ESVE Veterinary Endocrinology External Quality Assessment Scheme
ESVE REPORT

Release Month:	**May-18**
Release Number:	**012**
Species:	**Canine**

Overall Comments

General

This is the report of the twelth release of the ESVE EQA scheme. The efforts made by the participants to report their results were much appreciated. We had participation from 71 separate physical locations providing 476 analytical results. One registered participant did not return results for this release. The strength of a scheme such as this can only improve as more participants are recruited. If you are in contact with other laboratories that are generating veterinary endocrine analytical results that are not participants in the scheme, please encourage them to participate.

Although the numbers of participants within individual methodologies is still limted for some analytes, we can clearly seeing patterns of performance that should allow participants to get a feel for how their methods compare and in some cases that are raising questions that would be **best followed up by internal QC, reference range review and validation checks etc**

INSULIN: The data on this occasion continues to support previous concern that Siemems Immulite methods do not pick up canine insulin to the same extent that other methods can.

NEW STATISTICAL APPROACH FROM RELEASE 009 ONWARDS: Although we have low numbers of participants for some analytes, for others we now have sufficient to use more robust measures of mean and SD. From 009 onwards, the scheme uses a 10% trimmed (censored) set of analyte results to calcualte a robust trimmed mean and an appropriately adjusted standard deviation. The choice of 10% trimming means that analytes with n<20 participants (i.e., Oestradiol) will continue to be reviewed by traditional mean and standard deviation. Such an approach is common in EQA schemes and minimises the effect of very unusual results at the same time as retaining useful information about the distribution of the results submitted. The method used is that of Healy 1978 and 1979. The new statistical method has been retrospectively applied to results of previous releases for display in the participant report cummulative 6-cycle history window.

Healy (1979) Outliers in Clinical Chemistry Quality Control Schemes, Clinical Chemistry 25(5)675-677 http://clinchem.aaccjnls.org/content/25/5/675

Healy (1978) A mean difference standard deviation estimator in in symmetrically censored normal samples, Biometrika 65,643-646 https://doi.org/10.1093/biomet/65.3.643

We continue to be cautious with the public release of method names because of the limitations of so-far having only a small participant number but as was the case on previous releases we have highlighted a small number where it seems most relevant to do so.

NEW QUALITY COMPARATOR APPROACH FROM RELEASE 011 ONWARDS: The report now contains 2 approaches to the provision of "**quality goals**". For analytes that have had data published for biological variation (BV), it has been possible to determine "Allowable Total Error" (TEa) (see: http://vetbiologicalvariation.org/). TEa based Quality Specifications can be derived at "optimimal", "desirable", and "minimum" levels For those analytes for which TEa can be calculated from BV, participants will see a classification under the heading **"TEa (BV)"** that tells them whether their result (bias from the consensus mean) is within the the range for "optimal", "desirable" or "minimum" quality specifications or if the result falls outside the minimum specification ("Exceeds").

For those analytes for which BV has not been published, a different approach has been taken to derive candidate minimum quality specifications (cMQS) which was the maximum percentage bias from the consensus mean achieved by the closest 90% of analyses. Bias results for all participants, all releases and combined species were used in setting this cMQS.This specification will be reviewed and enhanced over time taking into account clinical relevance. They represent a "starting point" in quality specification for our scheme. Participants will see if their result is "Within" or "Exceeds" the cMQS under the heading **"cMQS-XX%"** where XX represents the combined Canine & Feline allowable bias for that analyte.

This Release

This was a canine serum pool.

Those of you familiar with other EQA schemes will recognise that the overall CV's we are seeing are high. By using robust measures for analytes with n>19, we are able to compare this scheme CV%'s to others more directly. On this release, 6 of 10 analytes were below 20% (Cortisol, Free T4, Fructosamine, Thyroxine, TSH, Creatinine) and 2 of these were below 10% (TSH, creatinine).A wide CV% makes sense for our peptide representative (Insulin) but it is concerning that we are seeing a high CV for steroids Progesterone, Oestradiol and Testosterone. However, all of these were at low concentartion in our sample. On a positive note, this release saw our best Cortisol, 2nd best Fructosamine, 2nd best Progesterone, 2nd best Testosterone and 3rd best TSH CV%. **It would be nice to believe that our scheme is having some impact!**

For those of you that are clinicians or that work closely with clinicians, these reports serve as a reminder to exercise caution in making significant clinical management decisions based on relatively modest differences in results and particulary when basing advice to third parties on laboratory results generated at locations or by equipment over which you have no control. Theoretically at least, we should feel relatively comfortable using literature reference ranges for steroids and non-species-specific analytes but these results indicate that we should be more cautious than we might expect to need to be. In this release a cortisol of 69 or 195 nmol/L could be obtained from the same sample depending on where the result originated.

As was the case in the previous releases and as has been the experience of the Michigan State University SCE EQUAS scheme, the range of results obtained for Oestradiol is tremendous. This is a notoriously difficult hormone to measure well which presents interpretative challenges.

Caution

It should be remembered that assays that are more commonly used may not turn out to be the ones that yield the most accurate results so at least for now, we may have to recognise that some of the methods with the most "outlying" results may not be the methods that are "wrong". Due to participant numbers, at present the target result for comparion is the All-method mean. It is accepted that this may be influenced by the distribution of methods. Where you method has several participants for a particular analyte, you should review your bias against that method mean.

Please note that the Method numbers bear no relationship to one another across analytes or releases. That is, for example, Immulite 1000, may be Method 1 for one analyte but Method 7 for another.

A simplistic way to check for the accuracy of your reconstitution of the freeze dried sample is to check if all your "SD Multiples" are consistently positive or consistently negative.

ESVE Veterinary Endocrinology External Quality Assessment Scheme
ESVE REPORT

Release Month:	**May-18**
Release Number:	**012**
Species:	**Canine**

Analytes:

Cortisol As was the case for previous releases, the overall range of results generated for cortisol continues to surprise; especially taking into account that this is not a species specific hormone and the general consensus among endocrinologists in the interpretation of cortisol results in suppression and stimulation tests. However, on this release there is much greater consensus than has been the case previously (CV% 12 compared to worst canine release 26%).It would be nice to believe we are successfully working towards a closer agreement among labs for this analyte - time will tell. In large human EQA schemes, CV for cortisol is 7-8% so we are getting closer now.

Fructosamine **The story for fructosamine is similar to the recent and much improved over previous performance** - we have had CV's as high as 39% (adj) in the past. However, at the extreme ends, the range of fructosamine results is still relatively wide when thinking about clinical application. Reference to the literature for diabetes diagnosis or monitoring cannot be recommended. Methods 6 (Cobas) , 2 (ABX) and 9 (Roche) are likely to be the same or similar sold under different (related company) names. These were also the brand names of methods used in the early 90's for the original veterinary fructosamine literature. Although there were only 2 participants there was also good agreement within Method 10 (Sentinel (Italy)).

Insulin As a peptide with some species differences, it is not too great a surprise to see variation in this analyte as different methods have different degrees of cross-reactivity between canine insulin and the method standards. This is an analyte where we should expect to see variation also in the reference ranges used by labs and clinicians should avoid textbook ranges for insulin and insulin:glucose ratios in reaching a diagnostic interpretation. **As has been the case in previous releases, the Immulite methods (Methods 7 and 8) yielded lower results than other methods.** The Immulite methods appear not to quantify low or normal insulin concentrations in dogs. That said, excluding the Immulite methods, there is reasonalbe consensus between 14 and 25 uIU/ml among the other methods. Several labs reported in pmol/L and their results were converted for statistical analysis to uU/ml using a human factor 7.175. One lab used an Equine insulin ELISA (Method 6).

Progesterone There was a range of results that would make interpretation difficult if the test was used for identifying pre-ovulatory luteinisation in bitches. However, given the low concentration in this sample, the consensus for the majority of labs is good and probably close to or within the intra-lab imprecision of many participants.

Thyroxine The adjusted all-method CV% achieved on this release was reasonable. However, the range of results obtained continues to surprise given that several participants would be reporting results below commonly used lower limits of the canine reference interval

Free T4 On a theoretical basis, the methods using dialysis or LC-MSMS should yield the Free T4 results closest to the true value. We had two participants use one dialysis method in this release (Method 3; 11.7 and 14.3 pmo/l) close to the consensus but one other dialysis method gave a different result (22.3 pmol/l). This release did not conatin any challenging conditions such as TgAA/T4AA suggesting several assays function well in "typical" serum. This may not be the case in atypical serum (T4AA, Non-thyroidal illness)

Oestradiol The variation in results obtained for Oestradiol is a well known phenomenon to anyone participating in the MSU/SCE EQUAS scheme. Methodologic and calibration differences along with poor low-end sensitivity have been considered to play their part. Some laboratories are using extraction procedures to improve their analyses. There should be considerable caution in interpreting oestradiol results against literature ranges particularly where oestradiol is being used in isolation to support diagnoses of adrenal dysfunction. As can be seen from the SD's for Methods 5, 6 and 7 laboratory environment/technique as well as assay method contributes significantly to the results generated. As a pooled sample of serum from many dogs, we would not expect the E2 concentration to be high as dogs that are in oestrus would be a low proportion of the dog samples included.

Testosterone This was our second best Testosterone CV so far and with the exception of 3 labs, reasonable consensus. Litetature based cut-off values for the detection of functional testicular tissue (e.g. 0.5 nmol/L) would suggest a reasonable proportion of intact males in our serum pool on this occasion.

TSH As is ususally the case, given the heavy industry reliance on automated platforms from a single supplier, the imprecision for TSH is impressive.

Creatinine The overall range of results is wide but this is heavily influence by only a small number of extreme results> In general there is good consensus (2nd best CV% so far)

Peter Graham, Program Coordinator, July 2018

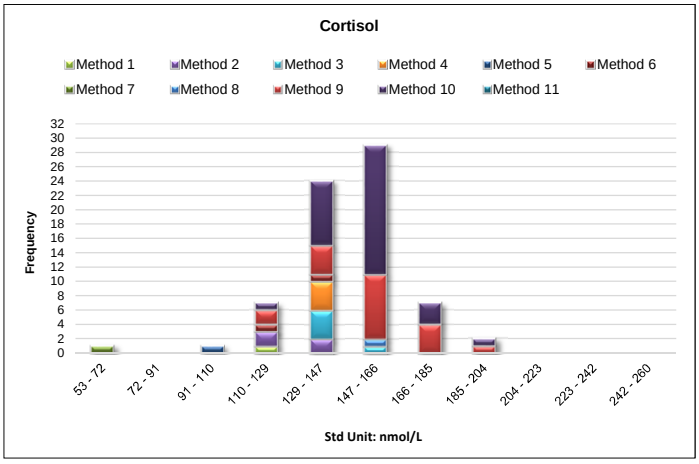
ESVE Veterinary Endocrinology External Quality Assessment Scheme
ESVE REPORT

Release Month:	**May-18**
Release Number:	**012**
Species:	**Canine**

Analyte results

Cortisol

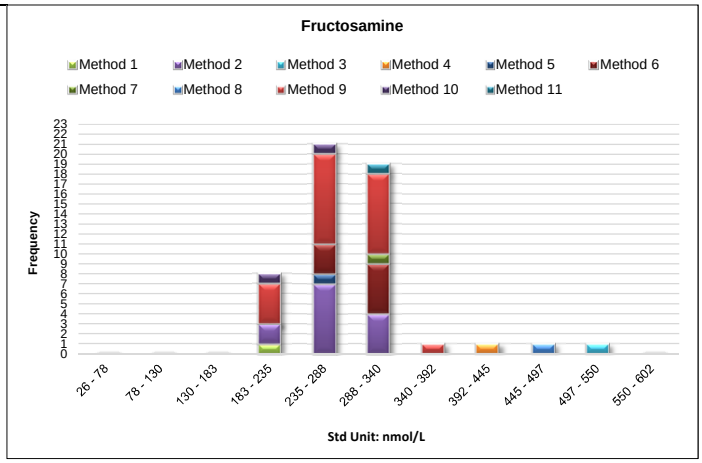
	n	Mean	StDev	%CV
Method 1	1	116		
Method 2	4	126	12.6	9.9
Method 3	5	143	8.3	5.8
Method 4	4	135	5.9	4.4
Method 5	1	98		
Method 6	2	127	22.4	17.7
Method 7	1	70		
Method 8	1	164		
Method 9	20	155	19.2	12.4
Method 10	32	153	13.2	8.6
Method 11	0			
Method 12	0			
		Trimmed	*Adjusted*	
All Methods	**71**	**148**	**18.1**	**12.2**



Note: Reported results ranged from 69 to 195 nmol/l.
Although Method 7 yeilded a low result compared to the consensus, it is reported against a lower reference range than most other labs

Fructosamine

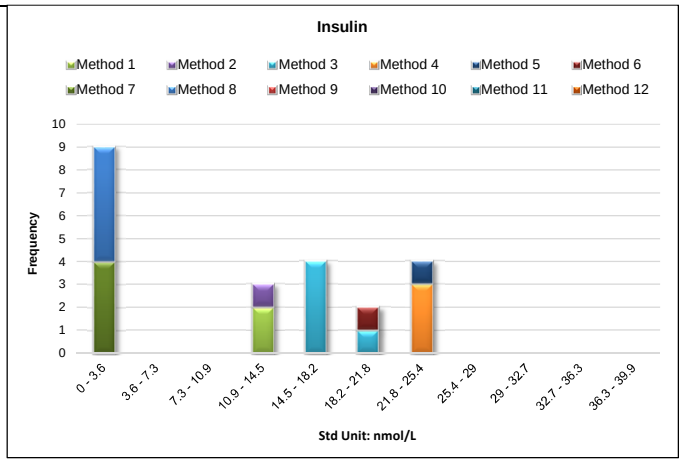
	n	Mean	StDev	%CV
Method 1	1	192		
Method 2	13	272	31.2	11.5
Method 3	1	541		
Method 4	1	395		
Method 5	1	237		
Method 6	8	285	25.3	8.9
Method 7	1	332		
Method 8	1	463		
Method 9	22	278	35.3	12.7
Method 10	2	238	10.5	4.4
Method 11	1	297		
Method 12	1	370		
		Trimmed	*Adjusted*	
All Methods	**53**	**282**	**43.5**	**15.4**



Note: Reported results ranged from 192 to 541 umol/L.

Insulin

	n	Mean	StDev	%CV
Method 1	2	14.4	0.12	0.8
Method 2	1	13.6		
Method 3	5	18.1	1.19	6.6
Method 4	3	22.9	1.62	7.0
Method 5	1	25.2		
Method 6	1	18.7		
Method 7	4	2.1	0.73	35.2
Method 8	5	3.0	0.40	13.3
Method 9	0			
Method 10	0			
Method 11	0			
Method 12	0			
		Trimmed	*Adjusted*	
All Methods	**22**	**12.1**	**9.86**	**81.5**



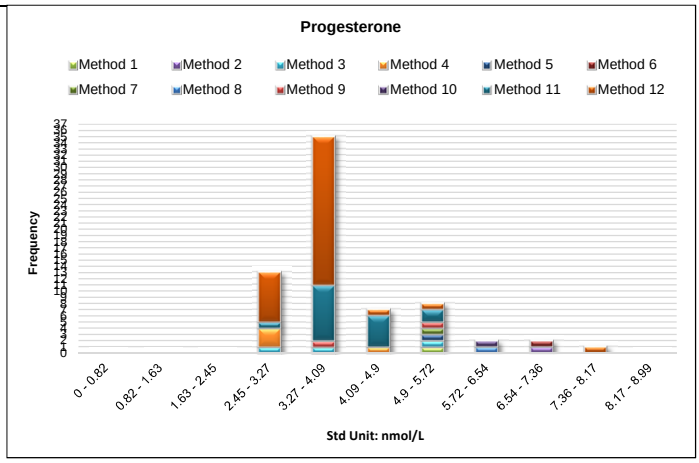
Note: Reported results ranged from below the limit of detection to 25 uU/ml
Methods 7 & 8 were Siemens Immulite. Three labs (Methods 7&8) commented that they knew their method was onl;y appropriate for horses

ESVE Veterinary Endocrinology External Quality Assessment Scheme
ESVE REPORT

Release Month:	**May-18**
Release Number:	**012**
Species:	**Canine**

Progesterone

	n	Mean	StDev	%CV
Method 1	1	5.2		
Method 2	1	7.3		
Method 3	3	3.8	1.08	28.8
Method 4	4	3.2	0.68	21.2
Method 5	1	5.0		
Method 6	1	6.9		
Method 7	1	5.2		
Method 8	1	5.9		
Method 9	2	4.2	1.12	26.5
Method 10	1	5.9		
Method 11	17	4.0	0.62	15.6
Method 12	35	3.7	0.87	23.8
		Trimmed	*Adjusted*	
All Methods	**68**	**3.9**	**0.85**	**21.9**

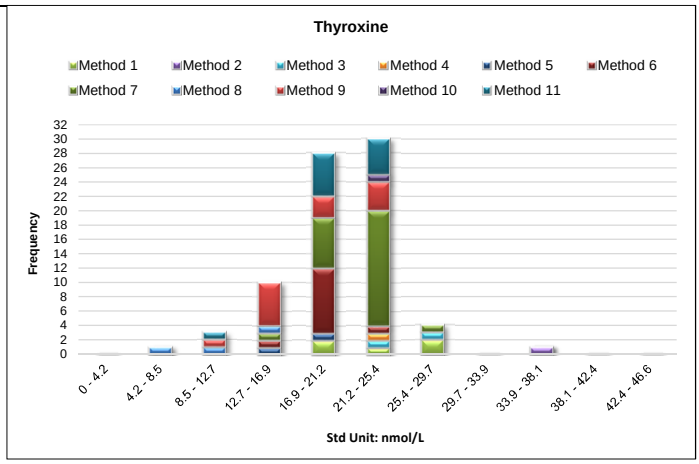


Note: Reported results ranged from 2.5 to 8 nmol/L
The most popular method (Method 12) was Siemens Immulite 2000; Method 11 was Immulite 1000

For statistical purposes, results lower than reportable limit have been converted to a value 0.5 x lowest reportable limit

Thyroxine

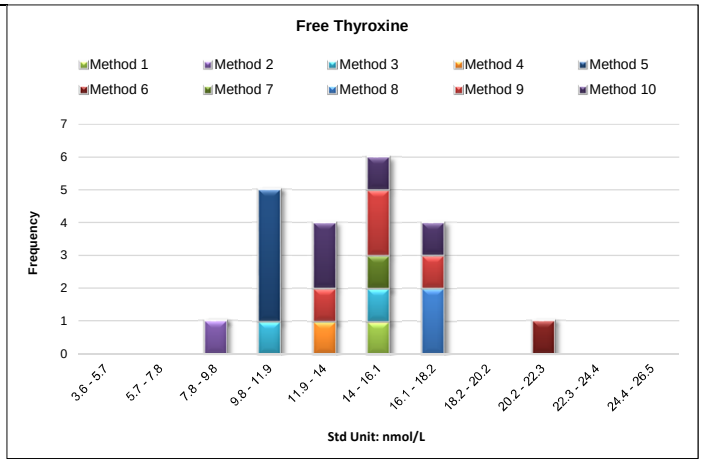
	n	Mean	StDev	%CV
Method 1	5	22.9	3.35	14.6
Method 2	1	34.7		
Method 3	2	25.5	2.37	9.3
Method 4	1	22.3		
Method 5	2	17.6	5.00	28.5
Method 6	11	19.4	2.11	10.9
Method 7	25	21.9	2.03	9.3
Method 8	3	11.2	4.32	38.6
Method 9	14	17.1	4.31	25.2
Method 10	1	25.4		
Method 11	12	19.9	3.46	17.4
Method 12	0			
		Trimmed	*Adjusted*	
All Methods	**77**	**20.3**	**3.85**	**19.0**



Note: Reported results ranged from "below the limit of detection" to 35 nmol/L.
Methods 5, 6 and 7 were "canine" methods (Immulite). Method 11 was a homologous assay (Thermo Microgenics DRI).

Free T4

	n	Mean	StDev	%CV
Method 1	1	14.2		
Method 2	1	8.4		
Method 3	2	13.0	1.84	14.1
Method 4	1	12.0		
Method 5	4	11.1	0.74	6.7
Method 6	1	22.3		
Method 7	1	14.8		
Method 8	1	14.8		
Method 9	2	17.4	0.93	5.4
Method 10	2	13.7	1.07	7.8
Method 11	4	14.8	1.55	10.5
Method 12	13	14.0	1.98	14.2
		Trimmed	*Adjusted*	
All Methods	**34**	**13.8**	**2.37**	**17.2**



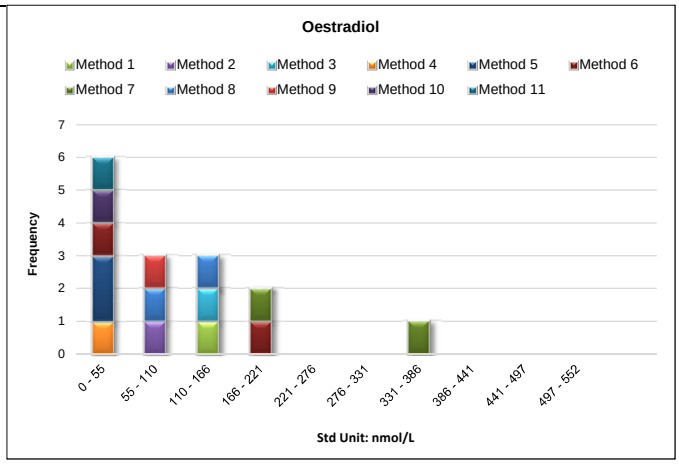
Note: Reported results ranged from 8 to 22 pmol/L.
A FT4 result by equilibrium dialysis was reported by 3 laboratories (Method 3; 11.7 and 14.3. Method 6; 22.3 pmol/l)
Methods 11 and 12 were "veterinary" methods. Method 7 was performed by LC-MSMS

ESVE Veterinary Endocrinology External Quality Assessment Scheme
ESVE REPORT

Release Month:	**May-18**
Release Number:	**012**
Species:	**Canine**

Oestradiol

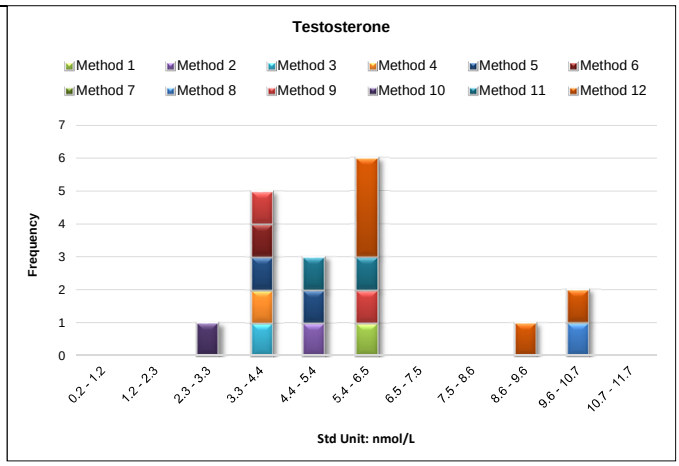
	n	Mean	StDev	%CV
Method 1	1	121		
Method 2	1	94		
Method 3	1	140		
Method 4	1	51		
Method 5	2	29	23.6	80.9
Method 6	2	101	103.2	102.2
Method 7	2	287	114.9	40.1
Method 8	2	130	33.7	26.0
Method 9	1	82		
Method 10	1	34		
Method 11	1	0		
Method 12	0			
All Methods	**15**	**108**	**94.6**	**87.6**



Note: Reported results ranged from "below the limit of detection" to 368 pmol/L.
Method 8 was "In-house RIA or EIA" so these results may not be directly comparable with one another.

Testosterone

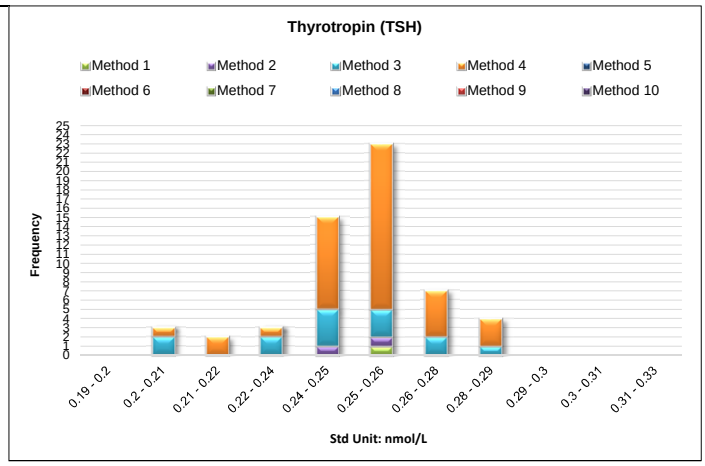
	n	Mean	StDev	%CV
Method 1	1	5.8		
Method 2	1	5.4		
Method 3	1	4.0		
Method 4	1	4.2		
Method 5	2	4.1	0.73	17.8
Method 6	1	4.0		
Method 7	3	4.2	0.22	5.3
Method 8	1	9.7		
Method 9	2	4.8	1.67	34.9
Method 10	1	2.9		
Method 11	2	5.4	0.64	11.8
Method 12	5	7.4	1.86	25.1
		Trimmed	*Adjusted*	
All Methods	**21**	**5.3**	**1.89**	**35.7**



Note: Reported results ranged from below 2.9 to 9.9 nmol/L

TSH

	n	Mean	StDev	%CV
Method 1	1	0.26		
Method 2	2	0.25	0.004	1.4
Method 3	14	0.24	0.025	10.0
Method 4	40	0.25	0.016	6.5
Method 5	0			
Method 6	0			
Method 7	0			
Method 8	0			
Method 9	0			
Method 10	0			
Method 11	0			
Method 12	0			
		Trimmed	*Adjusted*	
All Methods	**58**	**0.25**	**0.017**	**6.8**



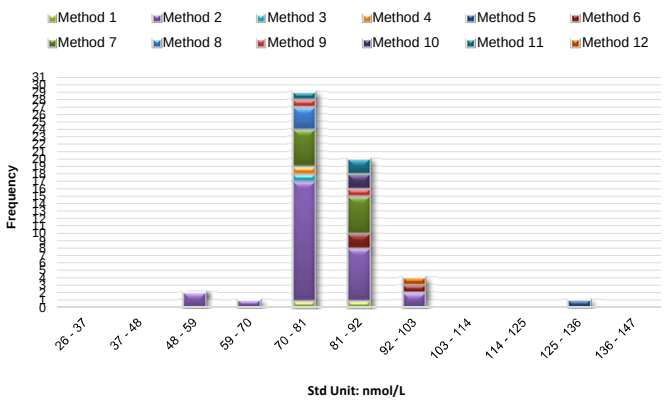
Note: Reported results ranged from 0.20 to 0.29 ng/ml. One result of 1ng/ml was exclued from statistical analysis
Methods 2, 3, and 4 represent the same manufacturer's chemiluminescent assay on 3 platforms (Siemens Immulite)

ESVE Veterinary Endocrinology External Quality Assessment Scheme
ESVE REPORT

Release Month:	**May-18**
Release Number:	**012**
Species:	**Canine**

Creatinine

	n	Mean	StDev	%CV
Method 1	2	79	4.1	5.2
Method 2	28	78	10.2	13.2
Method 3	1	80		
Method 4	1	76		
Method 5	1	126		
Method 6	3	90	8.7	9.6
Method 7	10	82	5.9	7.1
Method 8	3	79	0.4	0.6
Method 9	2	82	6.4	7.8
Method 10	2	86	1.5	1.8
Method 11	3	82	1.0	1.2
Method 12	1	93		
		Trimmed	*Adjusted*	
All Methods	**57**	**81**	**7.9**	**9.8**



Note: Reported results ranged from 53 to 126 umol/L.
Method 8 (with exceptional within method CV%) is an Enzymatic creatinine (non Jaffe)