

ESVE Veterinary Endocrinology External Quality Assessment Scheme

ESVE REPORT

Release Month:	Nov-18
Release Number:	013
Species:	Canine

Overall Comments

General

This is the report of the thirteenth 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 72 separate physical locations providing 538 analytical results. Four 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 limited for some analytes, we can clearly see 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 calculate 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 cumulative 6-cycle history window.

[Healy \(1979\) Outliers in Clinical Chemistry Quality Control Schemes, Clinical Chemistry 25\(5\)675-677](http://clinchem.aaccinls.org/content/25/5/675)
<http://clinchem.aaccinls.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)
<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 "optimal", "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 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. No quality goals have been set for PTH and ACTH.

This Release

This was a canine serum pool modified to create measurable concentrations of ACTH and PTH.

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 5 analytes were below 20% (Cortisol, 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. On a positive note, this release saw our best Progesterone and Testosterone CV's. **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 particularly 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 31 or 200 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 comparison 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.

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.

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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% 14.8 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% 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 8). 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 9 (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 5 and 6) 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 reasonable consensus between 11 and 34 uIU/ml among the other methods. Several labs reported in pmol/L and their results were converted for statistical analysis to uIU/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, this has our best progesterone CV so far. Consensus lay between 4 and 8 nmol/l with a small number of labs generating results outside this range. Given the method of manufacture of this release, high results should be unlikely.
- Thyroxine** The adjusted all-method CV% achieved on this release was reasonable. However, the range of results obtained continues to surprise. On this occasion there were several unusually high 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 with excellent precision (Method 3; 12 and 12.1 pmol/l) and one LC-MSMS (Method 11; 13pmol/L). The popular Immulite methods yielded lower results. This release did not contain 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 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 best Testosterone CV so far and with the exception of 3 or 4 labs, reasonable consensus. Literature 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 usually 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 influenced by only a small number of extreme results. In general there is good consensus between 70 and 100 umol/l
- ACTH** The inclusion of ACTH in this release was an experiment to see if we could include more labile hormones in our assessment. A significant proportion of sites generated results below their reportable limit. Although reportable limit varied between sites including on the same reagents, it is possible that some ACTH was lost in sample handling or processing. Some labs commented specifically on their sample handling after reconstitution. Eleven of the graphed results below 5 pg/ml were generated by the scheme's statistical approach of using 0.5 x lower reporting limit.
- PTH** As was the case for ACTH, the PTH inclusion was an experiment on labile peptides. There were only a small number of participants with 5 of them using either Immulite or Canine ELISA methods. There have been anecdotal reports that the Immulite does not detect canine PTH. This may be the case given the low results generated by the 2 Immulite participants.

Peter Graham, Program Coordinator, December 2018

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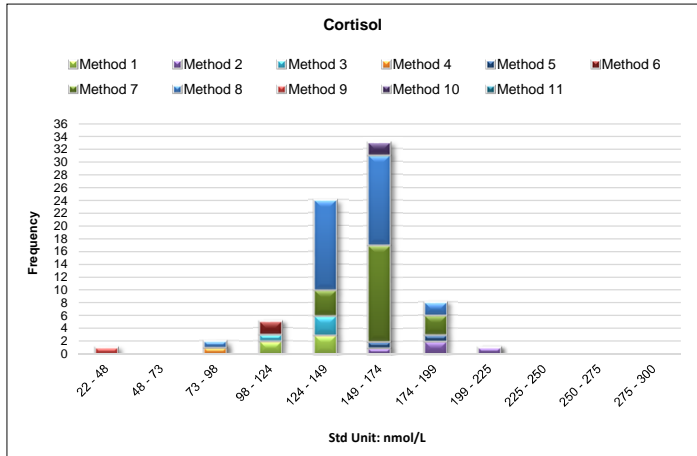
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Analyte results

Cortisol

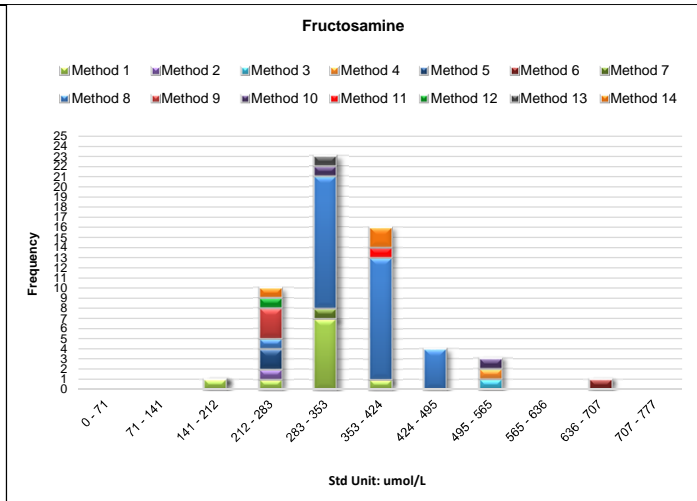
	n	Mean	StDev	%CV
Method 1	5	125	9.6	7.7
Method 2	4	185	12.3	6.7
Method 3	4	125	1.3	1.0
Method 4	1	97		
Method 5	2	184	15.4	8.3
Method 6	2	112	1.4	1.3
Method 7	22	161	14.8	9.2
Method 8	31	148	19.5	13.2
Method 9	1	31		
Method 10	2	152	1.4	0.9
Method 11	0			
Method 12	0			
All Methods	74	<i>Trimmed</i> 150	<i>Adjusted</i> 22.3	14.9



Note: Reported results ranged from 31 to 200nmol/L
Although Method 9 yielded a low result compared to the consensus, it is reported against a lower reference range than most other labs

Fructosamine

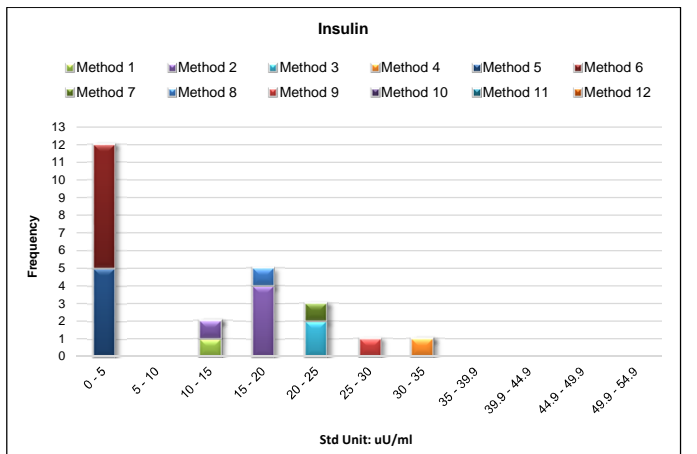
	n	Mean	StDev	%CV
Method 1	10	301	58.6	19.5
Method 2	1	236		
Method 3	1	512		
Method 4	1	500		
Method 5	2	252	2.1	0.8
Method 6	1	645		
Method 7	1	332		
Method 8	30	360	47.6	13.2
Method 9	3	240	19.9	8.3
Method 10	2	440	128.0	29.1
Method 11	1	393		
Method 12	1	265		
Method 13	1	337		
Method 14	3	338	65.4	19.4
All Methods	59	<i>Trimmed</i> 344	<i>Adjusted</i> 75.4	21.9



Note: Reported results ranged from 174 to 645umol/L
One result (25umol/l) was excluded from analysis, the participating lab reported a sample viscosity problem

Insulin

	n	Mean	StDev	%CV
Method 1	1	11.4		
Method 2	5	16.5	1.05	6.3
Method 3	2	22.5	0.71	3.1
Method 4	1	34.3		
Method 5	5	1.0	0.00	0.0
Method 6	7	1.2	0.42	36.4
Method 7	1	24.0		
Method 8	1	15.4		
Method 9	1	26.8		
Method 10	0			
Method 11	0			
Method 12	0			
All Methods	24	<i>Trimmed</i> 9.9	<i>Adjusted</i> 10.98	110.9



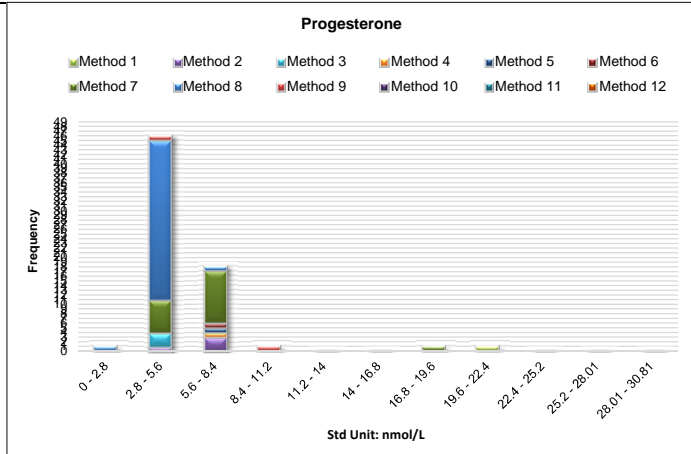
Note: Reported results ranged from below the limit of detection to 34.3uU/ml
Methods 5 & 6 were Siemens Immulite. Two labs (Methods 5&6) commented that they knew their method was only appropriate for horses

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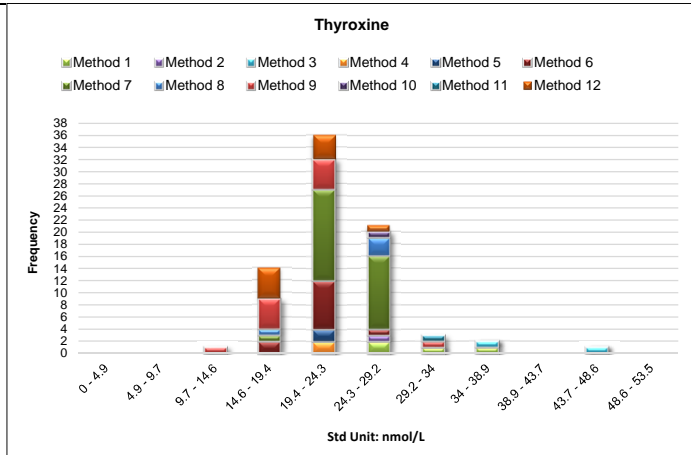
Progesterone				
	n	Mean	StDev	%CV
Method 1	1	21.1		
Method 2	4	6.3	1.04	16.5
Method 3	3	4.8	0.31	6.6
Method 4	1	8.0		
Method 5	1	7.7		
Method 6	1	6.0		
Method 7	19	6.4	3.06	47.9
Method 8	36	4.7	0.58	12.2
Method 9	2	7.4	3.18	42.7
Method 10	0			
Method 11	0			
Method 12	0			
All Methods	68	5.3	0.90	16.9



Note: Reported results ranged from below the limit of detection to 21.05nmol/L
The most popular method (Method 8) was Siemens Immulite 2000; Method 7 was Immulite 1000

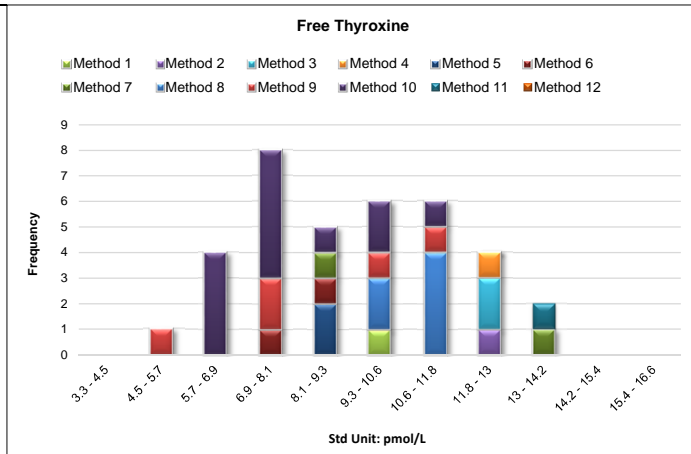
For statistical purposes, results lower than reportable limit have been converted to a value 0.5 x lowest reportable limit

Thyroxine				
	n	Mean	StDev	%CV
Method 1	4	31.1	4.73	15.2
Method 2	1	24.6		
Method 3	2	41.9	4.69	11.2
Method 4	2	20.5	0.71	3.4
Method 5	2	22.3	1.97	8.8
Method 6	11	20.9	2.01	9.6
Method 7	28	24.3	2.20	9.1
Method 8	4	24.5	4.51	18.4
Method 9	12	19.3	4.38	22.6
Method 10	1	27.0		
Method 11	1	32.2		
Method 12	10	20.2	2.47	12.2
All Methods	78	22.9	4.21	18.4



Note: Reported results ranged from 12.8 to 45.2nmol/L
Methods 5, 6 and 7 were "canine" methods (Immulite). Method 12 was a homologous assay (Thermo Microgenics DRI).

Free T4				
	n	Mean	StDev	%CV
Method 1	1	9.5		
Method 2	1	12.0		
Method 3	2	12.1	0.07	0.6
Method 4	1	12.0		
Method 5	2	8.5	0.39	4.6
Method 6	2	8.5	0.54	6.3
Method 7	2	11.3	3.22	28.4
Method 8	6	10.7	0.55	5.1
Method 9	5	8.3	2.53	30.4
Method 10	13	7.9	1.43	18.1
Method 11	1	13.0		
Method 12	0			
All Methods	36	9.3	2.27	24.4



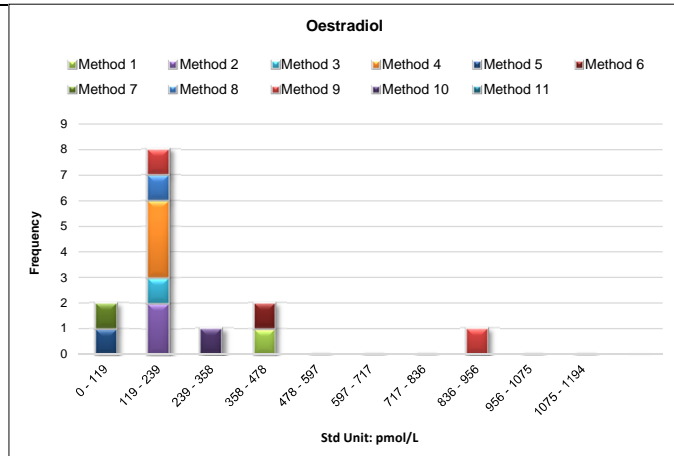
Note: Reported results ranged from 5.5 to 13.6pmol/l
A FT4 result by equilibrium dialysis was reported by 2 laboratories (Method 3; 12.0 and 12.1 pmol/l)
Methods 9 and 10 were "veterinary" methods. Method 11 was performed by LC-MSMS

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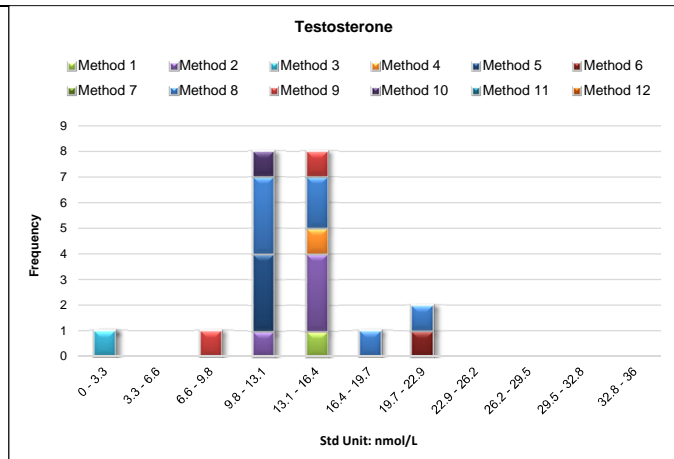
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Oestradiol				
	n	Mean	StDev	%CV
Method 1	1	460		
Method 2	2	196	39.9	20.4
Method 3	1	191		
Method 4	3	137	16.5	12.0
Method 5	1	104		
Method 6	1	415		
Method 7	1	118		
Method 8	1	123		
Method 9	2	553	491.2	88.8
Method 10	1	300		
Method 11	0			
Method 12	0			
All Methods	14	258	215.3	83.4



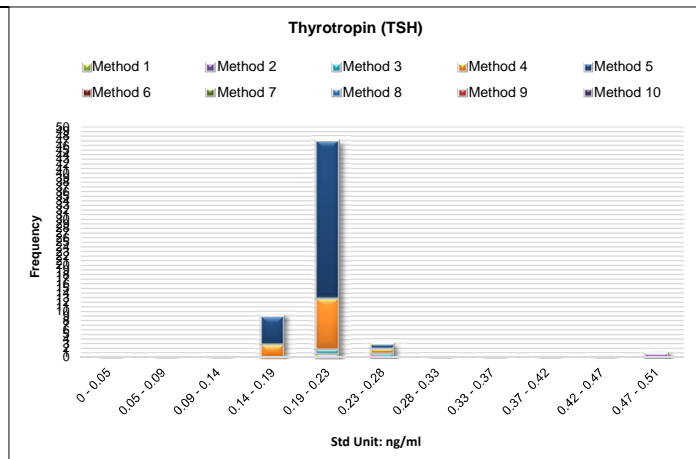
Note: Reported results ranged from 104 to 900pmol/l
Method 9 was "In-house RIA or EIA" so these results may not be directly comparable with one another.

Testosterone				
	n	Mean	StDev	%CV
Method 1	1	16.1		
Method 2	4	14.1	1.65	11.7
Method 3	1	0.1		
Method 4	1	15.0		
Method 5	3	12.7	0.49	3.8
Method 6	1	21.9		
Method 7	2	17.3	4.41	25.5
Method 8	7	15.2	3.25	21.5
Method 9	2	11.1	4.13	37.1
Method 10	1	12.1		
Method 11	0			
Method 12	0			
All Methods	23	<i>Trimmed</i> 14.3	<i>Adjusted</i> 3.27	22.9



Note: Reported results ranged from 0.1 to 21.9nmol/l

TSH				
	n	Mean	StDev	%CV
Method 1	1	0.19		
Method 2	1	0.48		
Method 3	2	0.24	0.030	12.6
Method 4	15	0.20	0.016	7.9
Method 5	41	0.20	0.016	8.1
Method 6	0			
Method 7	0			
Method 8	0			
Method 9	0			
Method 10	0			
Method 11	0			
Method 12	0			
All Methods	61	<i>Trimmed</i> 0.20	<i>Adjusted</i> 0.016	8.0



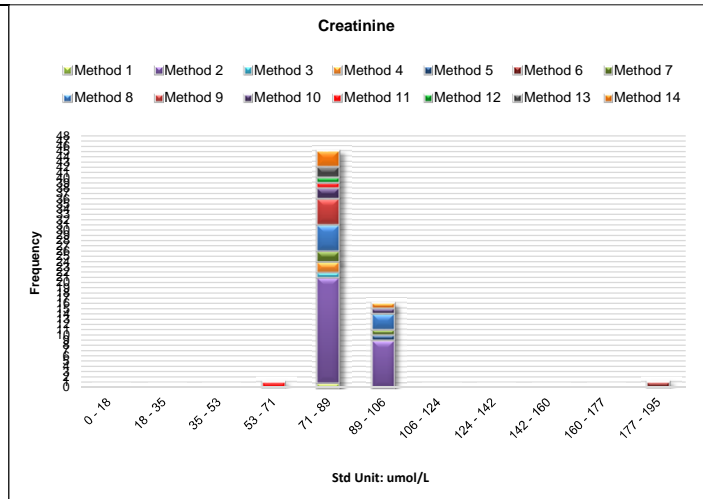
Note: Reported results ranged from 0.17 to 0.48ng/ml. One result of 0 was excluded from analysis.
Methods 3, 4 and 5 represent the same manufacturer's chemiluminescent assay on 3 platforms (Siemens Immulite)

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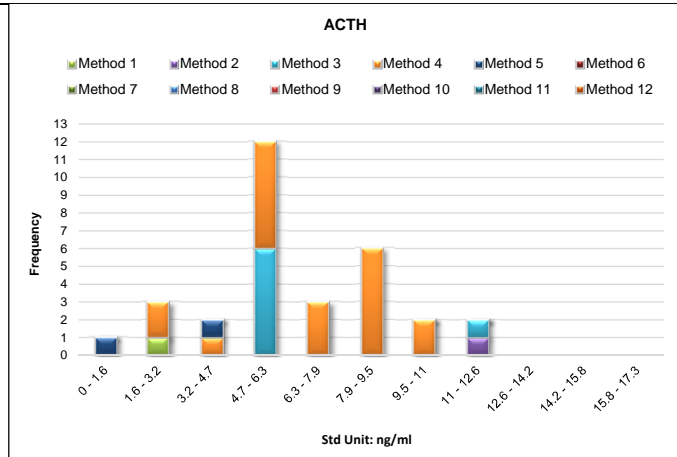
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Creatinine	n	Mean	StDev	%CV
Method 1	1	82		
Method 2	29	85	7.4	8.7
Method 3	1	86		
Method 4	2	80	0.0	0.0
Method 5	1	96		
Method 6	1	188		
Method 7	3	91	8.3	9.1
Method 8	8	87	4.7	5.4
Method 9	5	79	1.5	1.9
Method 10	3	84	12.1	14.4
Method 11	2	72	3.1	4.3
Method 12	1	87		
Method 13	2	86	1.5	1.8
Method 14	4	84	6.9	8.2
All Methods	63	<i>Trimmed</i> 85	<i>Adjusted</i> 7.6	8.9



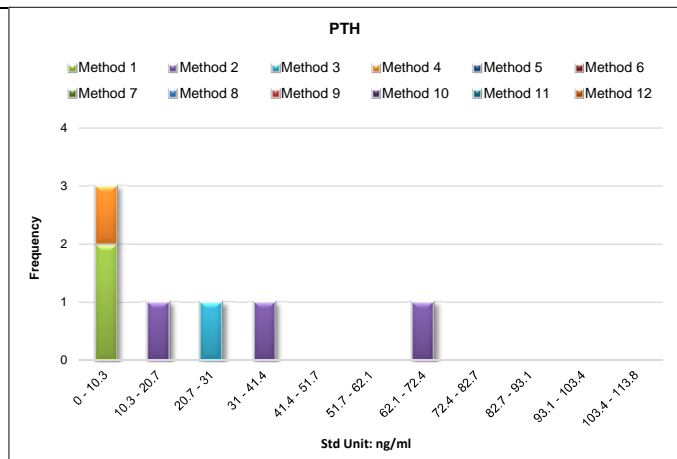
Note: Reported results ranged from 70 to 188umol/l
Method 9 (with exceptional within method CV%) is an Enzymatic creatinine (non Jaffe)

ACTH	n	Mean	StDev	%CV
Method 1	1	2.50		
Method 2	1	12.00		
Method 3	7	5.99	2.608	43.6
Method 4	20	6.88	2.398	34.9
Method 5	2	2.85	1.902	66.9
Method 6	0			
Method 7	0			
Method 8	0			
Method 9	0			
Method 10	0			
Method 11	0			
Method 12	0			
All Methods	31	<i>Trimmed</i> 6.40	<i>Adjusted</i> 2.880	45.0



Note: Reported results ranged from below the limit of detection to 12pg/ml
The most popular method (Method 4) was Immulite 2000. Method 3 is Immulite 1000.
Eleven of 31 labs reported a result below detection limit but this varied even within methods (<5 or <10 ng/ml on either Immulite 1000 or 2000)

PTH	n	Mean	StDev	%CV
Method 1	2	2	2.1	137.0
Method 2	3	40	28.0	69.5
Method 3	1	30		
Method 4	1	6		
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	23	25.0	109.1



Note: Reported results ranged from below the limit of detection to 69pg/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