

Evaluation Report

Eurolyser cCRP-VET 2nd generation test kit on solo and CUBE(-VET) Analyser

Locations

Location: Eurolyser Diagnostica GmbH
Operator: Michael Gruber
Date: January - April 2014

Specimens

The specimens used for analysis were taken from multiple sites and are frozen dog serum and plasma samples already used in previous validations. (Former cCRP-VET assay launched starting with LOT 0711-1 from July 2011.)

Equipment

- Eurolyser solo Analyser: Bc14577, Bc14578, Bc14579, Bc14580, Bc14581
 - Eurolyser CUBE Analyser with dedicated VET Software: Ca10627, Ca10628, Ca10629, Ca10630
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1. Introduction and scope

Designed for the quantitative determination of canine CRP in the point-of-care field, the Eurolyser cCRP-VET assay (Ref. no. VT0100) is now available in its 2nd generation. The first generation of the assay was based on a human CRP assay on which the affinity to dog samples was evaluated for each individual production LOT. Starting with production LOT 0414-1, a new antibody is used and the AB reagent is latex enhanced to improve the sensitivity and specificity of the cCRP-VET assay.

2. Comparison Studies

The comparison study is based on the correlation between the results of the SCIL/Gentian cCRP assay performed on a high end bench top clinical chemistry analyser CCA180 and the Eurolyser cCRP-VET assay.

On the GENTIAN cCRP method the canine serum or plasma sample is mixed with canine CRP immunoparticles. Canine CRP from the sample and the immunoparticles' anti-canine CRP aggregate. The complex particles created absorb light, and turbidimetric measurements of absorption are related to canine CRP concentration via interpolation on an established standard calibration curve.

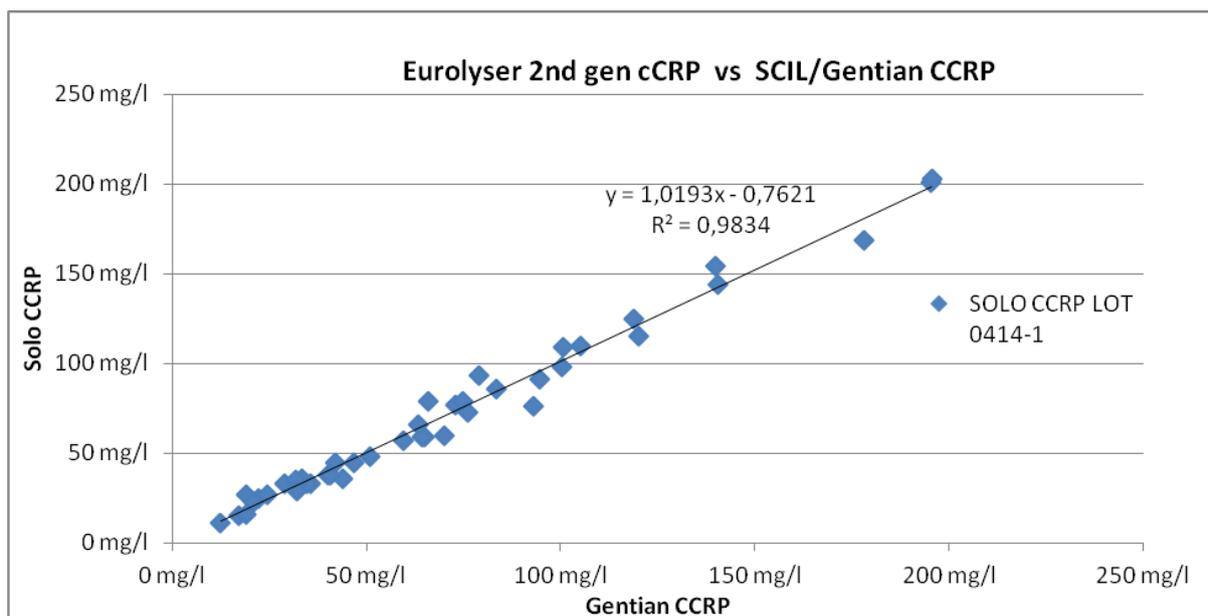
On the Eurolyser cCRP-VET method the buffer solution is pre-filled in the measuring cuvette and the immunoparticles (anti canine CRP) are pre-filled in the R2 cap.

The binding of the created complex when sample/buffer and immunoparticles are mixed together is measured in the laboratory photometer (solo or CUBE-VET Analyser) by measuring the reaction speed. The RFID card of the Eurolyser system is programmed with the LOT specific calibration curve and values of the photometer are interpolated with the calibration curve from the RFID card.

42 patient samples (lithium-heparin plasma and serum) have been tested with the Eurolyser cCRP-VET assay (LOT 0414-1) and with the Gentian cCRP assay LOT 1306402.

The acceptance criterion for this comparison study is a coefficient of determination $R^2 > 0.9$ obtained from linear regression between Eurolyser cCRP-VET and Gentian cCRP.

Sample	Type	GENTIAN CCRP on CCA180 LOT 1306402	Eurolyser cCRP-VET LOT 0414-1	Instrument
1	Li-hep	119 mg/l	125 mg/l	SOLO BC14577
2	Serum	51 mg/l	48 mg/l	SOLO BC14578
3	Li-hep	120 mg/l	115 mg/l	SOLO BC14579
4	Li-hep	44 mg/l	36 mg/l	SOLO BC14580
5	Li-hep	19 mg/l	27 mg/l	SOLO BC14581
6	Li-hep	22 mg/l	25 mg/l	CUBE Ca10627
7	Li-hep	65 mg/l	59 mg/l	CUBE Ca10628
8	Li-hep	29 mg/l	33 mg/l	CUBE Ca10629
9	Li-hep	95 mg/l	91 mg/l	CUBE Ca10630
10	Li-hep	100 mg/l	98 mg/l	SOLO BC14577
11	Li-hep	64 mg/l	59 mg/l	SOLO BC14578
12	Li-hep	73 mg/l	77 mg/l	SOLO BC14579
13	Li-hep	195 mg/l	201 mg/l	SOLO BC14580
14	Li-hep	17 mg/l	15 mg/l	SOLO BC14581
15	Li-hep	93 mg/l	76 mg/l	CUBE Ca10627
16	Li-hep	79 mg/l	93 mg/l	CUBE Ca10628
17	Serum	36 mg/l	33 mg/l	CUBE Ca10629
18	Serum	75 mg/l	79 mg/l	CUBE Ca10630
19	Serum	76 mg/l	73 mg/l	SOLO BC14577
20	Li-hep	196 mg/l	203 mg/l	SOLO BC14578
21	Li-hep	70 mg/l	60 mg/l	SOLO BC14579
22	Serum	66 mg/l	79 mg/l	SOLO BC14580
23	Serum	35 mg/l	33 mg/l	SOLO BC14581
24	Li-hep	19 mg/l	16 mg/l	CUBE Ca10627
25	Li-hep	60 mg/l	57 mg/l	CUBE Ca10628
26	Serum	33 mg/l	36 mg/l	CUBE Ca10629
27	Serum	83 mg/l	86 mg/l	CUBE Ca10630
28	Li-hep	32 mg/l	35 mg/l	SOLO BC14577
29	Li-hep	42 mg/l	45 mg/l	SOLO BC14578
30	Li-hep	24 mg/l	27 mg/l	SOLO BC14579
31	Li-hep	32 mg/l	29 mg/l	SOLO BC14580
32	Li-hep	40 mg/l	38 mg/l	SOLO BC14581
33	Serum	47 mg/l	45 mg/l	CUBE Ca10627
34	Serum	140 mg/l	154 mg/l	CUBE Ca10628
35	Serum	41 mg/l	38 mg/l	CUBE Ca10629
36	Unknown	12 mg/l	11 mg/l	CUBE Ca10630
37	Serum	105 mg/l	110 mg/l	SOLO BC14577
38	Serum	21 mg/l	23 mg/l	SOLO BC14578
39	Serum	178 mg/l	169 mg/l	SOLO BC14579
40	Serum	63 mg/l	66 mg/l	SOLO BC14580
41	Serum	140 mg/l	144 mg/l	SOLO BC14581
42	Serum	101 mg/l	109 mg/l	CUBE Ca10627



The result for the correlation between Eurolyser cCRP and Gentian cCRP is the linear regression function y (Solo cCRP) = 1.0193 x (Gentian cCRP) – 0.7621 and a $R^2 = 0.9834$.

3. Imprecision “within-run”

The imprecision “within-run” of Eurolyser cCRP-VET has been obtained through 20 measurements of 3 specimens at 3 different levels.

Seq	16 mg/l sample 14	33 mg/l sample 17	79 mg/l sample 18	Instruments
1	16 mg/l	31 mg/l	80 mg/l	SOLO BC14577
2	14 mg/l	30 mg/l	82 mg/l	SOLO BC14578
3	14 mg/l	28 mg/l	76 mg/l	SOLO BC14579
4	18 mg/l	33 mg/l	74 mg/l	SOLO BC14580
5	18 mg/l	32 mg/l	73 mg/l	SOLO BC14581
6	16 mg/l	30 mg/l	71 mg/l	CUBE Ca10627
7	17 mg/l	29 mg/l	72 mg/l	CUBE Ca10628
8	15 mg/l	28 mg/l	77 mg/l	CUBE Ca10629
9	18 mg/l	27 mg/l	78 mg/l	CUBE Ca10630
10	16 mg/l	33 mg/l	79 mg/l	SOLO BC14577
11	15 mg/l	30 mg/l	83 mg/l	SOLO BC14578
12	15 mg/l	31 mg/l	84 mg/l	SOLO BC14579
13	14 mg/l	29 mg/l	77 mg/l	SOLO BC14580
14	17 mg/l	33 mg/l	76 mg/l	SOLO BC14581
15	18 mg/l	31 mg/l	74 mg/l	SOLO BC14577
16	17 mg/l	30 mg/l	72 mg/l	SOLO BC14578
17	16 mg/l	29 mg/l	77 mg/l	SOLO BC14579
18	16 mg/l	28 mg/l	76 mg/l	SOLO BC14580
19	15 mg/l	32 mg/l	78 mg/l	SOLO BC14581
20	18 mg/l	30 mg/l	76 mg/l	CUBE Ca10627
mean	16 mg/l	30 mg/l	77 mg/l	
stabwn	1.42	1.77	3.60	
cv	8.82%	5.87%	4.70%	

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

8.82 % at 16 mg/l

5.87 % at 30 mg/l

4.70 % at 77 mg/l.

Due to the low sample volume of 5 µl it is mandatory to be aware of a careful pipetting procedure against good laboratory practice, in order to achieve this precision.

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

The imprecision “day-to-day” of Eurolyser cCRP-VET has been obtained through the measurement of 3 different calibrators on 5 consecutive days. The following results can be used in order to evaluate the reproducibility.

Summary	05.04.2014	to	10.04.2014	
Level	Calib. low 28.7	Calib. mid 75.8	Calib. high 150	
Unit	mg/l	mg/l	mg/l	Instrument
Day 1 - 1/3	31	77	133	SOLO BC14580
Day 1 - 2/3	29	84	166	SOLO BC14580
Day 1 - 3/3	31	76	155	SOLO BC14580
Day 2 - 1/3	33	71	144	SOLO BC14580
Day 2 - 2/3	29	79	149	SOLO BC14580
Day 2 - 3/3	27	81	153	SOLO BC14580
Day 3 - 1/3	28	82	151	SOLO BC14580
Day 3 - 2/3	33	79	152	SOLO BC14580
Day 3 - 3/3	34	74	166	SOLO BC14580
Day 4 - 1/3	28	75	167	SOLO BC14580
Day 4 - 2/3	27	74	155	SOLO BC14580
Day 4 - 3/3	29	78	163	SOLO BC14580
Day 5 - 1/3	32	77	152	SOLO BC14580
Day 5 - 2/3	33	81	151	SOLO BC14580
Day 5 - 3/3	31	85	150	SOLO BC14580
Mean	30.3	78.2	153.8	
Std	2.4	3.9	9.1	
%CV	7.9%	5.0%	5.9%	

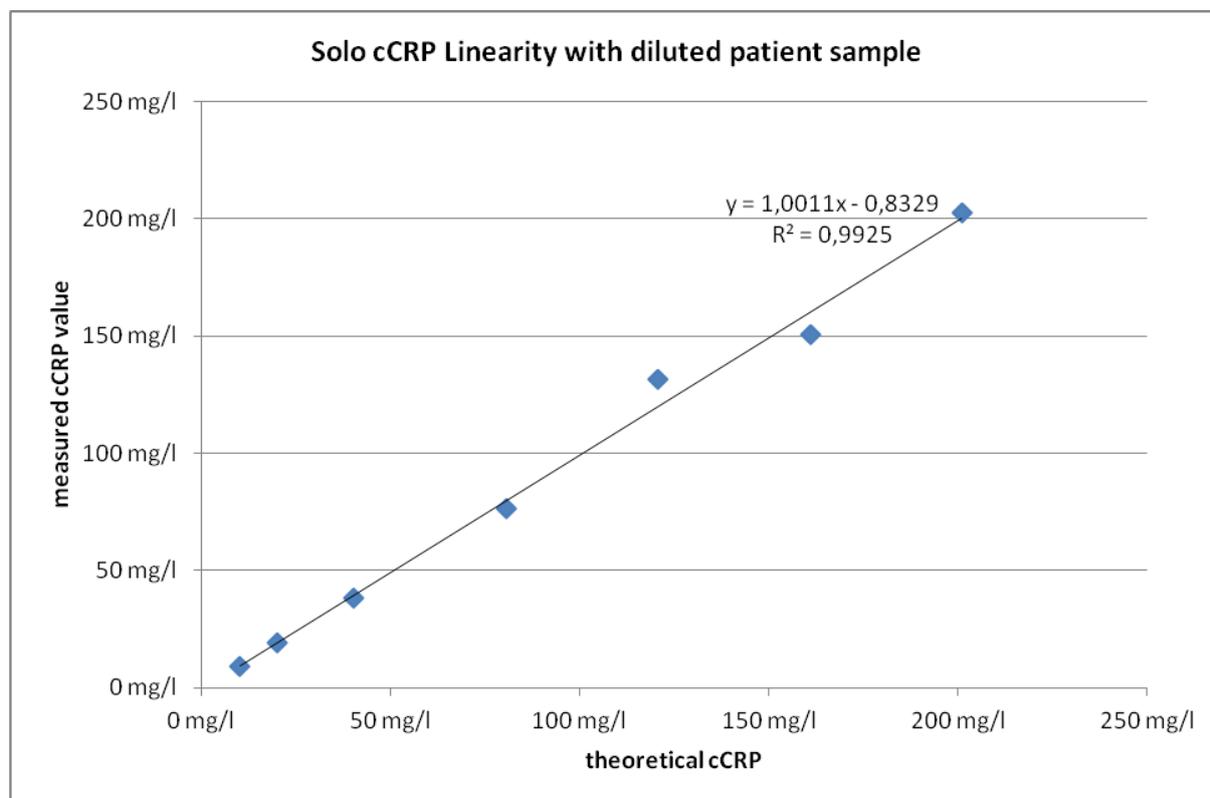
The results for the imprecision “day-to-day” of Eurolyser cCRP are as follows at three different levels:

Low (mean: 30.3 mg/l; n = 15):	CV = 7.9 %
Mid (mean: 78.2 mg/l; n = 15):	CV = 5.0 %
High (mean: 150 mg/l; n = 15):	CV = 5.9 %

5. Linearity Study

The highest patient sample has been 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.

	Sample 13	SOLO BC14577	SOLO BC14578	SOLO BC14579				
Dilution NaCL 0.9 %	Theoretical	meas 1	meas 2	meas 3	mean	stabwn	cv	Recovery
100	201 mg/l	191	206	210	202 mg/l	10.02	4.95%	101%
80	161 mg/l	149	159	144	151 mg/l	7.64	5.07%	94%
60	121 mg/l	125	133	137	132 mg/l	6.11	4.64%	109%
40	80 mg/l	81	74	75	77 mg/l	3.79	4.94%	95%
20	40 mg/l	36	38	41	38 mg/l	2.52	6.57%	95%
10	20 mg/l	22	18	17	19 mg/l	2.65	13.93%	95%
5	10 mg/l	11	9	8	9 mg/l	1.53	16.37%	93%



The result for the correlation between theoretical and recovered (measured) values for Eurolyser cCRP-VET is the linear regression function y (measured) = 1.0011 x (theoretical) + 0.8329 and an $R^2 = 0.9925$.

6. Interferences of the Eurolyser cCRP Assay

Canine CRP samples of about 27 mg/l (sample no. 30) were spiked with 5 g/l haemoglobin or 10 g/l intralipid. The samples were compared to control samples containing saline and water instead of haemoglobin and intralipid, respectively. None of the spiked samples demonstrated interference – defined as more than 10 % difference between the test sample and control sample.

Sample No 30	(27mg/l)	Control sample	Test sample	Recovery	Instrument
Intralipid	(10mg/ml)	25	27	108%	SOLO BC14579
Haemoglobin	(5mg/ml)	27	25	93%	SOLO BC14580

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

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

Reproducibility	16 mg/l sample 14	Sample 14 diluted to 50%	Sample 36 diluted to 50%	
1	16 mg/l	8 mg/l	4 mg/l	SOLO BC14577
2	14 mg/l	6 mg/l	6 mg/l	SOLO BC14578
3	14 mg/l	9 mg/l	7 mg/l	SOLO BC14579
4	18 mg/l	10 mg/l	3 mg/l	SOLO BC14580
5	18 mg/l	7 mg/l	3 mg/l	SOLO BC14581
6	16 mg/l	9 mg/l	6 mg/l	CUBE Ca10627
7	17 mg/l	11 mg/l	5 mg/l	CUBE Ca10628
8	15 mg/l	8 mg/l	2 mg/l	CUBE Ca10629
9	18 mg/l	7 mg/l	4 mg/l	CUBE Ca10630
10	16 mg/l	12 mg/l	3 mg/l	SOLO BC14577
11	15 mg/l	8 mg/l	4 mg/l	SOLO BC14578
12	15 mg/l	8 mg/l	5 mg/l	SOLO BC14579
13	14 mg/l	9 mg/l	4 mg/l	SOLO BC14580
14	17 mg/l	11 mg/l	5 mg/l	SOLO BC14581
15	18 mg/l	10 mg/l	4 mg/l	CUBE Ca10627
16	17 mg/l	9 mg/l	6 mg/l	CUBE Ca10628
17	16 mg/l	8 mg/l	4 mg/l	CUBE Ca10629
18	16 mg/l	8 mg/l	4 mg/l	CUBE Ca10630
19	15 mg/l	7 mg/l	6 mg/l	SOLO BC14577
20	18 mg/l	9 mg/l	4 mg/l	SOLO BC14578
mean	16.15	8.70	4 mg/l	
stabwn	1.42	1.53	1.28	
cv	8.82%	17.53%	28.68%	

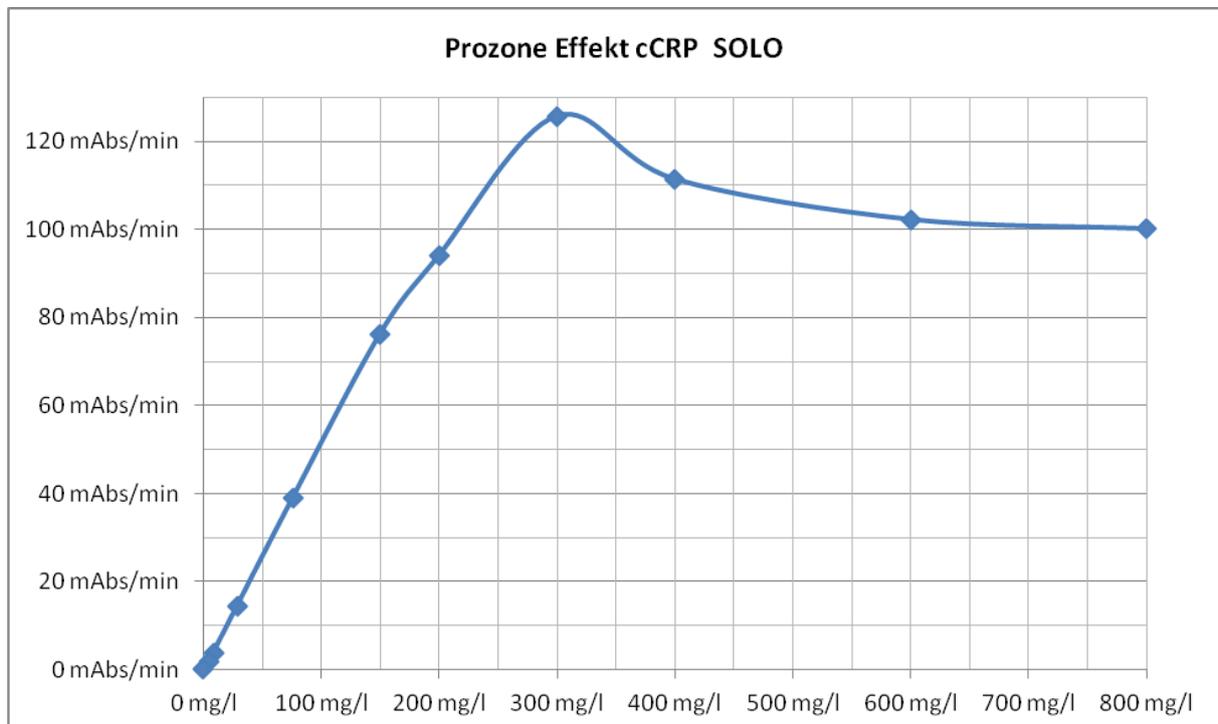
Estimated LOQ is set as 8 mg/l

8. Prozone Effect

Samples with a canine CRP concentration up to 800 mg/l return a value above 200 mg/l (upper limit of linearity range) and should be diluted for a rerun (4+1).

40 inc_190 sec Measinterval	
mg/l	mAbs/min
800 mg/l	100 mAbs/min
600 mg/l	102 mAbs/min
400 mg/l	111 mAbs/min
300 mg/l	126 mAbs/min
200 mg/l	94 mAbs/min
150 mg/l	76 mAbs/min
76 mg/l	39 mAbs/min
29 mg/l	14 mAbs/min
9 mg/l	4 mAbs/min
5 mg/l	2 mAbs/min
0 mg/l	0 mAbs/min

The following table shows the photometer raw data of high level life purified canine CRP:



9. Normal Values

Due to the higher sensitivity of the 2nd generation cCRP-VET assay and the lower LOQ the normal range can be reduced down to **10 mg/l** to increase the sensitivity of the assay.

It was tested if this reduced normal range can be confirmed if samples from potentially healthy dogs are measured. The target was to find none of those dogs with values above 10 mg/l

Potentially healthy dogs	Age in years	Gender	Breed	Eurolyser cCRP-VET	Instrument
1	2	Male	American bulldog	< 10 mg/l	SOLO BC14577
2	3	Male	Beagle	< 10 mg/l	SOLO BC14578
3	2	Female	Boxer	< 10 mg/l	SOLO BC14579
4	1	Male	Dalmatian	< 10 mg/l	SOLO BC14580
5	6	Male	Dobermann	< 10 mg/l	SOLO BC14581
6	6	Female	Elo	< 10 mg/l	CUBE Ca10627
7	2	Male	German Wirehaired pointer	< 10 mg/l	CUBE Ca10628
8	3	Male	Great Dane	< 10 mg/l	CUBE Ca10629
9	9	Female	Hanover Hound	< 10 mg/l	CUBE Ca10630
10	2	Male	Hovawart	< 10 mg/l	SOLO BC14577
11	2	Male	Irish setter	< 10 mg/l	SOLO BC14578
12	7	Male	Kromfohländer	< 10 mg/l	SOLO BC14579
13	3	Female	Labrador retriever	< 10 mg/l	SOLO BC14580
14	3	Male	German Sheperd dog	< 10 mg/l	SOLO BC14581
15	1	Male	Golden retriever	< 10 mg/l	CUBE Ca10627
16	1	Male	Labrador retriever	< 10 mg/l	CUBE Ca10628
17	2	Female	Boxer	< 10 mg/l	CUBE Ca10629
18	3	Male	German Sheperd dog	< 10 mg/l	CUBE Ca10630
19	2	Female	American bulldog	< 10 mg/l	SOLO BC14577
20	1	Male	Dobermann	< 10 mg/l	SOLO BC14578
21	1	Male	Dobermann	< 10 mg/l	SOLO BC14579
22	6	Male	Hanover Hound	< 10 mg/l	SOLO BC14580
23	6	Male	Manchester terrier	< 10 mg/l	SOLO BC14581
24	5	Female	German Sheperd dog	< 10 mg/l	CUBE Ca10627
25	5	Female	Münsterländer	< 10 mg/l	CUBE Ca10628
26	7	Male	Rottweiler	< 10 mg/l	CUBE Ca10629
27	8	Male	Hanover Hound	< 10 mg/l	CUBE Ca10630
28	9	Female	Golden retriever	< 10 mg/l	SOLO BC14577
29	3	Male	Labrador retriever	< 10 mg/l	SOLO BC14578
30	2	Male	Rottweiler	< 10 mg/l	SOLO BC14579
31	2	Female	Labrador retriever	< 10 mg/l	SOLO BC14580
32	1	Male	German Wirehaired pointer	< 10 mg/l	SOLO BC14578
33	4	Male	Dalmatian	< 10 mg/l	SOLO BC14579
34	5	Female	Beagle	< 10 mg/l	SOLO BC14580
35	6	Male	Labrador retriever	< 10 mg/l	SOLO BC14581

36	6	Male	German Sheperd dog	< 10 mg/l	CUBE Ca10627
37	2	Female	Golden retriever	< 10 mg/l	SOLO BC14578
38	3	Male	Labrador retriever	< 10 mg/l	SOLO BC14579
39	3	Male	German Sheperd dog	< 10 mg/l	SOLO BC14580
40	3	Female	Pomeranian	< 10 mg/l	SOLO BC14581
41	4	Male	Rottweiler	< 10 mg/l	CUBE Ca10627
42	6	Female	Labrador retriever	< 10 mg/l	SOLO BC14578
43	2	Female	Russian Toy	< 10 mg/l	SOLO BC14579
44	2	Male	Tyrolean Hound	< 10 mg/l	SOLO BC14580
45	7	Male	Golden retriever	< 10 mg/l	SOLO BC14581
46	2	Male	Labrador retriever	< 10 mg/l	CUBE Ca10627