

ESVE Veterinary Endocrinology External Quality Assessment Scheme

ESVE REPORT

Release Month:	Dec-13
Release Number:	003

Overall Commentary

General This is the report of the third release of the ESVE EQA scheme. The send out was delayed from November in to December 2013 because we were selecting certain sample types with which to make the pooled EQA material. It took longer than expected to identify sufficient of the required sample type to make the total volume required. Unfortunately, this also meant that EQA samples arrived around the end-of-year holidays and not all made it to their destination on the first attempt. The efforts made by the participants to report their results were much appreciated. We had participation from 27 separate physical locations providing 165 analytical results. The strength of a scheme such as this can only improve as more participants are recruited. Given the numbers of participants within individual methodologies it would still be difficult to draw strong conclusions from much of the data at this stage. However, even by our 3rd release I believe participants that have been in the scheme from the beginning should already be getting a feel for the performance characteristics of their chosen methods.

As was the case for the previous release, it should be remembered also 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".

It does not appear that participants had difficulty with the accuracy of reconstituting the freeze dried samples. A simplistic way to check for this yourself is to check if all your "SD Multiples" are consistently positive or consistently negative.

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.

As was the case in the last 2 releases, the range of values generated for Cortisol and Fructosamine are dramatic (see below) and we have yet further support for the likelihood that some of the methods used for insulin do not work for dog in the baseline range. Whether by co-incidence or as a consequence of this program we are seeing an improvement in the variation of fructosamine results over the past 2 releases.

The range of results obtained for Oestradiol is tremendous, but less of a surprise given the historic experience of the Michigan State University SCE EQUAS scheme. This is a notoriously difficult hormone to measure well.

On this release we attempted to construct a serum pool that had TSH closer to the diagnostic cut-off, and that would present some challenges to the Free T4 methods. The pool was also Thyroglobulin antibody (TgAA) positive and contained a low proportion of T4-autoantibody. The pool also contained added Oestradiol to ensure there would be a low but measurable amount present.

We have not previously released method names because of the limitations of so-far having only a small participant number. However, on this release we have highlighted two Method names (see Insulin and Free T4)

Canine TSH The results generated for TSH are quite tight with an overall CV of around 10%. All methods reported are a variation of a single manufacturer's product range. The upper limit of the manufacturer's "Expected range" is around 0.4 and literature and several labs suggest a diagnostic cut-off of around 0.6 to 0.7. All participants generated results in this "equivocal" zone and consequently would all have delivered in similar clinical conclusions.

Cortisol As was the case for both previous releases, the range of results generated for cortisol was a real 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. Overall CV was close to 20%. In large human EQA schemes, CV for cortisol is 7-8%.

Fructosamine The range of Fructosamine results also continues to surprise. Although the overall CV is around 12% and a great improvement of the previous 2 releases (25% and 32% respectively), the overall range of individual results is surprising particularly if textbook and literature tables are used to support the interpretation of these results in diabetic monitoring.

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 be wary to avoid textbook ranges (for insulin but also where appropriate insulin:glucose ratios) in reaching a diagnostic interpretation. Method 9 is the Immulite 1000 method which has yielded similar low results in all 3 releases (Method 7 in 001, Method 6 in 002). Based on 3 sets of EQA results alone it does not appear this method is not suitable for baseline canine and feline insulin. However, spike/recovery or other studies may be helpful in determining whether this method is usable with reduced reference ranges or not.

Progesterone This sample contained very little progesterone and so was a challenge to the low end sensitivity of the methods. Around 40% of participants indicated that the result was below their limit of detection. The majority of the remainder reported a very tight range of results. One laboratory provided a borderline luteal result although the same method in another lab yielded a result close to the mean.

Thyroxine The variation in results obtained was greater than seen in previous releases (CV=36%, 10.3% in 001, 16% in 002). The likely explanation for this is the inclusion of TgAA including low amounts of T4-AA. Because of the potential for effects of T4AA on immunoassays, it is not possible to confirm what the true TT4 result is likely to have been. It does appear that different methods may be differentially affected by T4AA

Free T4 A wide range of results were returned for Free T4 (CV 56.7%) and as was the case for Total T4, I suspect the reason for such variation was the inclusion of TgAA including low amounts of T4-AA. Part of the rationale for including auto-antibody in this release was to challenge Free T4 methods. Future releases will check free T4 results in antibody negative serum. On a theoretical basis, the methods using dialysis or 2-step immunoseparation should yield the results closest to the true value. The mean of the FT4d and 2-Step methods in this release was 11.7pmol/L. The methods yielding the 3 highest results were variations of Siemens "Veterinary Free T4"

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Oestradiol The variation in results obtained for Oestradiol is a well known phenomenon to anyone participating in the MSU/SCE EQUAS scheme. One result was excluded from analysis for being too extreme. 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. Unfortunately, it was not possible to assess the impact of clinical diagnosis of such disparate results as only a very few participants provided their interpretative guidance values. 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.

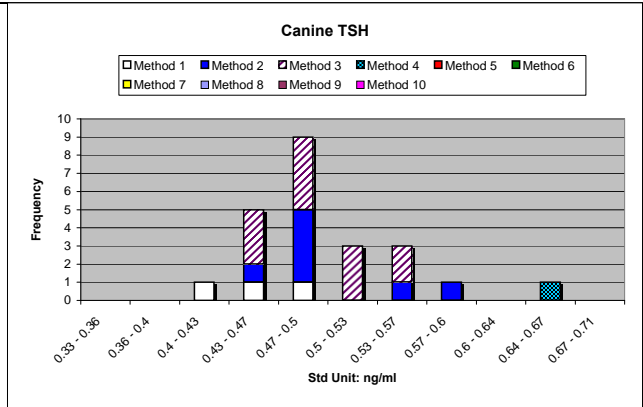
Testosterone Four participants volunteered Testosterone results. Three were able to generate a detectable result. There was certainly a low level in the sample but interestingly there were results both above and below 0.5nmol/L a cut-off that has been used for determining the likely presence of functional testicular tissue. Cut-offs were not provided by the participants for this interpretative purpose so it is not clear whether there would be a risk of diagnostic error with this range of results.

Peter Graham, Program Coordinator, February 2014

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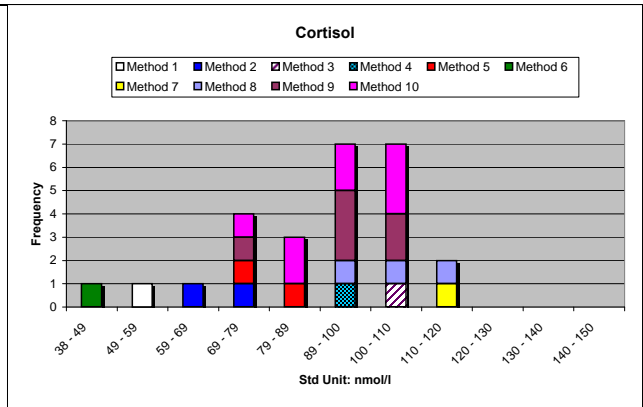
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Canine TSH				
	n	Mean	StDev	%CV
Method 1	3	0.44	0.030	6.9
Method 2	7	0.51	0.052	10.3
Method 3	13	0.50	0.033	6.7
Method 4	1	0.64		
Method 5	0			
Method 6	0			
Method 7	0			
Method 8	0			
Method 9	0			
Method 10	0			
All Methods	24	0.50	0.052	10.4



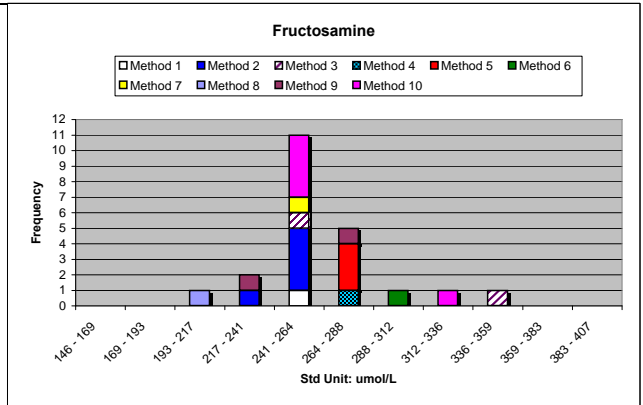
Note: Methods 1 to 4 are different versions of a single manufacturer's product

Cortisol				
	n	Mean	StDev	%CV
Method 1	1	89		
Method 2	2	68	4.4	6.4
Method 3	1	101		
Method 4	1	92		
Method 5	2	77	6.6	8.6
Method 6	1	46		
Method 7	1	114		
Method 8	3	106	8.4	7.9
Method 9	6	94	11.8	12.7
Method 10	8	93	10.0	10.7
All Methods	26	89	17.5	19.7



Note: Reported results ranged from 46 to 114 nmol/L

Fructosamine				
	n	Mean	StDev	%CV
Method 1	1	265		
Method 2	5	254	9.4	3.7
Method 3	2	305	72.9	23.9
Method 4	1	284		
Method 5	3	268	5.2	1.9
Method 6	1	303		
Method 7	1	263		
Method 8	1	198		
Method 9	2	249	43.8	17.6
Method 10	5	265	28	10.7
All Methods	22	264	32	12.1



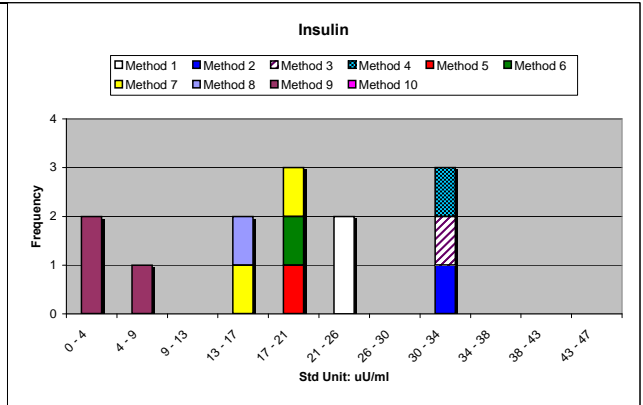
Note: Reported results ranged from 198 to 356 nmol/L

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

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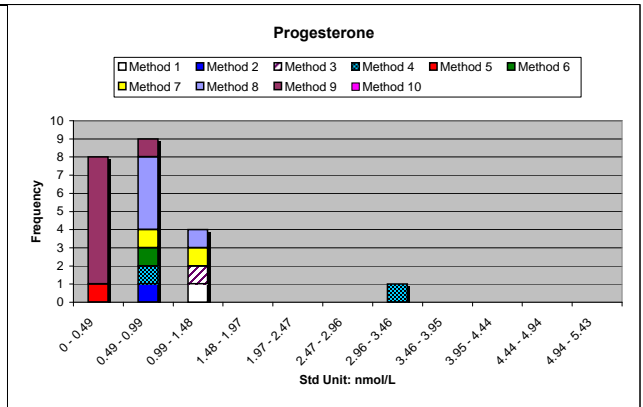
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Insulin				
	n	Mean	StDev	%CV
Method 1	2	23.0	0.0	0.1
Method 2	1	30.0		
Method 3	1	31.5		
Method 4	1	30.5		
Method 5	1	20.9		
Method 6	1	18.6		
Method 7	2	15.7	2.1	13.7
Method 8	1	16.6		
Method 9	3	3.6	1.0	28.9
Method 10	0			
All Methods	13	18.0	9.90	55.0



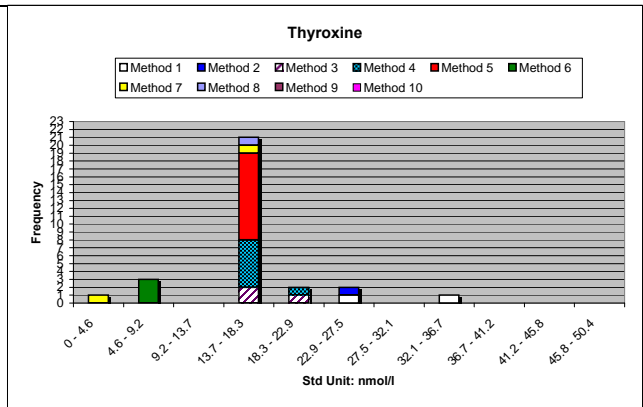
Note: A conversion factor of 101 was used for the 2 participating laboratories that reported in "ug/L Canine insulin"
A conversion factor of 0.101 for 1 that reported in "ng/L Equine insulin"

Progesterone				
	n	Mean	StDev	%CV
Method 1	1	1.21		
Method 2	1	0.50		
Method 3	1	1.00		
Method 4	2	2.13	1.664	78.1
Method 5	1	0.02		
Method 6	1	0.90		
Method 7	2	0.95	0.189	19.9
Method 8	5	0.87	0.220	25.3
Method 9	8	0.37	0.147	39.9
Method 10	0			
All Methods	22	0.80	0.660	82.5



Note: 9 laboratories declared their result to be below their methods' limit of detection

Thyroxine				
	n	Mean	StDev	%CV
Method 1	2	29.4	6.21	21.1
Method 2	1	23.8		
Method 3	3	17.2	2.63	15.3
Method 4	7	16.1	2.16	13.3
Method 5	11	16.0	0.84	5.2
Method 6	3	6.5	0.00	0.0
Method 7	2	8.5	7.48	87.9
Method 8	1	15.6		
Method 9	0			
Method 10	0			
All Methods	30	15.9	5.73	36.0



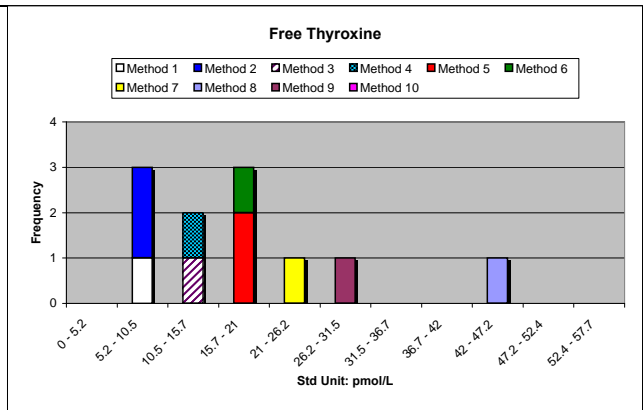
Note: 4 laboratories declared their result to be below their methods' limit of detection

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

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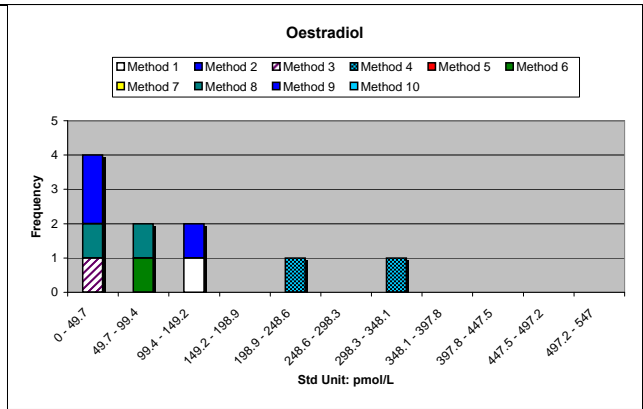
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Free T4				
	n	Mean	StDev	%CV
Method 1	1	9.8		
Method 2	2	7.4	0.15	2.1
Method 3	1	13.5		
Method 4	1	11.8		
Method 5	2	18.5	0.69	3.8
Method 6	1	19.8		
Method 7	1	23.0		
Method 8	1	42.3		
Method 9	1	27.8		
Method 10	0			
All Methods	11	18.2	10.32	56.7



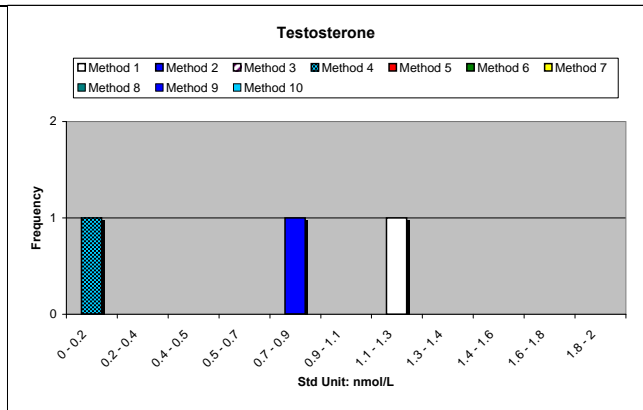
Note: Reported results ranged from 7.4 to 42.3 pmol/L
The mean of the FT4d and 2-Step methods in this release was 11.7pmol/L (see commentary)

Oestradiol				
	n	Mean	StDev	%CV
Method 1	1	121.1		
Method 2	1	122.0		
Method 3	1	9.2		
Method 4	2	280.9	84.49	30.1
Method 5	0			
Method 6	1	72.3		
Method 7	1	161.5		
Method 8	2	48.4	17.73	36.7
Method 9	2	26.6	4.95	18.6
Method 10	0			
All Methods	12	110.5	96.41	87.2



Note: One result of 1501 pmol/L was excluded from analysis. Included results ranged from <18.3 to 341 pmol/L

Testosterone				
	n	Mean	StDev	%CV
Method 1	1	1.20		
Method 2	1	0.76		
Method 3	0			
Method 4	1	0.00		
Method 5	0			
Method 6	0			
Method 7	0			
Method 8	0			
Method 9	0			
Method 10	0			
All Methods	4	0.60	0.500	83.3



Note: 1 Laboratory reported an undetectable concentration

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