ESVE Veterinary Endocrinology External Quality Assessment Scheme
ESVE REPORT

Release Month:	**May-17**
Release Number:	**010**

Overall Commentary

General

This is the report of the tenth release of the ESVE EQA scheme. The efforts made by the participants to report their results were much appreciated. We had participation from 49 separate physical locations providing 400 analytical results. Two registered participants 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, we are already 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: We now have a sufficient number of participants to state more empahtically that Siemems Immulite methods do not pick up canine insulin. Clinical anecdotes support missed insulinoma diagnoses with this method (Sue Foster, Murdoch Australia). Research papers suggest stimulated (greatly increased) concentrations may be detected (Zeugswetter 2012, JVECC) and the clinically relevant increase in equine insulin appears to be sucessfully detected

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 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. From release 010, the new statistical method has been 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.

This Release

This was a 25% concentrated otherwise unadulterated canine serum pool.

Those of you familiar with other EQA schemes will recognise that the overall CV's we are seeing are high. To some extent this is due the scheme using raw CV%'s and comparing them to human schemes that use robust measures of dispersion. Now that robust measures have been implemented for analytes with n>19, we will be able to compare this scheme CV%'s to others more directly. On this release, Cortisol, Total T4 and Progesterone adjusted CV's are below 10%. A wide CV% makes sense for our peptide representative (insulin) but it is concerning that we are seeing a high CV for steroids Oestradiol and Testosterone. On a positive note, this release saw our best Cortisol, Thyroxine and Progesterone CV's. For Fructosamine, this is our 3rd best CV%.

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 48 or 241 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".

Please note that the Method numbers bear no relationship to one another across analytes. 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.

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, when focussing on the majority of results rather than the extremes, the performance looks reasonable and is much improved over previous releases. This is our second best cortisol CV yet at 9.7% (adj; 18.8% raw). 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 close now we are using trimmed and adjusted means and SD's..

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Fructosamine **The story for fructosamine is much improved over many previous releases** - we have had canine CV's as high as 39% (adj) in the past. However, the range of fructosamine results is still relatively wide and reference to the literature for diabetes diagnosis or monitoring cannot be recommended. Of 26 participants that provided an upper reference limt for canine fructosamine, 21 reported a result above that limit. There was no relationship between the result reported and the upper limit of the reference ranges used (Slope -0.051, R-sq <0.0007) suggesting comparison to local ranges and cut-off's may still be problematic. Methods 5 (Cobas) , 2 (ABX) and 10 (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 they had only 2 participants each there was good agreement within Method 9 (Randox) and Method 11 (Sentinal (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 (n=13; Methods 11 and 12) yielded much lower results than other methods (all <2.5uU/ml).** The Immulite methods do not appear to quantify low or normal insulin concentrations in dogs.Several labs reported in pmol/L and their results were converted for statistical analysis to uU/ml using a human factor 7.175 from the manufacturer's package insert (Methods 1 and 12). One lab used an Equine insulin ELISA (Method 7) and their ng/L result was converted to uU/ml using a manufacturer supplied factor of 0.101

Progesterone There was a wide range of results but the performance was good (CV 6.6%) when the most extreme results were removed for robust statistical analysis. However, despite the relatively narrow CV, the variation in results is concerning because of the very divergent advice that would be given when used for e.g., the timing of mating using the princicple of pre-ovulatory luteinisation in dogs.

Thyroxine The adjusted all-method CV% achieved on this release was excellent. However, the range of reuslts obtained continues to surprise - the most extreme reslts would cause a divergent recommendation with regard to thyroid function. Methods 7 (Immulite 1000 Canine TT4) yelded a CV below 10%.

Free T4 On a theoretical basis, the methods using dialysis should yield the Free T4 results closest to the true value. Unfortunately, we have only two participant using such a method in this release (Method 2; 10.2 and 20.2 pmo/l) and their results were at opposite ends of the range of reported values.

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. Interestingly, once again, one ELISA (Method 7) yeilded both the second highest and second lowest results confirming that laboratory environment/technique as well as assay method contributes significantly to the results generated.

Testosterone This was our second best Testosterone CV so far. All results on this occasion would be diagnostically consistent with the presence of testicular tissue (based on a cut-off of 0.5nmol/L).

TSH All methods yielded close agreement across laboratories with a couple of outlying results form the Immulite 2000 (0.54 ng/ml) and Immulite 1000 (Excluded 1ng/ml). The one non-Immulite method (Method 1) also agreed with the Immulite methods.

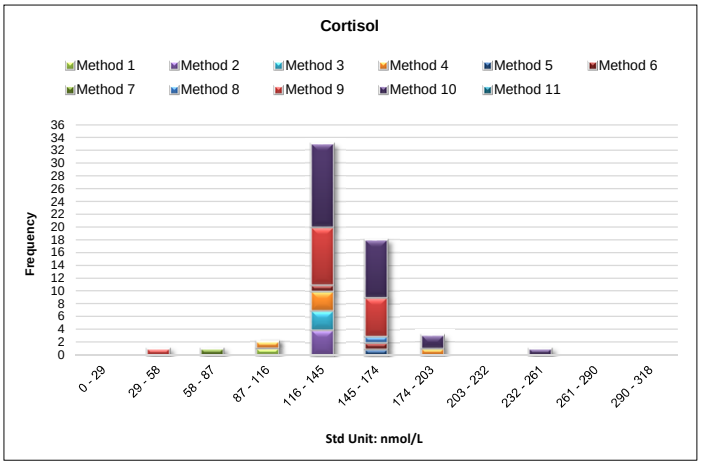
Creatinine Method type (compensated vs uncompensated Jaffe vs Enzymatic) did not have a consistent efect on the results obtained although those reported as enzymatic were generaly in the lower part of the distribution. All but 2 results were within their laboratory's reference intervals. If these results had been used to stage canine chronic renal disease using the IRIS guidelines, 35 would be Stage 1, and 10 Stage 2.

Peter Graham, Program Coordinator, February 2017

Analyte results

Cortisol

	n	Mean	StDev	%CV
Method 1	1	110		
Method 2	4	123	9.1	7.4
Method 3	3	136	1.1	0.8
Method 4	5	137	30.8	22.5
Method 5	1	157		
Method 6	2	133	19.3	14.5
Method 7	1	76		
Method 8	1	156		
Method 9	16	134	25.2	18.8
Method 10	25	151	25.9	17.2
Method 11	0			
Method 12	0			
		Trimmed	*Adjusted*	
All Methods	**59**	**140**	**13.6**	**9.7**



Note: Reported results ranged from 48 to 241 nmol/l.

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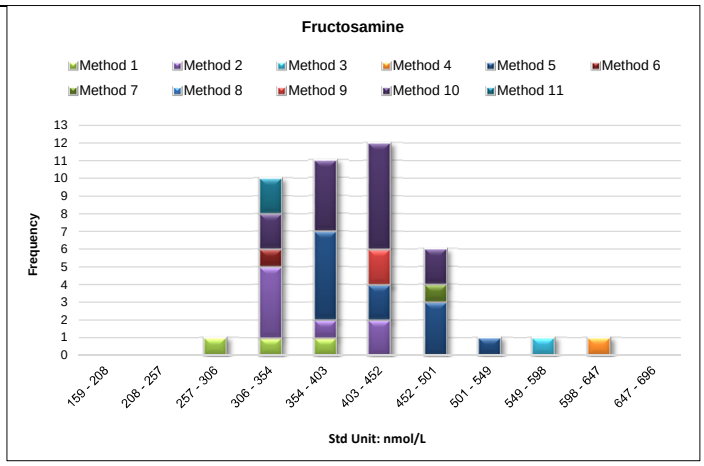
Fructosamine

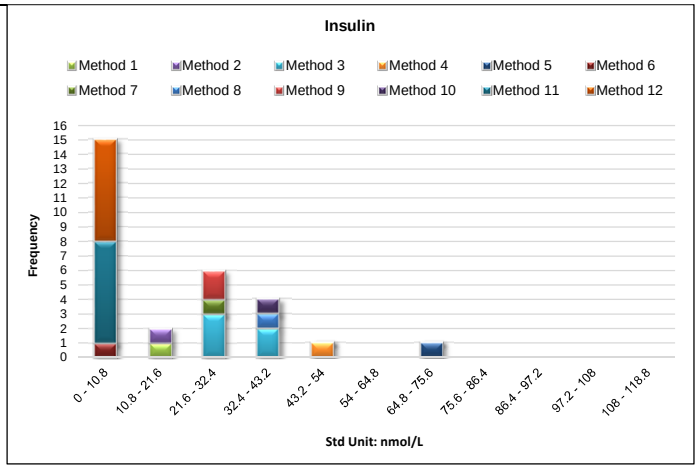
	n	Mean	StDev	%CV
Method 1	3	333	48.5	14.6
Method 2	7	358	42.1	11.8
Method 3	1	588		
Method 4	1	613		
Method 5	11	420	47.1	11.2
Method 6	1	339		
Method 7	1	487		
Method 8	0			
Method 9	2	410	2.8	0.7
Method 10	14	409	39.4	9.6
Method 11	2	318	3.1	1.0
Method 12	0			
		Trimmed	*Adjusted*	
All Methods	**44**	**396**	**73.3**	**18.5**



Note: Reported results ranged from 288 to 613 umol/L. One result (1635umol/l) was excluded from analysis.

Insulin

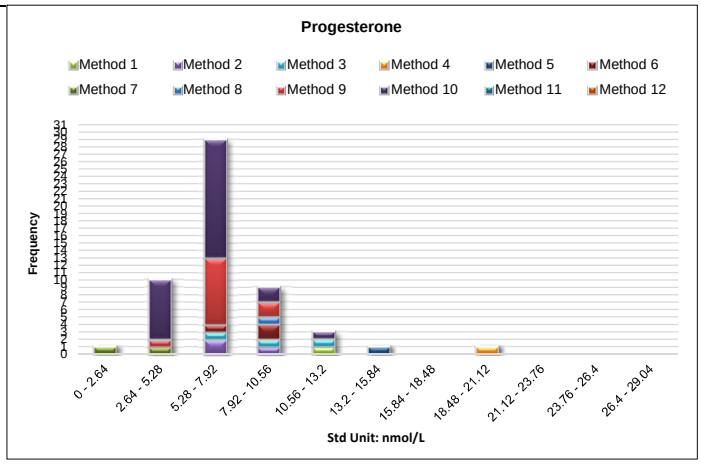
	n	Mean	StDev	%CV
Method 1	1	21.0		
Method 2	1	18.5		
Method 3	5	31.3	4.54	14.5
Method 4	1	46.0		
Method 5	1	73.0		
Method 6	1	4.7		
Method 7	1	29.4		
Method 8	1	42.0		
Method 9	2	23.6	0.93	3.9
Method 10	1	40.1		
Method 11	6	1.0	0.00	0.0
Method 12	7	2.2	0.13	5.8
		Trimmed	*Adjusted*	
All Methods	**28**	**16.4**	**18.12**	**110.5**



Note: Reported results ranged from <2 to 73 uU/ml
Methods 11 & 12 were Siemens Immulite. One lab (Method 9) commented that they knew their method was only validated for equine samples

Progesterone

	n	Mean	StDev	%CV
Method 1	1	11.2		
Method 2	3	7.2	1.13	15.6
Method 3	3	9.5	3.88	40.9
Method 4	1	19.0		
Method 5	1	13.8		
Method 6	3	9.0	0.96	10.7
Method 7	2	3.0	2.21	74.6
Method 8	1	10.0		
Method 9	12	6.5	1.18	18.2
Method 10	27	6.3	1.60	25.5
Method 11	0			
Method 12	0			
		Trimmed	*Adjusted*	
All Methods	**54**	**6.9**	**0.46**	**6.6**



Note: Reported results ranged from 1.4 to 19 nmol/L
The most popular method (Method 10) was Siemens Immulite 2000; Method 9 was Immulite 1000

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

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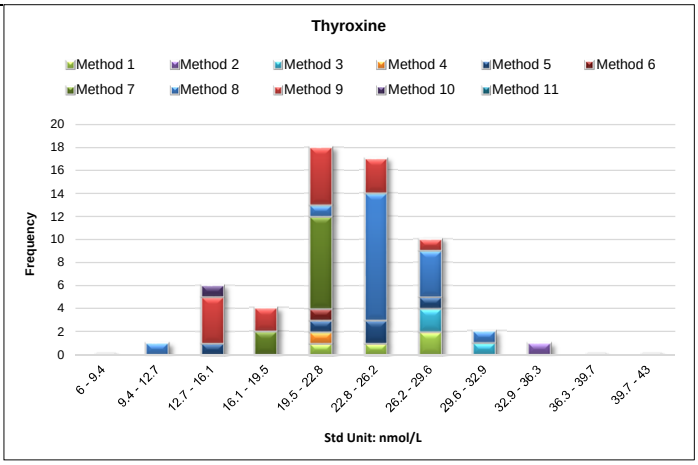
Thyroxine

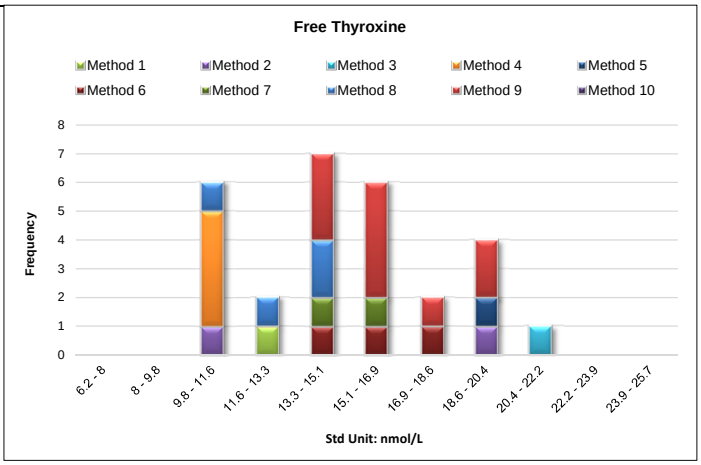
	n	Mean	StDev	%CV
Method 1	3	23.9	2.45	10.3
Method 2	1	33.5		
Method 3	3	28.8	2.92	10.1
Method 4	1	22.7		
Method 5	6	23.0	5.31	23.0
Method 6	1	20.2		
Method 7	10	20.3	1.36	6.7
Method 8	18	25.0	3.97	15.9
Method 9	15	20.5	4.11	20.0
Method 10	1	13.8		
Method 11	0			
Method 12	0			
		Trimmed	*Adjusted*	
All Methods	**59**	**22.8**	**0.70**	**3.1**



Note: Reported results ranged from 11 to 33 nmol/L.
Methods 6, 7 and 8 were "canine" methods (Immulite). Method 5 was a homologous assay (Microgenics DRI).

Free T4

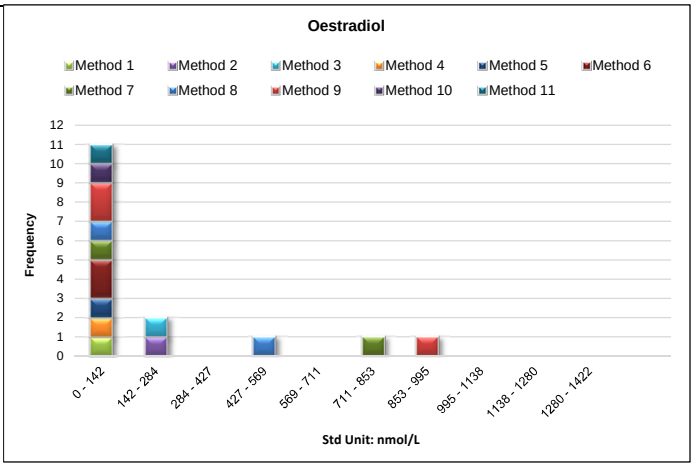
	n	Mean	StDev	%CV
Method 1	1	13.1		
Method 2	2	15.2	7.07	46.5
Method 3	1	22.0		
Method 4	4	10.9	0.39	3.6
Method 5	1	20.3		
Method 6	3	16.1	1.69	10.5
Method 7	2	14.8	0.49	3.3
Method 8	4	13.2	1.67	12.7
Method 9	10	16.3	1.73	10.6
Method 10	0			
Method 11	0			
Method 12	0			
		Trimmed	*Adjusted*	
All Methods	**28**	**15.0**	**3.34**	**22.3**



Note: Reported results ranged from 10.2 to 15.7 pmol/L.
A FT4 result by equilibrium dialysis was reported by 2 laboratories (Method 2; 10.2 and 20.2 pmol/l)
Methods 8 and 9 were "veterinary" methods. Method 5 was performed by LC-MSMS

Oestradiol

	n	Mean	StDev	%CV
Method 1	1	55		
Method 2	1	146		
Method 3	1	185		
Method 4	1	52		
Method 5	1	9		
Method 6	2	82	15.5	19.0
Method 7	2	407	528.9	130.0
Method 8	2	293	227.1	77.4
Method 9	3	404	478.8	118.4
Method 10	1	83		
Method 11	1	53		
Method 12	0			
All Methods	**16**	**210**	**278.4**	**132.6**



Note: Reported results ranged from <18.3 to 957 pmol/L.
Method 9 was "In-house RIA or EIA" so these results are not directly comparable.

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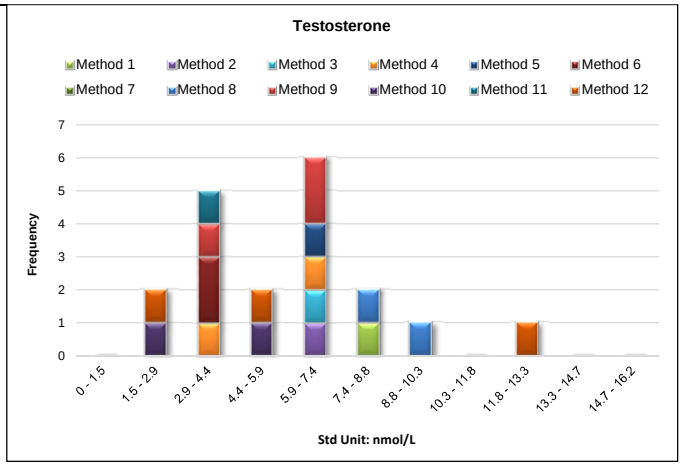
Testosterone

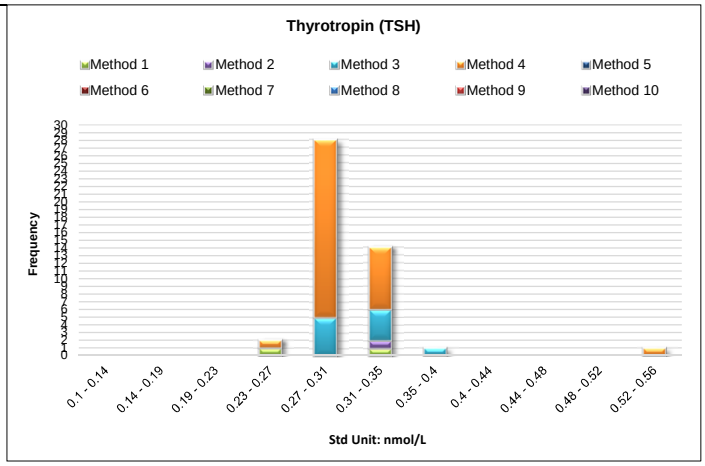
	n	Mean	StDev	%CV
Method 1	1	8.1		
Method 2	1	6.3		
Method 3	1	6.6		
Method 4	2	5.1	1.37	26.7
Method 5	1	6.0		
Method 6	2	4.3	0.15	3.5
Method 7	1	4.4		
Method 8	2	8.7	0.69	7.9
Method 9	3	5.3	1.87	35.0
Method 10	2	3.9	1.86	47.8
Method 11	1	4.2		
Method 12	3	7.0	4.91	70.2
		Trimmed	*Adjusted*	
All Methods	**20**	**5.7**	**2.17**	**38.1**



Note: Reported results ranged from 2.6 to 12.4 nmol/L

TSH

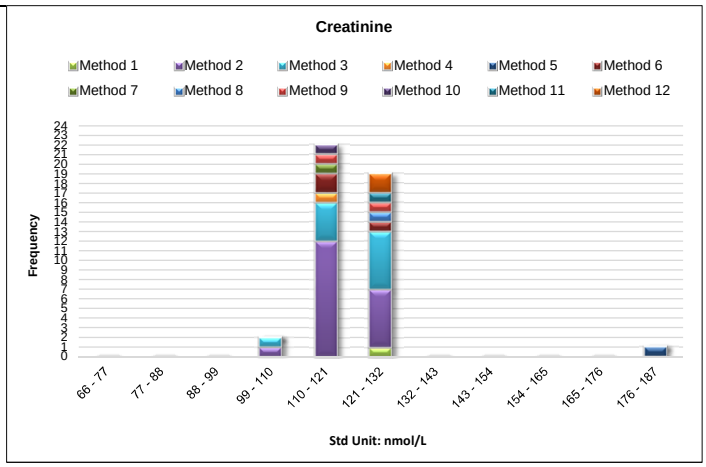
	n	Mean	StDev	%CV
Method 1	2	0.30	0.035	
Method 2	1	0.32		
Method 3	10	0.32	0.019	6.0
Method 4	33	0.31	0.045	14.4
Method 5	0			
Method 6	0			
Method 7	0			
Method 8	0			
Method 9	0			
Method 10	0			
Method 11	0			
Method 12	0			
		Trimmed	*Adjusted*	
All Methods	**47**	**0.31**	**0.076**	**24.5**



Note: Reported results ranged from 0.26 to 0.54 ng/ml. One result was excluded from statistical analysis (Method 3: 1ng/ml)
Methods 2, 3, and 4 represent the same manufacturer's chemiluminescent assay on 3 platforms (Siemens Immulite)

Creatinine

	n	Mean	StDev	%CV
Method 1	1	123		
Method 2	19	119	7.1	6.0
Method 3	11	119	6.7	5.6
Method 4	1	118		
Method 5	1	179		
Method 6	3	118	3.7	3.1
Method 7	1	114		
Method 8	1	127		
Method 9	2	121	0.7	0.6
Method 10	1	116		
Method 11	1	123		
Method 12	2	126	4.9	3.9
		Trimmed	*Adjusted*	
All Methods	**45**	**120**	**32.8**	**27.3**



Note: Reported results ranged from 108 to 179 umol/L. One additonal result (Method 6; 15 umol/L) was excluded from statistical analysis