

# ESVE Veterinary Endocrinology External Quality Assessment Scheme

## ESVE REPORT

Release Month: **May-16**  
Release Number: **008**

### Overall Commentary

- General** This is the report of the eighth release of the ESVE EQA scheme. The efforts made by the participants to report their results were much appreciated. We had participation from 44 separate physical locations providing 276 analytical results. Only 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 the numbers of participants within individual methodologies is still limited, 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
- 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 feline serum pool which was concentrated by 25% to increase measured analyte concentrations.
- This was the third feline release of the scheme. Those of you familiar with other EQA schemes will recognise that the overall CV's we are seeing are high. On this release, Cortisol, Total T4, Free T4, TSH and Creatinine CV's are below 20%. A wide CV% makes more sense for our peptide representative (insulin) but it is concerning that we are seeing a high CV for Fructosamine. On a positive note, this release saw our second best Fructosamine CV and the lowest CV's for Free T4 and Creatinine so far.
- 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 71 or 220 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.

### Analytes

- Cortisol** As was the case for previous releases, the 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, this is our third best cortisol CV yet at 19.1% and similar to our first feline release (002). 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%.
- Fructosamine** The range of fructosamine results is wide, the overall CV is high and reference to the literature for diabetes diagnosis or monitoring cannot be recommended. However, all 23 participants that provided an upper reference limit for feline fructosamine agreed that the concentration in this release was around their upper limit or above. That said, there was no relationship between the result reported and the upper limit of the reference ranges used (R-sq 0.08) suggesting comparison to local ranges and cut-off's may still be problematic. Method 5 (Cobas) again showed a really good method CV on this occasion and Methods 2 (ABX) and 8 (Roche) gave similar results to one another over relatively narrow CV's. All 3 of these methods 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
- 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 feline 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 but also where appropriate insulin:glucose ratios) in reaching a diagnostic interpretation. As has been the case in previous releases, the Immulite methods (n=6; Methods 7 and 8) yielded much lower results than other methods (all except one less than 4uU/ml). One lab indicated that they would not normally use this method for cats but were comfortable that it was valid for equine samples. Two labs reported in pmol/L and their results were converted for statistical analysis to uU/ml using human factors 7.175 and 7.217 from manufacturers' package inserts.
- Progesterone** There was an exceptionally wide range of results but we have seen a wide CV for feline progesterone before (86% Release 002). It is intriguing that the RIA and LC-MSMS methods were the ones giving the higher results compared to the EIA and chemiluminescent methods. Perhaps protein-binding of feline progesterone is having a differential effect across the methods.
- Thyroxine** The all-method CV% achieved on this release was our 3rd best so far. Methods 1 (Tosoh AIA), 4 (Microgenics DRI), 5 (Immulite 1 Canine TT4), 7 (Immulite 2000 Canine TT4) yielded CV's below 10%.

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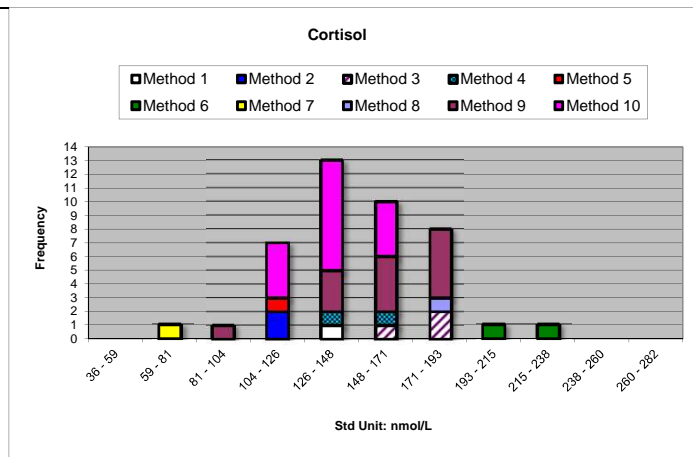
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- Free T4** On a theoretical basis, the methods using dialysis should yield the Free T4 results closest to the true value. Unfortunately, we have only one participant using such a method in this release (Method 1; 25 pmol/l). However, on this occasion, the all-method CV is our best ever of the scheme so far.
- 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.
- Testosterone** This was our worst Testosterone CV so far. The values cover a range that would cause diagnostic discordance when investigating for the presence of testicular tissue. The one participant lab using LC-MSMS methodology did not detect testosterone in this sample.
- TSH** The 3 Immulite methods yielded close agreement across laboratories. Several laboratories have their upper reference limit at 0.15ng/ml for cats in the Immulite assay. This sample concentration was set around that limit. One non-Immulite method (Method 1) appears to be more canine specific than the Immulite. It has consistently yielded higher TSH results than Immulite for canine samples, but yielded a lower result on the feline sample in this release. One result was excluded from statistical analysis because of the lack of a conversion factor for feline TSH between uU/ml and ng/ml. The result was around the centre of that method's feline reference limits.
- Creatinine** The creatinine concentration was set towards the level considered to be clinically significant azotaemia by several authors. There was not a clear effect of methodological type (Uncompensated vs compensated Jaffe and enzymatic). However the method with an extremely low CV was enzymatic (Method 10 Roche CREA Plus). As was the case for fructosamine, there was not a relationship between the creatinine result and the upper limit of the feline reference interval (R-sq= 0.0001) based on reference limits provided by 23 participants. All except the one excluded result would yield an IRIS classification of Stage 2 in combination with dilute urine and appropriate clinical findings.

Peter Graham, Program Coordinator, July 2016

Cortisol				
	n	Mean	StDev	%CV
Method 1	1	135		
Method 2	2	115	6.1	5.3
Method 3	3	173	5.0	2.9
Method 4	2	146	4.2	2.9
Method 5	1	124		
Method 6	2	214	8.4	3.9
Method 7	1	71		
Method 8	1	184		
Method 9	13	157	24.4	15.6
Method 10	16	138	12.1	8.8
Method 11	0			
Method 12	0			
<b>All Methods</b>	<b>42</b>	<b>148</b>	<b>28.3</b>	<b>19.1</b>



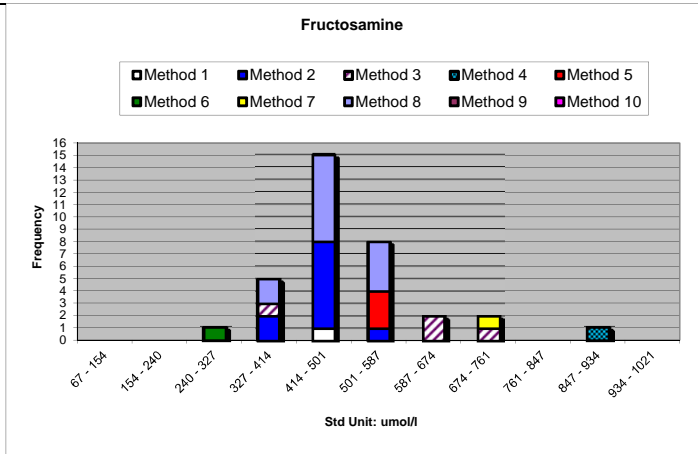
**Note:** Reported results ranged from 71 to 220 nmol/l.

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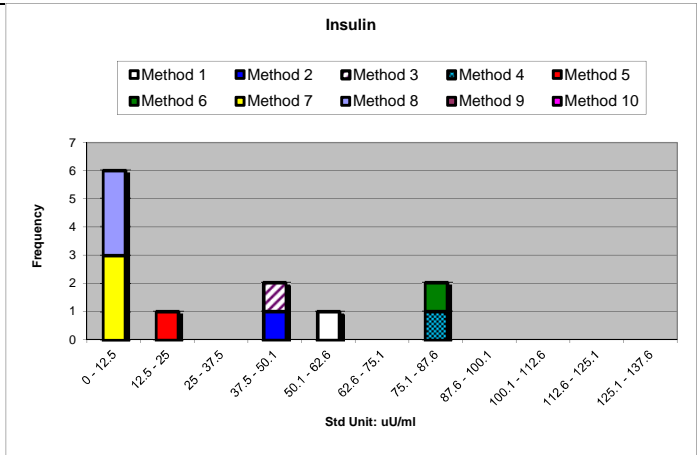
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Fructosamine				
	n	Mean	StDev	%CV
Method 1	1	457		
Method 2	10	455	42.6	9.4
Method 3	4	594	128.2	21.6
Method 4	1	902		
Method 5	3	517	20.6	4.0
Method 6	1	324		
Method 7	1	698		
Method 8	13	474	69.0	14.6
Method 9	0			
Method 10	0			
Method 11	0			
Method 12	0			
<b>All Methods</b>	<b>34</b>	<b>501</b>	<b>113.2</b>	<b>22.6</b>



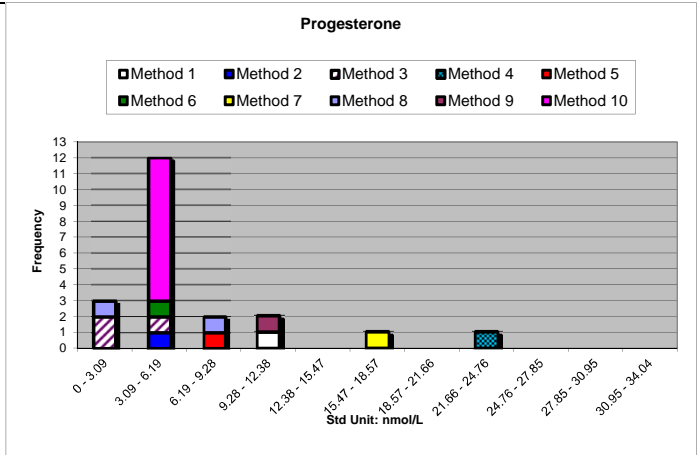
**Note:** Reported results ranged from 324 to 902 umol/L

Insulin				
	n	Mean	StDev	%CV
Method 1	1	60.5		
Method 2	1	40.3		
Method 3	1	42.0		
Method 4	1	86.0		
Method 5	1	14.3		
Method 6	1	84.0		
Method 7	3	3.2	0.4	12.1
Method 8	3	4.3	2.1	48.0
Method 9	0			
Method 10	0			
Method 11	0			
Method 12	0			
<b>All Methods</b>	<b>12</b>	<b>29</b>	<b>32.5</b>	<b>111.7</b>



**Note:** Reported results ranged from 2.6 to 86 uU/ml  
Methods 7 & 8 were Siemens Immulite. One lab (Method 8) commented that they knew their method was only validated for equine samples

Progesterone				
	n	Mean	StDev	%CV
Method 1	1	9.3		
Method 2	1	5.0		
Method 3	3	3.1	2.49	79.4
Method 4	1	22.0		
Method 5	1	8.1		
Method 6	1	4.2		
Method 7	1	17.8		
Method 8	2	3.9	3.64	92.4
Method 9	1	11.8		
Method 10	9	4.8	0.34	7.1
Method 11	17	4.4	0.50	11.4
Method 12	0			
<b>All Methods</b>	<b>38</b>	<b>5.6</b>	<b>3.91</b>	<b>69.8</b>



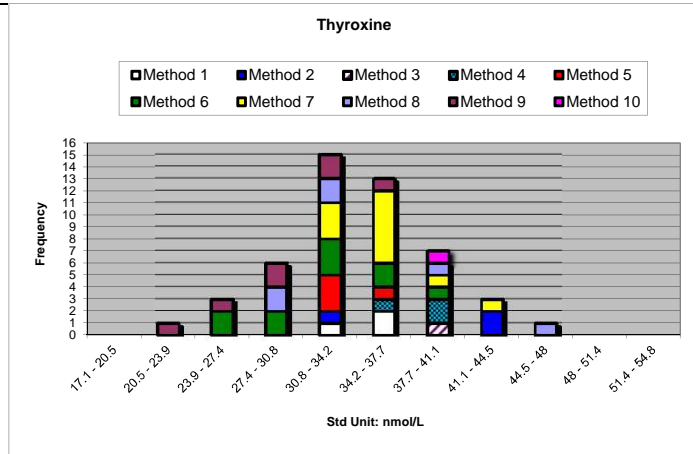
**Note:** Reported results ranged from 1.4 to 22 nmol/L  
The 2 highest results (Methods 4 and 7) were the only RIA results. The third highest was an LC-MSMS (Method 9; 11.8 nmol/L)  
One lab (Method 6) indicated that they knew their method was only validated for canine samples.

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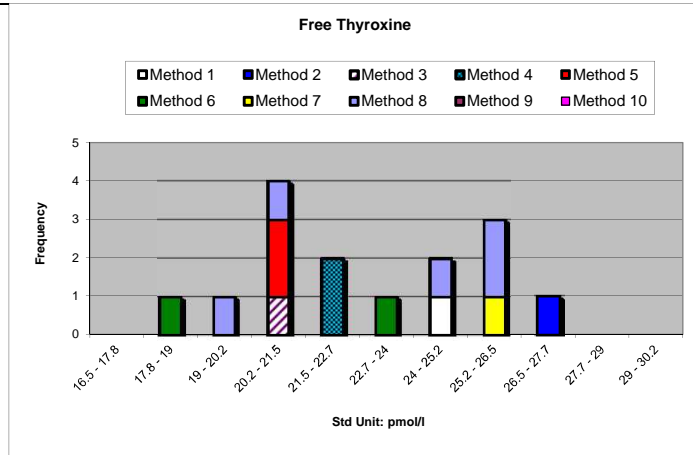
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Thyroxine				
	n	Mean	StDev	%CV
Method 1	3	35.1	1.22	3.5
Method 2	3	39.4	5.17	13.1
Method 3	1	41.0		
Method 4	3	38.2	2.44	6.4
Method 5	4	33.8	1.63	4.8
Method 6	10	31.4	4.33	13.8
Method 7	11	36.2	3.11	8.6
Method 8	6	34.2	7.01	20.5
Method 9	7	29.5	4.44	15.1
Method 10	1	38.9		
Method 11	0			
Method 12	0			
<b>All Methods</b>	<b>49</b>	<b>34.2</b>	<b>4.97</b>	<b>14.5</b>



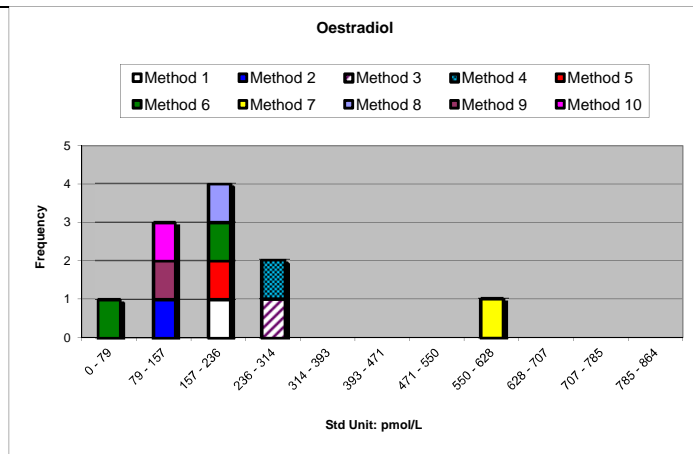
**Note:** Reported results ranged from 22 to 44.9 nmol/L.  
Methods 5, 6 and 7 were "canine" methods. Method 4 is a homologous assay.

Free T4				
	n	Mean	StDev	%CV
Method 1	1	25.0		
Method 2	1	27.0		
Method 3	1	21.1		
Method 4	2	21.9	0.64	2.9
Method 5	2	20.6	0.01	0.0
Method 6	2	20.9	3.04	14.6
Method 7	1	25.7		
Method 8	5	23.1	2.83	12.3
Method 9	0			
Method 10	0			
Method 11	0			
Method 12	0			
<b>All Methods</b>	<b>15</b>	<b>22.7</b>	<b>2.60</b>	<b>11.5</b>



**Note:** Reported results ranged from 18.7 to 27.0 pmol/L.  
A FT4 result by equilibrium dialysis was reported by one laboratory (Method 1; 25 pmol/l)  
Methods 7 and 8 were "veterinary" methods. Method 3 was performed by LC-MSMS

Oestradiol				
	n	Mean	StDev	%CV
Method 1	1	158		
Method 2	1	149		
Method 3	1	259		
Method 4	1	236		
Method 5	1	199		
Method 6	2	140	130.8	93.8
Method 7	1	571		
Method 8	1	184		
Method 9	1	135		
Method 10	1	95		
Method 11	1	200		
Method 12	0			
<b>All Methods</b>	<b>12</b>	<b>205</b>	<b>130.1</b>	<b>63.5</b>



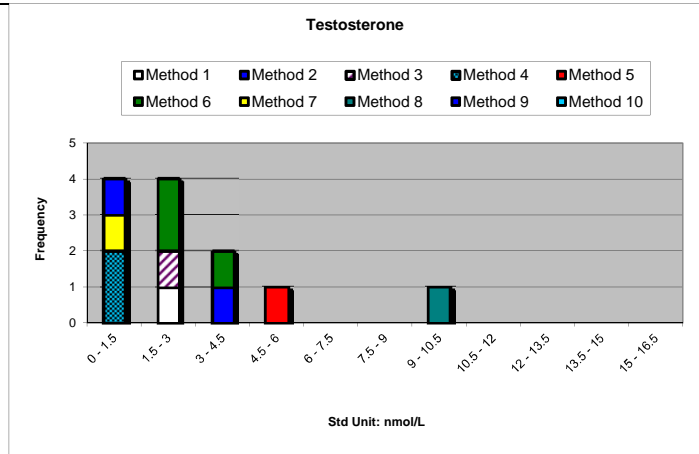
**Note:** Reported results ranged from 47 to 571 pmol/L.

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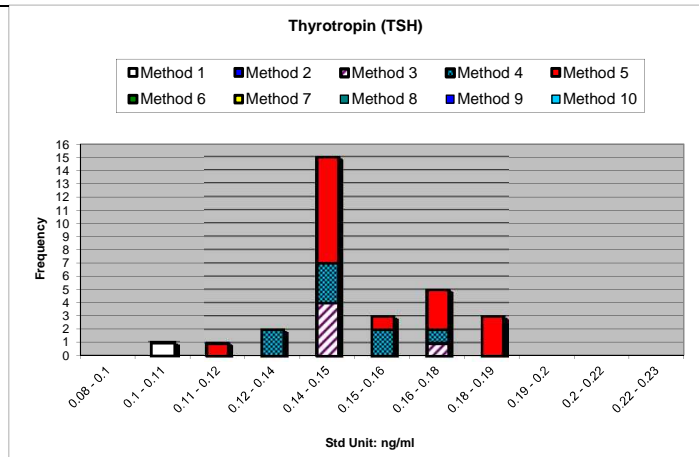
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Testosterone				
	n	Mean	StDev	%CV
Method 1	1	2.9		
Method 2	1	3.1		
Method 3	1	2.1		
Method 4	2	1.1	0.10	8.4
Method 5	1	5.6		
Method 6	3	2.2	0.72	32.2
Method 7	1	0.0		
Method 8	1	10.0		
Method 9	1	0.8		
Method 10	0			
Method 11	0			
Method 12	0			
<b>All Methods</b>	<b>12</b>	<b>2.8</b>	<b>2.69</b>	<b>96.1</b>



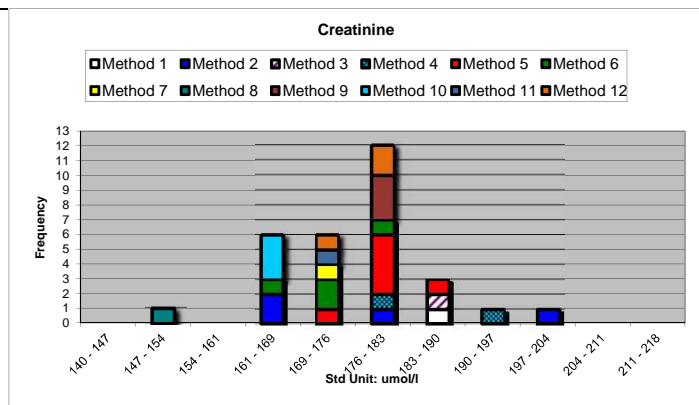
**Note:** Reported results ranged from 0 (undetectable) 10 nmol/L. One lab (Method 4) indicated that they knew their method was only validated for canine samples.

TSH				
	n	Mean	StDev	%CV
Method 1	1	0.10		
Method 2	0			
Method 3	5	0.15	0.014	9.3
Method 4	8	0.15	0.013	9.2
Method 5	16	0.16	0.021	13.6
Method 6	0			
Method 7	0			
Method 8	0			
Method 9	0			
Method 10	0			
Method 11	0			
Method 12	0			
<b>All Methods</b>	<b>31</b>	<b>0.15</b>	<b>0.020</b>	<b>13.3</b>



**Note:** Reported results ranged from 0.1 to 0.19 ng/ml. One result was reported in uIU/ml (Method 2) and excluded due to a lack of conversion factor (0.25 fel ref range 0.04 -0.44 uIU/ml). Methods 3, 4 and 5 represent the same manufacturer's chemiluminescent assay on 3 platforms (Siemens Immulite)

Creatinine				
	n	Mean	StDev	%CV
Method 1	1	187		
Method 2	4	177	16.7	9.4
Method 3	1	186		
Method 4	2	183	10.0	5.5
Method 5	6	179	6.0	3.3
Method 6	4	171	5.7	3.3
Method 7	1	169		
Method 8	1	154		
Method 9	3	179	1.7	0.9
Method 10	3	168	0.6	0.3
Method 11	1	173		
Method 12	3	175	3.7	2.1
<b>All Methods</b>	<b>31</b>	<b>176</b>	<b>9.2</b>	<b>5.2</b>



**Note:** Reported results ranged from 105 to 201 umol/L. One result (Method 9; 105 umol/L) was excluded from statistical analysis (c), (uc), (enz) and (ns) refer to Compensated Jaffe, Uncompensated Jaffe, Enzymatic and not-specified methods respectively (c) Methods 2 & 6; (uc) Methods 1, 5, 9 & 12; (enz) Methods 3, 7 & 10; (ns) Methods 4, 8 & 11