the test programming and the calibration data.

The RF-ID card is supplied to the analyzer and the instrument is programmed according the instruction for use. Before measurement, cuvette and ERS cap are equilibrated to room temperature for at least 10 minutes. Afterwards, the sample (volume of 20 µl or 5 µl, respectively) is transferred into the cuvettes’ reagent using an appropriate pipette. The ERS cap is placed onto the cuvette and the combined device is applied to the smart instrument. The fully automated measurement takes about 6 minutes.

When using a sample volume of 20 µl, the linearity is limited to 8 µg/dl. In order to extend the linear range by a factor of 4 (up to 32 µg/dl), the EUROLYSER-SOLO system offers an option for pipetting a sample volume of 5 µl.

Linear range: 0.5 – 32 µg/dl.

Immulite 1000/canine Total T4

For method comparison analysis, the Siemens Immulite 1000 system with the Siemens canine Total T4 test kit was chosen. The canine Total T4 assay is a solid-phase, chemiluminescent competitive immunoassay. The system is the usually used for veterinarian T4 measurements and therefore selected as the best reference system on market today.

Samples described above were used in a parallel analysis according to the manufacturers' instruction for use. A volume of 30 µl serum was used[2,6].

For higher concentrated samples with Total T4 values above 6 µg/dl, dilutions were used. A dilution of 1:4 (3 parts diluents/1 part sample) was made with a pooled serum samples (5 samples) having a low total T4 concentration. To estimate the Total T4 concentration the pooled sample was analyzed three times (value: 0.68 µg/dl). Results were corrected prior its usage for data analysis.

Reference values

Since the evaluation of the EUROLYSER-SOLO system was scope of this study, normal values had not been evaluated in detail. Several publications are available defining the reference values of common species using different immunological methods. Table 1 show that normal values of dogs and cats are in a similar ranging from 1.0 to 4.5 µg/dl. The data were derived from publications as well as from companies offering veterinarian Total T4 assays.

Table 1: Reference values for Total T4 from published sources [µg/dl].

Species	Singh, 1997[2]	Lien, 2008[4]	Immulite canine T4[6]	Idexx Snap Test[8]	Laboklin[7]
Dog	1.0 – 3.1	-	1.3 – 4.5	1.3 – 2.9	1.3 – 4.5
Cat	0.8 – 3.2	0.9 – 3.1	-	0.5 – 2.0	0.9 – 2.9

Limitations

The assay is optimized for the determination of Total T4 in serum and plasma. The use of whole blood is not possible. In rare cases, animals may have autoantibodies which interfere with the assay and result in low test values.

Specificity and Interferences

Test system had been analyzed for various interferences. Components with chemical structure similar to that of thyroxine and certain concurrently used components were tested for possible cross reactivity in the thyroxine assay. The % cross reactivity was determined as the percent of equivalent Total T4 concentration observed when tested concentration of the cross reactant was added to a T4 negative serum.

Compound	**Conc. Tested [µg/dl]**	**% cross reactivity**
Triiodothyronine (T3)	10	3.2*
Triiodothyroacetic Acid	10	0.5*
Tetraiodothyroacetic Acid	10	25.3*
3,5-Diiodotyronone	10000	0
3,5-Diiodotyrosine	10000	0
Iodotyrosine	10000	0
Methimazole	10000	0
Phenylbutazone	10000	0
Phenytoin	10000	0
Propylthiouracil	10000	0
Tyrosine	10000	0
Acetaminophen	10000	0
Acetylsalicylic Acid	10000	0

* *The tested concentrations greatly exceed the normal serum concentrations of these components. Therefore the cross reactivity is not clinically significant.*

In addition, following probably causes of interference had been analyzed and found not to interfere significantly:

Bilirubin	up to 30 mg/dl
Hemoglobin	up to 800 mg/dl
Triglycerides	up to 1000 mg/dl
Cholesterol	up to 400 mg/dl

Results and discussion

Precision

Since the EUROLYSER-SOLO system comprises from single-use-units, only a run-to-run precision analysis is suitable. Assay precision was determined by assaying three clinical serum samples derived from cats or dogs. At least 10 replicates had been performed. The samples had been selected that different concentration levels were covered.

Table 2: Precision of EUROLYSER-SOLO Total T4 assay.

Sample	Sample volume [µl]	Species	Replicates	Mean [µg/dl]	SD [µg/dl]	CV [%]
1	20	Cat	14	7.98	0.35	4.4
2	20	Cat	10	1.93	0.08	4.1
3	20	Dog	10	1.03	0.04	3.8
1	5	Cat	5	7.79	0.27	3.5

The result of the run-to-run assay precision is shown in table 2. The variation coefficient (CV) ranges from 3.8-4.4 % when using 20 µl sample volume.

To further analyze whether samples with a concentration of around 8 µg/dl were found reproducibly using both instrument modes, sample number 1 was reanalyzed using the reduced sample volume of 5 µl. 5 replicates were found with a standard deviation of 3.5%. Mean vales were 7.98 µg/dl (20 µl sample volume) and 7.79 µg/dl (5 µl sample volume), respectively. The difference between the mean values was found to be 2.44 % demonstrating the high assay precision at the junction point of both assay modes. In conclusion table 1 show that the EUROLYSER-SOLO Total T4 assay is highly reproducible with different concentrations and precise using both modes of the system.

Method comparison

In total, serum samples derived from 48 dogs and from 43 cats with Total T4 concentration ranging from approximately 0.5 to 20 µg/dl had been chosen for this method validation. As reference system for the EUROLYSER-SOLO analyzer, Siemens Immulite canine Total T4 assay had been chosen. According to the scope of the EUROLYSER-SOLO system, 20 µl volume had been used for samples concentrated below 8 µg/dl, 5 µl sample volume had been used for samples with a concentration of higher than 8 µg/dl. Similarly for measurements with the Siemens Immulite system, samples having concentrations higher than 6 µg/dl had been diluted 1:4 (3 parts diluent/1 part sample) before measurement. For dilution a low concentrated pooled serum sample had been used, results were corrected before data analysis.

Data were subjected to a linear regression analysis, which is shown in Figure 1. Fig 1A shows the analysis of the cat-specific data, Fig. 1B the analysis of data obtained with the dog samples. Finally, Fig 3C combines all data.

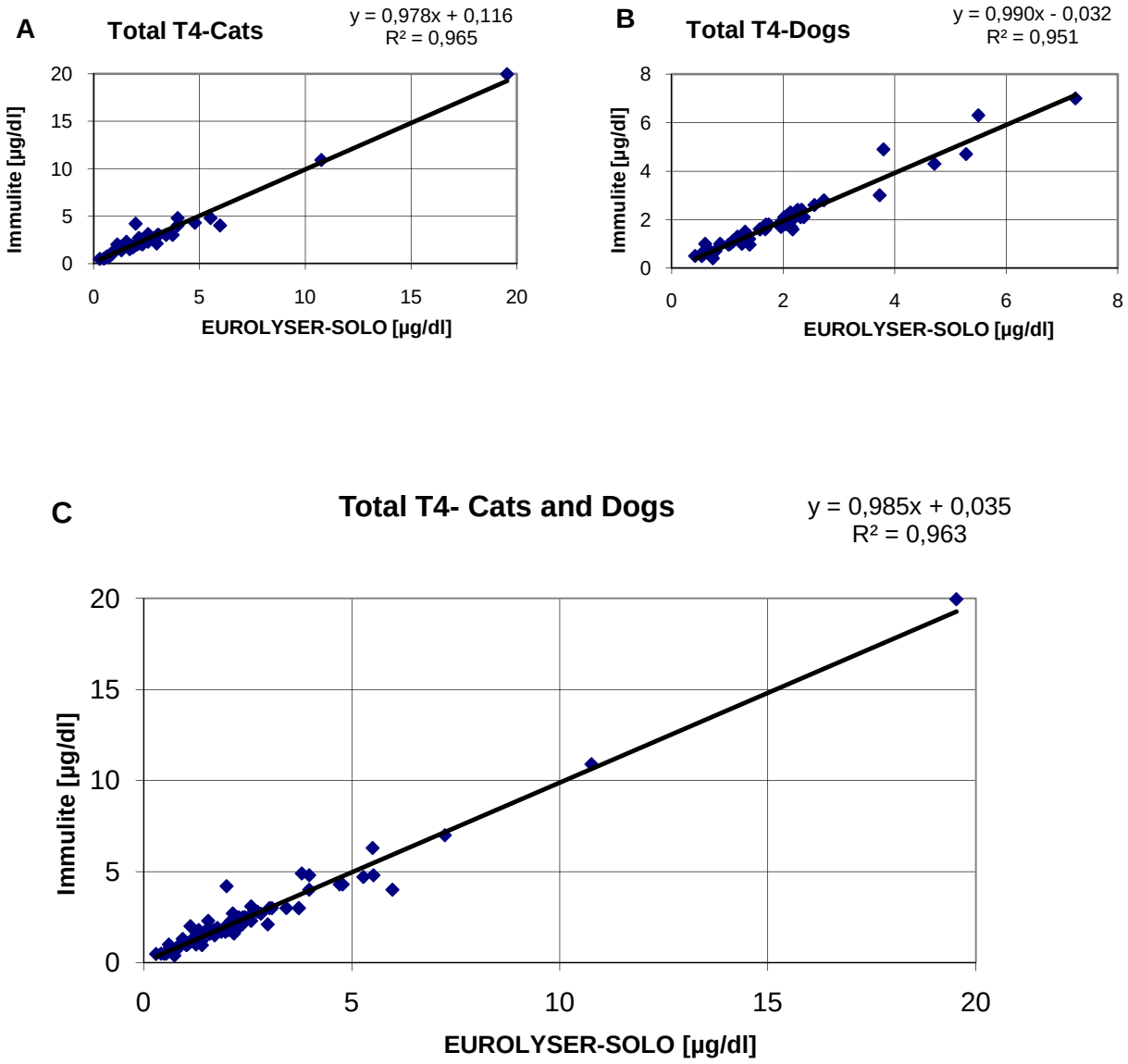

Figure 1: Method comparison of EUROLYSER-SOLO Total T4 assay. A: Dogs; B: Cats; C: Dogs and Cats.

The analysis shows that both assays correlate very well for cats as well as for dogs. In all cases the value of correlation coefficient R^2 was higher than 0.95 demonstrating a high correlation between both assay systems. Mean values of all samples was 2.38 µg/dl for both groups, respectively.

Linearity

Three samples derived from two cats and one dog were diluted to the lower detection limit of the EUROLYSER-SOLO Total T4 assay. All samples were measured in duplicates using 20 µl sample volume (with one exception). The result (mean of the duplicates) is shown in table 3.

Table 3: Linearity of EUROLYSER-SOLO Total T4 assay.

Dilution factor	Sample 1: Cat		Sample 2: Cat		Sample 3: Dog	
	Result [µg/dl]	Recovery [%]	Result [µg/dl]	Recovery [%]	Result [µg/dl]	Recovery [%]
1:1	5,25		8,94*		4,87	
1:2	2,58	98	4,62	103	2,41	99
1:4	1,40	99	2,29	102	1,28	105
1:8	0,79	105	1,18	105	0,7	113
1:16	0,48	132	0,64	112		

* sample volume: 5µl.

The data shown in table 2 demonstrate recovery rates of 95-105 %. If samples were diluted to concentration of 0.7 µg/dl or lower to the end of the measuring range of 0.5 µg/dl recovery values are slightly higher. In summary the data show that the Total T4 assay is linear with respect to the indicated measuring range.

Summary

Goal of this study was the validation of the new veterinary Total T4 assay assigned for analysis with the EUROLYSER-SOLO analyzer. In summary the study demonstrates that the EUROLYSER-SOLO total T4 assay

- is **precise** at different concentrations
- is **linear** over the wide range of 0.5 – 32 µg/dl
- has an **excellent correlation** when compared to the present gold standard (Immulite canine Total T4/Siemens)

Neuried, 2011-04-27 Dr. Volker Schlüter

Literature

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