ESVE Veterinary Endocrinology External Quality Assessment Scheme
ESVE REPORT

Release Month:	**May-19**
Release Number:	**014**
Species:	**Canine**

Overall Comments

General

This is the report of the fourteenth release of the ESVE EQA scheme. Welcome to new participants! The efforts made by participants to report their results were much appreciated. We had participation from 76 separate physical locations providing 566 analytical results. **Disappointingly six registered participants did not return results for this release causing unecessary expenditure on sample preparation and shipping**. 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. It was pleasing to see the participation of a few quality-conscious sites using in-clinic analysers.

Although the numbers of participants within individual methodologies is still limited 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**

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.

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.

Statisitics

Although we have low numbers of participants for some analytes, for others we have sufficient to use robust measures of mean and SD. 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.

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

Quality Goals

The report 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). These are 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. No quality goals have been set for PTH and ACTH.

This Release

This was an unmodified 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 other schemes more directly. On this release, 7 analytes had CV% at or below 20% (Cortisol, Free T4, Fructosamine, Progesterone, 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 when we see a high CV for non peptides.

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 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 42 or126 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 your 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.

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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 sometimes been the case previously (CV% 13 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.

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% 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. In this report Roche and Cobas methods have been reported as a single method (Method 11). These were also the brand names of methods used in the early 90's for the original veterinary fructosamine literature. A reasonable all-method CV% has been maintianed despite some labs having to change reagents. In some markets it appears that RocheCobas reagents are no longer available for open-channel application on other brands of analyser and about a third of these reagent users have moved to other methods.

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. One lab reported in pmol/L and their results were converted for statistical analysis to uU/ml using a human factor 7.175. Two labs used an Equine insulin ELISA (Method 10). All-method CV% has improved although this may reflect the labs that failed to return results being mostly Immulite insulin users.

Progesterone This sample was of low progesterone concentration and there a reasonable agreement in results. The oddly high results we have seen on previous releases were not apparent on this occasion hopefully implying that methodology has been corrected, modified or replaced since the last release.

Thyroxine The adjusted all-method CV% achieved on this release was reasonable. However, the range of results obtained continues to surprise particularly in respect of common lower reference limits used for the diagnosis of hypothyroidism. On this occasion there were several unusually low results.

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 (13.6 and 19.5) and one LC-MSMS (Method 11; 13pmol/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, NTI)

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 with more than one participant, 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 and most labs agreed by generating low or below detection limit results (12 of 18).

Testosterone This was a low Testosterone serum pool making it challenging to achieve good CVs.

TSH As has been the case in previous releases, the heavy rleiance among labs on automated platforms from a single supplier contributes to the impreesive all-method CV. This release saw the paricipation of 3 non-Immulite methods including 2 in-clinic methods. Unfortunately, for both the in-clinic methods, their limit of detection (0.25ng/ml) was close to and slightly higher than the concensus mean and so it was not clear how well thiese methods might otherwise agree with the reference lab equipment.

Creatinine The overall range of results is wide but this is heavily influenced by only a small number of extreme results. In general there is good consensus between 70 and 100 unmol/l

ACTH This sample was not modified to contain measurable quantities of labile ACTH so not surprisingly, most labs generated "undetectable" results.

PTH This sample was not modified to contain measurable quantities of labile PTH. Several labs generated low or undetecable results. so not surprisingly, most labs generated "undetectable" results.

Peter Graham, Program Coordinator, July 2019 (Updated December 2019)

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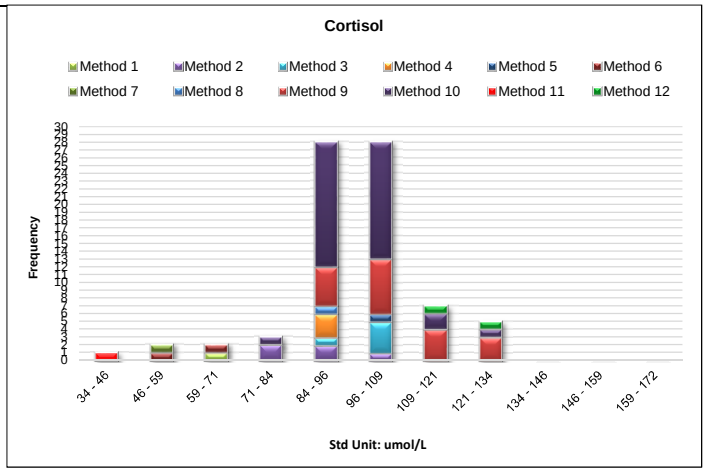
Analyte results

Cortisol

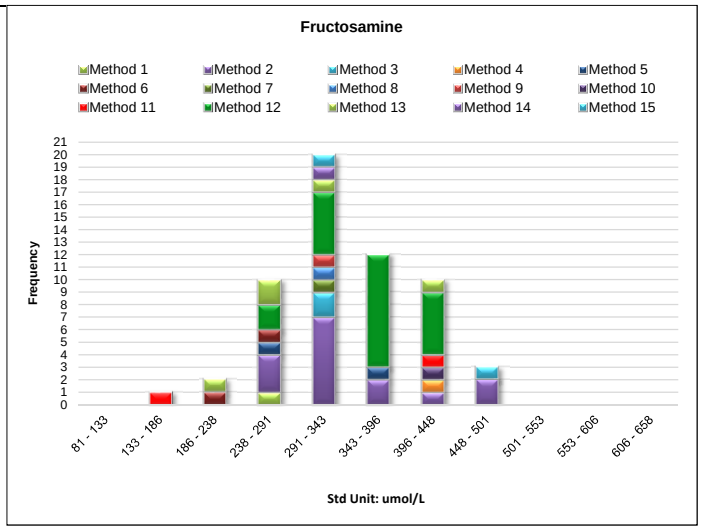
	n	Mean	StDev	%CV
Method 1	1	69		
Method 2	5	88	10.8	12.2
Method 3	5	101	2.9	2.9
Method 4	3	90	1.9	2.1
Method 5	1	107		
Method 6	2	56	10.2	18.3
Method 7	1	58		
Method 8	1	95		
Method 9	17	106	12.8	12.1
Method 10	35	96	8.2	8.5
Method 11	1	42		
Method 12	1	125		
		Trimmed	*Adjusted*	
All Methods	**73**	**97**	**12.6**	**13.0**



Note: Reported results ranged from 42 to 126nmol/L
Although Method 11 yeilded a low result compared to the consensus, it is reported against a lower reference range than most other labs
Methods 7 & 8 were in-clinc analysers

Fructosamine

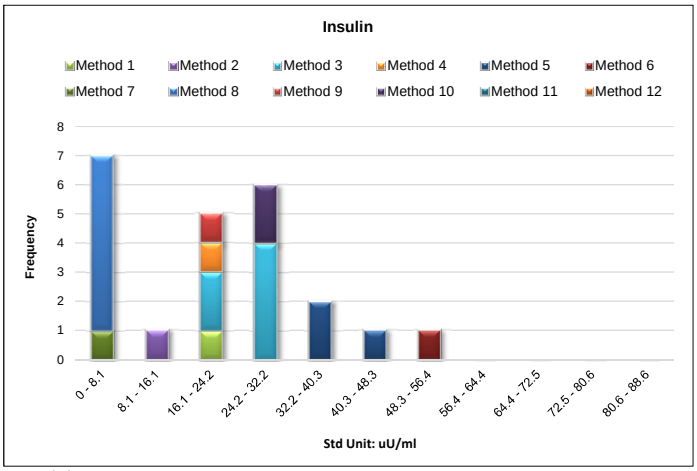
	n	Mean	StDev	%CV
Method 1	1	265		
Method 2	15	350	61.2	17.5
Method 3	2	305	15.9	5.2
Method 4	1	444		
Method 5	2	328	66.5	20.3
Method 6	2	243	18.4	7.6
Method 7	1	315		
Method 8	1	301		
Method 9	1	397		
Method 10	2	273	188.5	69.0
Method 11	20	370	51.1	13.8
Method 12	5	294	78.2	26.5
Method 13	1	314		
Method 14	2	406	105.4	26.0
Method 15	3	364	39.6	10.9
		Trimmed	*Adjusted*	
All Methods	**59**	**346**	**66.1**	**19.1**



Note: Reported results ranged from 140 to 490umol/L
Method 6 was an in-clinic analyser method. Method 12 was Roche/Cobas

Insulin

	n	Mean	StDev	%CV
Method 1	1	19.0		
Method 2	1	15.8		
Method 3	6	25.1	1.89	7.5
Method 4	1	23.6		
Method 5	3	39.3	2.52	6.4
Method 6	1	54.7		
Method 7	1	1.0		
Method 8	6	1.3	0.65	51.1
Method 9	1	23.3		
Method 10	2	25.9	1.32	5.1
Method 11	1	25.0		
Method 12	0			
		Trimmed	*Adjusted*	
All Methods	**24**	**19.8**	**15.09**	**76.2**



Note: Reported results ranged from below the limit of detection to 54.7uU/ml
Methods 7 & 8 were Siemens Immulite. Two labs (Methods 7&8) commented that they knew their method was only appropriate for horses

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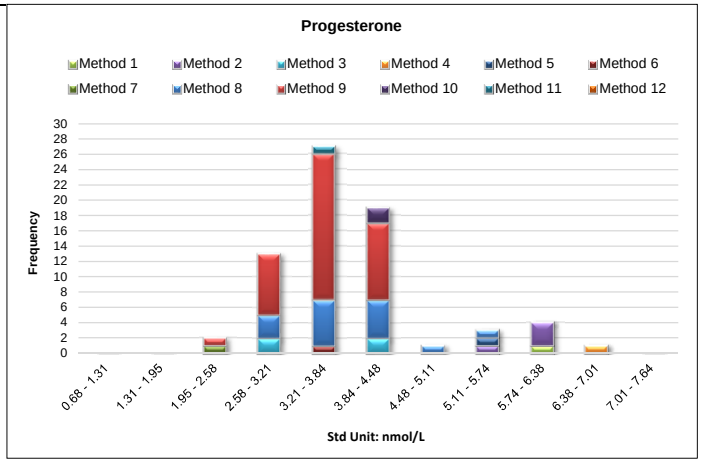
Progesterone

	n	Mean	StDev	%CV
Method 1	1	6.2		
Method 2	4	5.9	0.32	5.4
Method 3	4	3.4	0.82	23.8
Method 4	1	6.5		
Method 5	1	5.2		
Method 6	1	3.8		
Method 7	1	2.3		
Method 8	14	3.8	0.73	19.4
Method 9	38	3.5	0.40	11.4
Method 10	2	3.9	0.02	0.5
Method 11	1	3.8		
Method 12	0			
		Trimmed	*Adjusted*	
All Methods	**68**	**3.8**	**0.76**	**20.0**

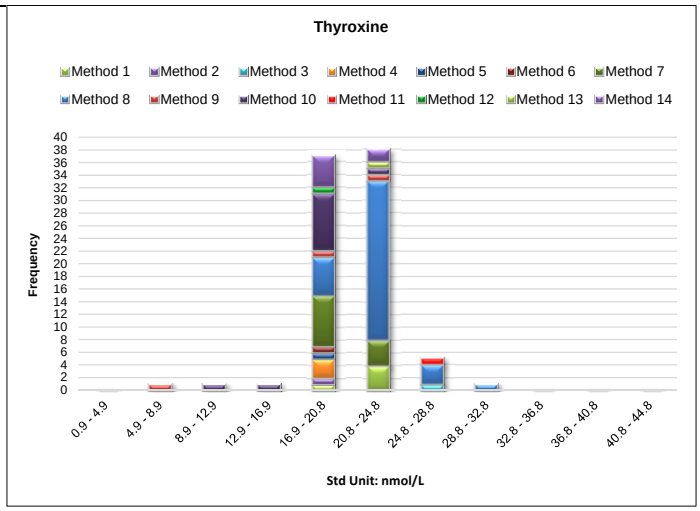


Note: Reported results ranged from 2.28 to 6.5nmol/L
The most popular method (Method 9) was Siemens Immulite 2000; Method 8 was Immulite 1000

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	22.2	1.52	6.9
Method 2	1	19.2		
Method 3	1	25.7		
Method 4	3	19.4	1.38	7.1
Method 5	1	17.4		
Method 6	1	18.0		
Method 7	2	18.9	1.19	6.3
Method 8	10	20.3	1.43	7.0
Method 9	35	22.7	2.64	11.6
Method 10	2	14.1	11.74	83.3
Method 11	12	17.6	2.13	12.1
Method 12	1	27.0		
Method 13	1	23.0		
Method 14	6	19.8	2.64	13.4
		Trimmed	*Adjusted*	
All Methods	**81**	**21.0**	**2.91**	**13.9**



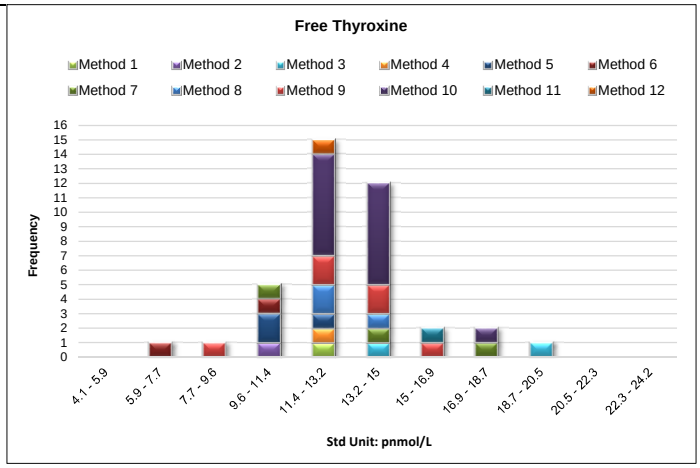
Note: Reported results ranged from <11.5 to 32.4nmol/L
Methods 7 and 8 were "canine" methods (Immulite). Method 14 was a homologous assay (Thermo Microgenics DRI). Methods 5 & 6 were in-clinc analysers

Free T4

	n	Mean	StDev	%CV
Method 1	1	12.1		
Method 2	1	10.7		
Method 3	2	16.6	4.17	25.2
Method 4	1	12.1		
Method 5	3	11.4	1.25	11.0
Method 6	2	8.9	2.23	25.0
Method 7	2	16.2	2.93	18.1
Method 8	3	13.3	0.44	3.3
Method 9	5	13.0	2.49	19.1
Method 10	15	13.8	1.40	10.2
Method 11	1	12.9		
Method 12	0			
		Trimmed	*Adjusted*	
All Methods	**36**	**13.3**	**2.03**	**15.3**

		SD multiple	Classification	cMQS-40%
Your result	13.6	0.15	<2SD	Within
Your 2nd result	NONE		NONE	

Your Method Method 3 Antech FT4D
Your 2nd Method



Note: Reported results ranged from 7.3 to 19.5pmol/l
A FT4 result by equilibrium dialysis was reported by 3 laboratories (Method 3; 13.6 and 19.5 and Method 11; 15.6 pmol/l)
Methods 9 and 10 were "veterinary" methods. Method 12 was performed by LC-MSMS

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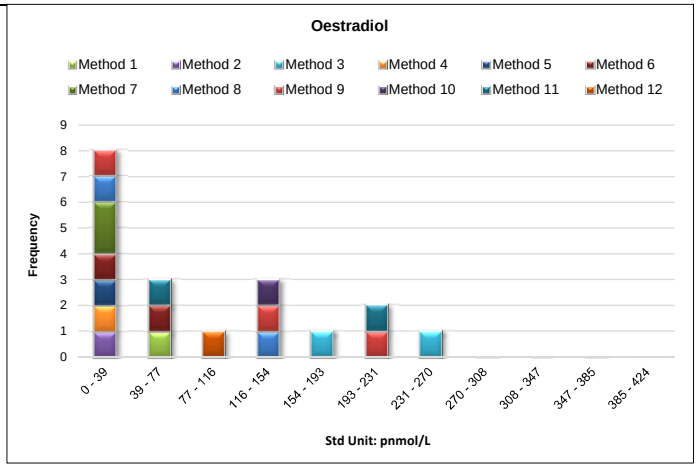
Oestradiol

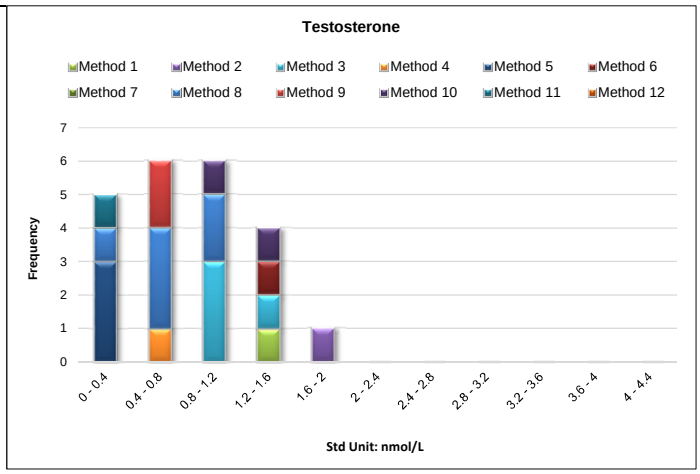
	n	Mean	StDev	%CV
Method 1	1	55		
Method 2	1	0		
Method 3	2	213	62.4	29.3
Method 4	1	10		
Method 5	1	25		
Method 6	2	38	41.1	108.0
Method 7	2	4	2.1	53.0
Method 8	2	89	72.6	81.6
Method 9	2	76	56.3	74.3
Method 10	1	127		
Method 11	2	131	122.5	93.5
Method 12	1	97		
All Methods	**18**	**79**	**77.5**	**98.1**



Note: Reported results ranged from below the limit of detection to 257pmol/l
Method 11 was "In-house RIA or EIA" so these results may not be directly comparable with one another.

Testosterone

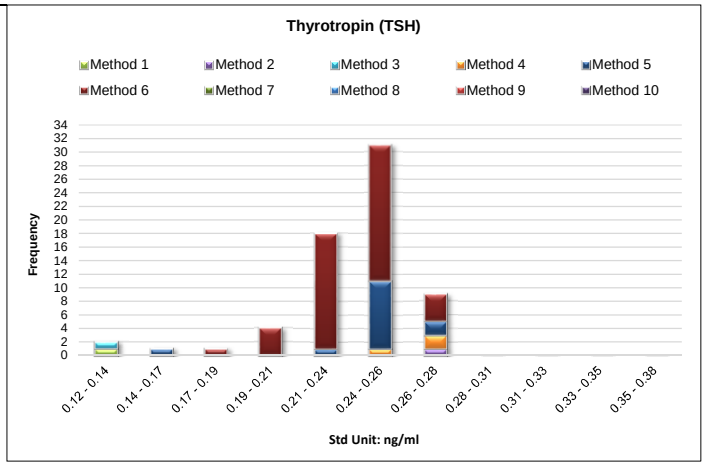
	n	Mean	StDev	%CV
Method 1	1	1.5		
Method 2	1	1.7		
Method 3	4	1.2	0.27	23.1
Method 4	1	0.5		
Method 5	3	0.3	0.06	17.3
Method 6	1	1.4		
Method 7	2	2.1	0.84	40.7
Method 8	6	0.6	0.37	56.5
Method 9	1	0.5		
Method 10	2	1.3	0.40	30.9
Method 11	1	0.0		
Method 12	0			
		Trimmed	*Adjusted*	
All Methods	**23**	**0.9**	**0.61**	**67.8**



Note: Reported results ranged from 0 to 2.7nmol/l

TSH

	n	Mean	StDev	%CV
Method 1	1	0.1		
Method 2	1	0.3		
Method 3	1	0.1		
Method 4	3	0.3	0.02	5.9
Method 5	12	0.2	0.03	12.3
Method 6	46	0.2	0.02	7.8
Method 7	0			
Method 8	0			
Method 9	0			
Method 10	0			
Method 11	0			
Method 12	0			
		Trimmed	*Adjusted*	
All Methods	**64**	**0.24**	**0.022**	**9.2**



Note: Reported results ranged from below the limit of detection (<0.25ng/ml) to 0.28ng/ml.
Methods 4, 5 and 6 represent the same manufacturer's chemiluminescent assay on 3 platforms (Siemens Immulite). Methods 1 & 3 were in-clinic analysers

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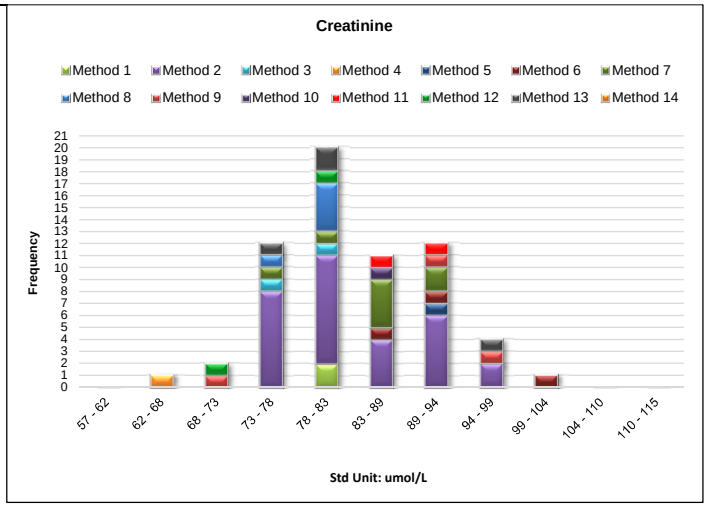
Creatinine

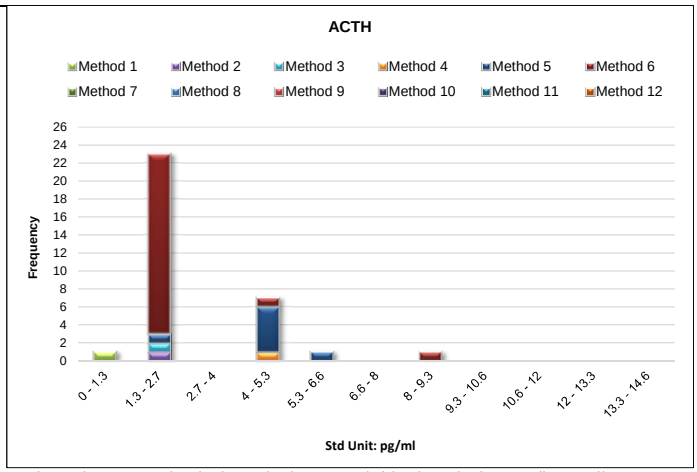
	n	Mean	StDev	%CV
Method 1	2	80.7	2.13	2.6
Method 2	28	83.1	6.63	8.0
Method 3	2	76.5	3.54	4.6
Method 4	1	64.5		
Method 5	1	93.5		
Method 6	3	92.2	6.68	7.2
Method 7	8	86.2	4.38	5.1
Method 8	5	79.4	3.37	4.2
Method 9	3	85.4	12.69	14.9
Method 10	1	84.0		
Method 11	2	87.9	4.34	4.9
Method 12	2	77.3	6.88	8.9
Method 13	4	84.3	7.09	8.4
Method 14	0			
		Trimmed	*Adjusted*	
All Methods	**62**	**83**	**7.3**	**8.8**



Note: Reported results ranged from 65 to 100umol/l
Methods 8 and 12 were an Enzymatic creatinine (non Jaffe) methods. Method 3 was an in-clinic analyser

ACTH

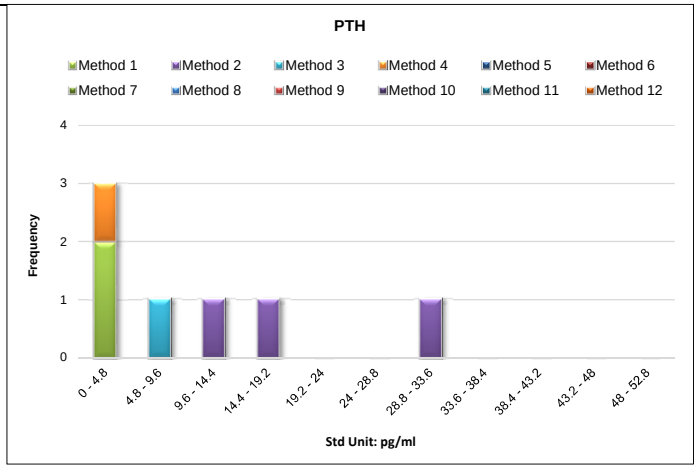
	n	Mean	StDev	%CV
Method 1	1	0.2		
Method 2	1	2.5		
Method 3	1	2.5		
Method 4	1	4.0		
Method 5	6	4.6	1.02	22.3
Method 6	22	2.9	1.48	50.7
Method 7	0			
Method 8	0			
Method 9	0			
Method 10	0			
Method 11	0			
Method 12	0			
		Trimmed	*Adjusted*	
All Methods	**32**	**3.10**	**0.890**	**28.7**



Note: All but 3 results were below their assay limit of detection. (Numeric results were 2.5 (Method 3: Cobas), 5 & 9 pg/ml (Both Method 6 Immulite 2000))
The most popular method (Method 6) was Immulite 2000. Method 5 is Immulite 1000.

PTH

	n	Mean	StDev	%CV
Method 1	2	1.5	2.12	141.0
Method 2	3	20.0	10.82	54.1
Method 3	1	6.7		
Method 4	1	2.5		
Method 5	0			
Method 6	0			
Method 7	0			
Method 8	0			
Method 9	0			
Method 10	0			
Method 11	0			
Method 12	0			
All Methods	**7**	**10**	**11.2**	**108.5**



Note: Reported results ranged from below the limit of detection (2 labs; Methods 1 and 4) to 32pg/ml
Method 1 was Immulite 2000. Method 2 was a canine ELISA.

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