EVALUATION REPORT OF THE AIA-360 AND THE ANALYSIS OF SAMPLES FROM DOGS AND CATS

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INTRODUCTION

Biochemical analyses are now becoming a reality in routine veterinary medicine and they are in daily practice including point of care testing. However, the analyses are not an easy accomplishment in particular in the endocrinology field. Thus hormone analyses are done in a specialised laboratory because most are performed with validated methods and expensive analysers. However, hormone testing using immunoenzymetric methodology exists for human analysis and there is now a potential interest in veterinary medicine. We have tested the AIA-360 (Tosoh Bioscience) by comparing results obtained in plasma and serum of dogs and cats with that of methods already utilised in veterinary practice.

I. OBJECTIVE OF THE STUDY

To compare the results obtained on the AIA-360 for the following analytes:
Total T4
FreeT4
Insulin
Cortisol
Progesterone
Troponin I
with that of a method used in a specialised routine veterinary laboratory at “Le Laboratoire de Biochimie de l’Ecole Nationale Vétérinaire de Lyon”.

II. MATERIALS AND METHODS

II.1. SAMPLES

The aliquots of serum or heparinised plasma from the dogs and cats (whether ill or not) where collected from the reference laboratory: “Le Laboratoire de Biochimie de l’Ecole Nationale Vétérinaire de Lyon” and the “Laboratoire de Biologie Médicale Vétérinaire, Ecole Nationale Vétérinaire de Toulouse. These specimens were preserved at -18°C and underwent a maximum of 1 freeze/thawing cycle.

The samples were analysed for the following analytes:

- Total T4 (TT4): 103 specimens from dogs and 19 from cats
- FT4: 37 specimens from dogs on which TSH was measured + 30 other specimens on which only TT4 was measured.
- Cortisol: 101 specimens from dogs
- Insulin: 67 specimens from dogs
- cTnI: 73 specimens from dogs
- Progesteron: 33 specimens from dogs

II.2. DOSAGES

II.2.1. Reference Methods

The methods used for the measurement of the hormones were the same as used in the reference Laboratory (Le Laboratoire de Biochimie de l'Ecole Nationale Vétérinaire de Lyon).

- Cortisol: RIA kit, DPC TK CO 1 (Diagnostic Products Corporation, La Garenne Colombes, France)
- Insulin: RIA kit, DiaSorin P2796, (DiaSorin SA, Antony, France) and HI 14K (Linco Research, St Charles, USA)
- Thyroxine (Total T4): RIA kit, DiaSorin CA 1535M et DPC Immulite canine T4 (chemiluminescence)
- Progesterone: RIA kit, DPC Immulite (Diagnostic Products Corporation)

Plasma Troponin I was measured using the Bayer Advia Centaur (chemiluminescence) by "Le Laboratoire de Biochimie du Centre Hospitalier universitaire de Toulouse Rangueil. This method has been validated for the measurement of dog samples.

III.2.2. Measurement on the AIA-360

After defrosting in a water bath at 37°C, the specimens were centrifuged for 3 minutes, right before analysis the supernatant was transferred into an Eppendorf tube. All the specimens were analysed according to the procedure given by Tosoh Bioscience. The daily check was carried out everyday before the beginning of the analysis and all the results were satisfactory (Appendix 2). The ranges, except for that of Progesterone which was carried out thereafter, were performed in duplicate the day of the start-up by the

technician. The controls for TT4 presented a slightly high values and the level 3 controls (high values) gave values outside the reference interval during subsequent days of experimentation. After a telephone call with the Hot Line of TOSOH, the reference range was recalculated and all controls gave results within these ranges. In accordance with the instructions of TOSOH, apart from the days of calibration, at least one control was carried out on each day for each analyte. Disregarding the slight deviation for high TT4 controls, corrected afterwards as indicated previously, all the results of the control samples gave results conforming to the interval of the target values provided by TOSOH.

II.3. STATISTICAL ANALYSIS

The statistical analyses were carried out using Excel and Analyse It software. The correlation methods used were Spearman correlation or Kendall correlation when the number of results was lower than 30. The methods were compared using Passing Bablock regressions.

III. RESULTS

III.1. PRACTICAL ANALYSIS

No specific difficulty was encountered in the practical realisation of the tests.

Twice a cup was not recognised but afterwards these cups could be used without any problem.

After the conversation with the Hot Line of TOSOH Bioscience and the analysis of the first results of TT4, it was decided to implement, parallel to the realisation of a new range of TT4, a decontamination procedure (appendix 3). Although being slightly time-consuming, this procedure, which apparently is done only when necessary, requires neither competence nor special material except products usually used in laboratories (hydrochloric acid and bleach).

The achievement of the ranges is done while following the instructions without difficulties, and the validity period of the ranges is sufficient (3 months)

III.2. CORTISOL RESULTS

III.2.1. Distribution of the Plasma Values of the Specimens

The cortisol results of the 101 specimens of dogs varied from 5 to 1138 µmol/L with the reference method. The sensitivity of this method is 5 µmol/L; the usual values go from 250 µmol/L for basal cortisol to 500 µmol/L for the cortisol post stimulation with ACTH. 67 samples had cortisol concentrations of <250 µmol/l, 23 between 250 and 500 µmol/l and 11 >500 µmol/L.

The interval of the plasmatic concentrations of the tested samples thus corresponds to the interval of the plasmatic, physiological and pathological concentrations, observed under the conditions of the current veterinary surgeon practice.

III.2.2. Comparison of Results

The results show a very good correlation between those obtained with the DPC kit and the AIA-360 (fig 1). The correlation coefficient (confidence interval 95% between brackets) is 0.96 [0.94-0.97], p<0.0001). The interval of the actual values with the AIA varies from 11 to 1364 µmol/l. Only one sample, whose cortisol measured by the DPC was that of 1094 µmol/l gave a result higher than the assay range (approximately 1650 nmol/l).This was not a problem in so far as these two values were clinically considered the same.

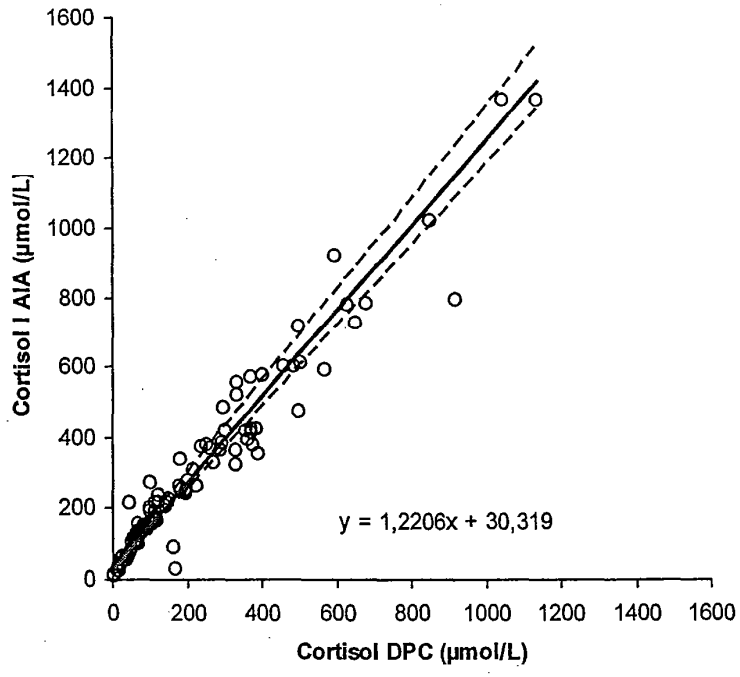

Figure 1: Comparison of the cortisol results obtained from 101 canine plasma samples with DPC and AIA-360 (TOSOH Bioscience) (Passing Bablock regression).
___ Trendline, ---------- 95% confidence interval.

III.2.3. Clinical Interpretation

The cortisol was measured in dogs within the framework of the diagnosis of 2 endocrinopathies: hypo- and hypercorticism. Isolated cortisol values have little value, considering the very diurnal character of the cortisol secretion, and in fact dynamic tests (stimulation of ACTH or reducing with the dexamethasone) are employed.

During hypocorticism, the values of the cortisol before and after stimulation are very low: the sensitivity of the measured values on the AIA makes it possible to detect these cases. During hypercorticism, one observes often high basic cortisol (>250 µmol/L), and cortisol value of >500 µmol/L after stimulation with ACTH. If the same decision thresholds are applied to the cortisolemies measured with the AIA, all the samples whose cortisolemies are higher than the reference values by the reference method are classed in the same way by the AIA. On the other hand, 7 plasmas whose cortisolemies were inferior then 250 µmol/L and 6 whose cortisolemies lie between 250 and 500 µmol/L with the DPC had cortisolemies of >250 and 500 µmol/L respectively with the AIA (cf Table 1).

CORTISOL	DPC	CORTISOL AIA (µmol/L)		
(µmol/L)	n	<250	250≤ -<500	>500
<250	67	60	7	0
250≤ -<500	23	0	17	6
>500 µmol/L	11	0	0	11

Table 1: Comparison of the clinical classifications of the values of cortisol between DPC and AIA if the same values thresholds are applied. CortDPC = cortisol measured on DPC; CortAIA = cortisol measured on the AIA-360

If one applies to the DPC thresholds values the multiplying coefficient of the Passing Bablock regression (y = 1.22x), the threshold values of the AIA become 305 and 610 µmol/L. With these new reference value limits, only 4 specimens are classified in different clinical categories with the two methods: 3 whose cortisol measured by DPC is <250 µmol/l have a cortisol value on the AIA of >305 µmol/L (CortDPC 185, 216 and 238 µmol/L versus CortAIA 336, 307 and 372 µmol/L respectively). A specimen whose cortisol measured by DPC is >500 µmol//L (568 µmol/L) has a cortisol measured on the AIA lower than 610 µmol/L; the result obtained with the AIA (592 µmol/L) is however very close to this threshold and analytically equivalent taking into account the inaccuracy of the method.

CORTISOL	DPC	CORTISOL AIA (µmol/L)		
([µmol/L)	n	< 305	305< - <610	>610
<250	67	64	3	0
250≤ -<500	23	0	23	0
>500 µmol/L	11	0	1	10

Table 2: Comparison of the clinical classifications of the cortisol values between DPC and AIA if the value limits are adapted according to the Passing Bablock regression.

III.2.4. Conclusion

The AIA-360 from TOSOH gives results of cortisol completely comparable with those obtained with Immulite from DPC. It can be used in routine by using the limits of 305 and 610 µmol/L as reference interval.

III.3. TOTAL T4 RESULTS

III.3.1. Distribution of the Specimen Plasma Values

The total thyroxine of 63 dogs and 19 cats measured with the DiaSorin method went from 2 to 111 nmol/L and from 16 to 260 nmol/L respectively. The sensitivity of this method is 2 nmol/L. In dogs, 32, 19, 26 and 4 specimens had a total thyroxine of below 20 nmol/L (regarded as low), ranging between 20 and 25 nmol/L (regarded as "doubtful"), ranging between 25 and 50 nmol/L (usual values in dogs) and higher than 50 nmol/L respectively. In cats, 13 and 6 specimens had a total thyroxine of lower than 50 nmol/L (usual values in the cat) and higher than 50 nmol/L respectively. The interval of the plasma concentrations of the tested samples thus corresponds to the plasma intervals, physiological and pathological observable concentrations under the conditions of the current veterinary surgeon practice.

We also obtained 40 specimens of dogs whose total thyroxine had been measured using the DPC kit; sensitivity of this method is 6 nmol/L. Twenty seven, 5 and 8 samples had a total thyroxine respectively of less than has 20 nmol/L (regarded as low), ranging between 20 and 25 nmol/L (regarded as "doubtful"), ranging between 25 and 50 nmol/L (usual values in the dog).

III.3.2. Comparison of Results

With Dogs

The results show an average correlation between those obtained with the DiaSorin kit and the AIA-360 (fig 2). The correlation coefficient (confidence interval 95% between brackets) is 0,60 ([0,43-0,73], p<0.0001). The interval of the observed values with the AIA goes from below sensitivity to 79,8 nmol/L, while the variation with DiaSorin is from 2 to 111 nmol/L. Eleven samples, whose thyroxine measured by DiaSorin varied from 2 to 21 nmol/L, gave with the AIA a result lower than assay high, that is to say < 3.9 nmol/l.

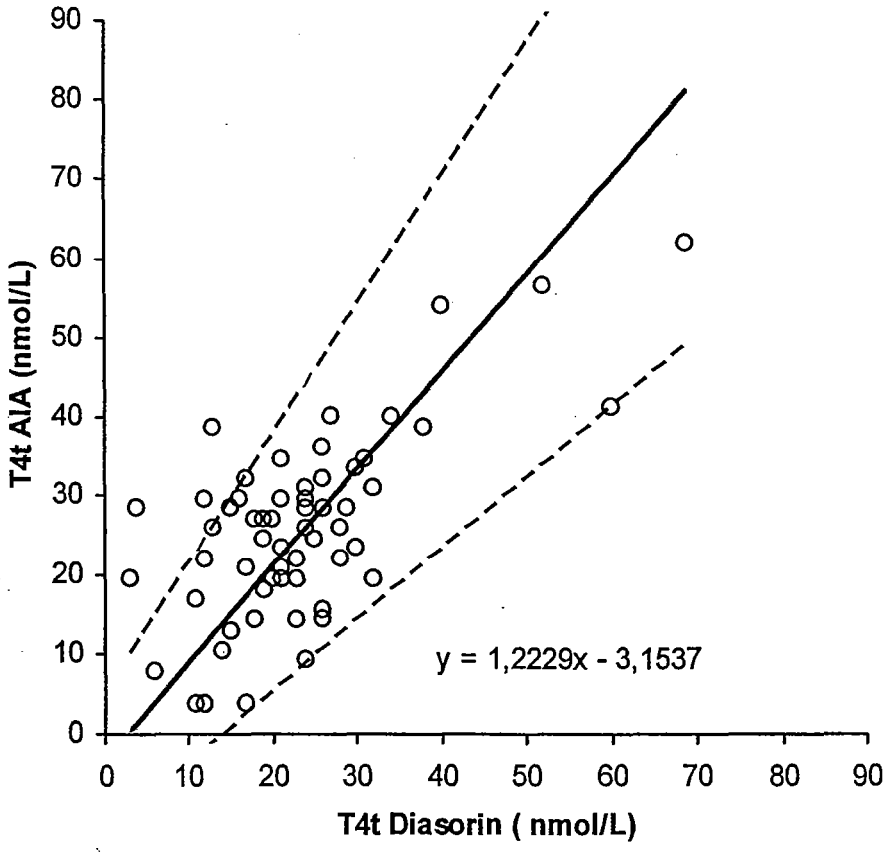

Figure 2: Comparison of the Total thyroxine results obtained on 63 plasma samples from dogs measured with the DiaSorin kit and with the AIA-360.
___ Trendline, ---------- 95% confidence interval.

This is why we carried out a second comparison with the DPC kit, more expensive but considered as being one of the best methods in dogs, on specimens whose total thyroxine varied between < 6 and 34 nmol/l with the DPC. One observed a very good correlation between the DPC and the AIA (figure 3). The correlation coefficient is 0,93 ([0,87-0,96], p <0.0001).

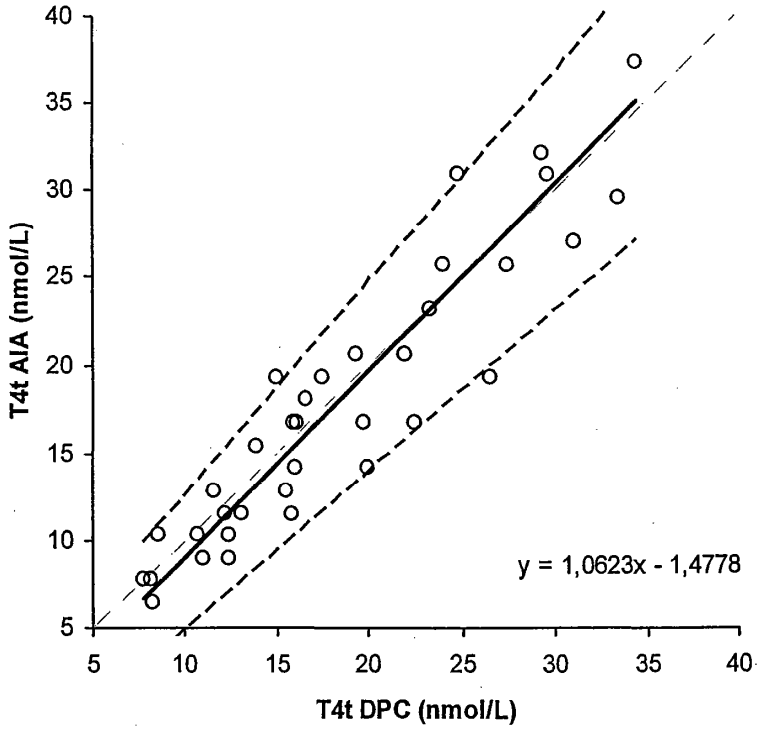

Figure 3: Comparison of the results for Total Thyroxine obtained from 40 plasma dog samples measured with the DPC kit and the AIA-360. (Passing Bablock regression).
___ Trendline, ---------- 95% confidence interval.

In Cats

The results show a correct correlation between those obtained with the DiaSorin kit and the AIA-360 (fig 4). The regression coefficient is 0,99 ([0,49-1,26], p<0.02).

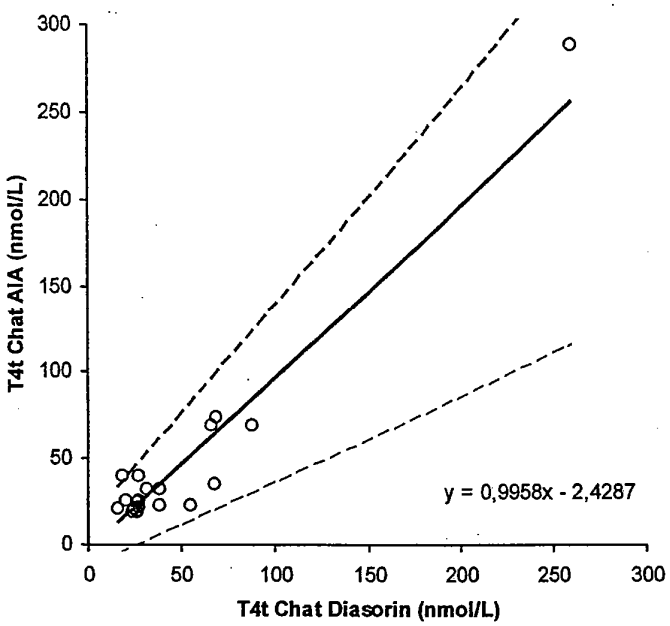

Figure 4: Comparison of the results for Total Thyroxine obtained from 19 feline plasma samples measured with the DiaSorin kit and the AIA-360. (Passing Bablock regression).
___ Trendline, ---------- 95% confidence interval.

III.3.3. Clinical Interpretation

In Dogs

Thyroxin was measured within the framework of the diagnosis and the follow-up of the treatment of a principal endocrinopathy, hypothyroidism. At the time of this endocrinopathy, the values of thyroxine can be very low in dogs, and in particular much lower than the actual values in humans. The interval of the values obtained on the AIA, and its correct correlation are included in the values. The interpretation of the total thyroxine is made difficult by the fact that at the time of in particular chronic affections, low values are observed without them being associated to a functional deficit of the hypothalamo-hypohyso-thyroid axis: one speaks about pseudo hypothyroidism. This is why there is a zone of values known as "doubtful" between 20 and 25 nmol/L, although the reference values in healthy animals lie between 25 and 50 nmol/L.

We have used the same value limits, which are consistent with the coefficients obtained by the Spearman regressions, that it performs the DiaSorin-AIA (0,96) or DPC-AIA (1,06) comparisons.

Fourteen samples, whose total thyroxine was measured by DiaSorin were < 20 nmol/L, are classified by the AIA in the doubtful zone (n=6) or within the reference range (n=8) (table 4). The largest change was observed in a sample whose thyroxine measured by DiaSorin is 13 nmol/L versus 37 by the AIA. In the same way, the 19 samples considered doubtful by DiaSorin, 3 have a thyroxine <20 nmol/L (and even < than the detection limit for one of them) and 6 ranging between 25 and 50 nmol/L according to the AIA. Lastly, among the 26 samples classed as normal by DiaSorin, 16 are also classified like such by the AIA, while 2 have a measured thyroxine <20 nmol/L (14 and 18 nmol/L), 7 between 20 and 25 nmol/L and 1 >50 (56 nmol/L).

TT4 DiaSorin (nmol/L)	n	TT4 AIA(nmol/L)			
		<20	20 ≤ - <25	25<- <50	> 50
<20	32	18	6	8	0
20 ≤ - <25	19	3	10	6	0
25<- <50	26	2	7	16	1
> 50	4	0	0	2	2

Table 4: Comparison of the clinical classification of the Thyroxine values between DiaSorin and AIA in dogs. TT4Diasorin = Thyroxin measured by DiaSorin; TT4AIA = Thyroxin measured on AIA

When one compares the results obtained in the standard low values interval between the DPC and the AIA, only 1 specimen identified with a low thyroxine by the DPC is classified

as doubtful by the AIA; however, the values obtained by the 2 methods are very close and can even be considered as identical taking into account the precision of the methods (CV of 10% in this measurement range for the DPC, and 8% according to the manual of TOSOH): TT4DPC = 19,4 nmol/L versus TT4AIA = 20,6 nmol/L. Two samples, whose measured TT4 were 20 and 22,5 nmol/L, are classified in the low range by the AIA (TT4AIA of 14,2 and 16,7 respectively). Lastly, on the 8 samples whose TT4 measured by the DPC was within the reference range (25 - 50 nmol/L), all except one are properly classified (TT4AIA 19,3 nmol/L versus TT4DPC 25,6 nmol/L).

TT4 DPC (nmol/L)	n	TT4 AIA (nmol/L)		
		<20	20< - <25	25<- <50
<20	27	26	1	0
20<_ - <25	5	2	3	0
25<- <50	8	1		7

Table 5: Comparison of clinical classifications of the thyroxin values between DPC and AIA in dogs. TT4DPC = thyroxin measured on DPC; TT4AIA = thyroxin measured on AIA

In Cats

Contrary to dogs, the principal indication for the measurement of thyroxine in cats is the diagnosis of a hyperthyroidism. At the time of this affection, the diagnosis is done by the evidence of thyroxine higher than the upper reference limit, specifically 50 nmol/L. All the cats classified as healthy by DiaSorin are also classed like such by the AIA. In 6 cats classified with hyperthyroidism by DiaSorin, 4 are also classed like such by the AIA, while 2 others are regarded as having thyroxine within the reference range (TT4Dias 55 and 68 nmol/L versus TT4AIA, 23 and 35 nmol/L).

	TT4AIA <50 nmol/L	TT4AIA >50 nmol/
2 TT4Dias <50 nmol/L (n=13)	13	0
TT4Di$_a$s >50 nmol/L (n=6)	2	4

Table 6: Comparison of the clinical classifications of the thyroxine values between DiaSorin and AIA in cats. TT4Dias = thyroxine measured on DiaSorin; TT4AIA thyroxine measured on AIA

III.4. Conclusion

The correlation between AIA and DiaSorin is not satisfactory in the dog; it merits to be studied on a larger number of samples on cats. However, with the DPC kit considered as superior, the AIA-360 of TOSOH gives completely comparable results of canine thyroxine, including in the low range of TT4 which are often a difficulty in veterinary medicine. This comparison DPC=AIA did not take place in the cat due to on the one hand the correct

results with DiaSorin in this species, and on the other hand the time and cost which this complementary study would have required.
The AIA can thus be used in routine for the measurement of TT4 in dogs and cats.

III.4. RESULTS FOR FREE T4 (FT4)

III.4.1. Distribution of Concentration Values of Plasma Specimens

We did not have specimens in which the free thyroxine had been measured as such methods are not used in routine in veterinary medicine. On the other hand, for 37 specimens, we had the plasma concentration of TSH besides the total thyroxine measured with the kit DiaSorin. These two measurements enabled us to report the T4/TSH ratio which decreases (<75) when the animal has hypothyroidism. Fourteen specimens presented such a reduction in T4/TSH ratio. We also measured the FT4 in 30 other specimens for which we had the total thyroxine value.

III.4.2. Comparison of the Results

The plasmatic concentration in FT4 correlates overall with the total thyroxine. The correlation coefficient (confidence interval 95% between brackets) is 0,42 ([0,20-0,60], p<0,001). We unfortunately could not repeat the comparison with the specimens on which the total thyroxine had already been measured with the DPC kit; moreover, we did not have TSH measurements on these specimens.

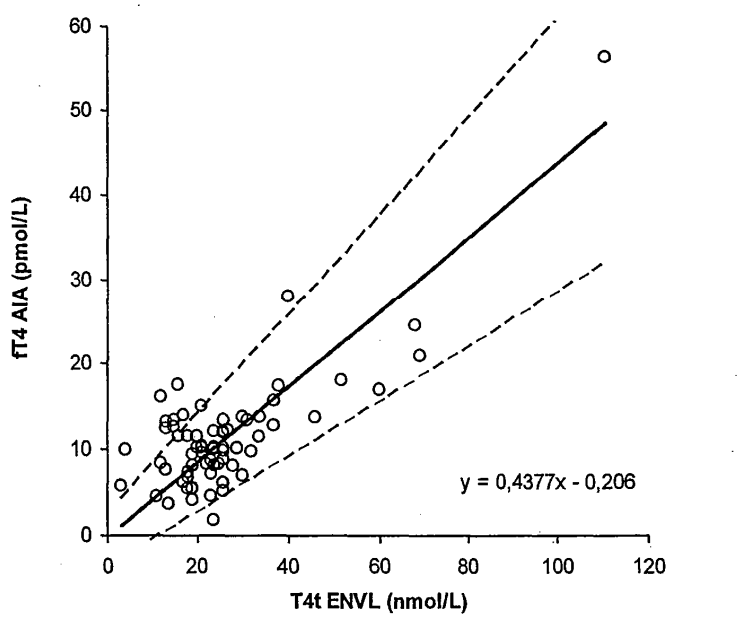

Figure 5: Comparison between the results of total thyroxine and free thyroxine obtained on 67 plasma of dogs with the DiaSorin kit and the AIA-360 (TOSOH Bioscience) (Passing Bablock regression). _____ Trendline, ---------- confidence interval 95%.

III.4.3. Clinical Interpretation

The values obtained with the AIA are completely comparable with the reference ranges obtained with other methods of FT4 measurement in literature and covers all values obtained in animals with hypothyroidism, pseudohypothyroid and euthyroid (up to 4.8 ng/dL). In an interesting way, the FT4 was measured as lower then the measurement limit of the AIA in 7 dogs for which a study of the TT4/TSH ratio is strongly in favour of hypothyroidism (varying from 1,4 to 35). It was lower than this limit in no other specimens. We miss the clinical data allowing us to diagnose pseudohypothyroidism.

III.4.4. Conclusion

The AIA-360 gives FT4 results completely comparable with the literature data, overall correlates with the total thyroxine, which is consistent with the physiology of the thyroid hormones. A FT4 lower than the measurement limit seems to be a good indicator of hypothyroidism, which is very interesting in the canine species giving the difficulties of diagnosing this affection in this species. It would be desirable to carry out studies of populations of healthy dogs, pseudohypothyroidism and hypothyroidism while having all the clinical and biological data making it possible to correctly classify these under populations in order to determine the exact FT4 sensitivity and specificity of the measurement with the AIA in the diagnosis of the hypothyroidism in dogs.

III.5. INSULIN RESULTS

III.5.1. Distribution of Concentration Values of Plasma Specimens

The results of insulin in the 27 specimens of dogs varied between 3 to 263 mU/L with the reference method. The sensitivity of this method is 2 mU/L; the usual values go from 25 to 50 mU/L. 6 specimens had an insulin value considered very high (>110 mU/L). We could not know which of these specimens corresponded to follow-ups of glycaemia on animals with diabetics treatments by exogenic insulin. The interval of the plasma concentrations of the samples tested thus corresponds to the interval of the plasma concentrations, physiological and pathological, observable under the conditions of the current veterinary surgeon practice.

III.5.2. Comparison of Results

The results show a poor correlation between those obtained with the DiaSorin kit and the AIA-360 (fig 6). The correlation coefficient (confidence interval 95% between brackets) is 0,51 [0,27-0,91], $p<0.001$). The interval of the reference values with the AIA varies from lower then the measurement limit (2 specimens which insulin measured by the DiaSorin kit was 10 and 3 mU/L, i.e. lower than the reference range) to 175 mU/L.

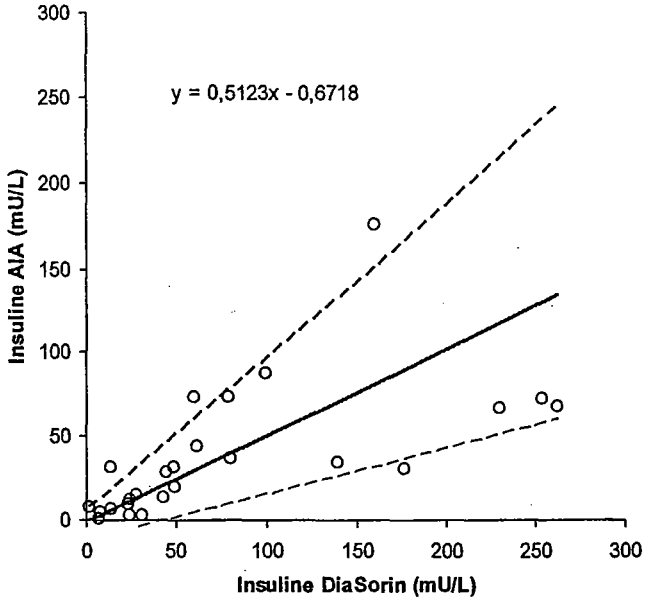

Figure 6: Comparison between the insulin results obtained from 25 dog plasmas with DiaSorin kit and the AIA-360 (TOSOH Bioscience) (Passing Bablock regression).
____ Trendline, ---------- confidence interval 95%.

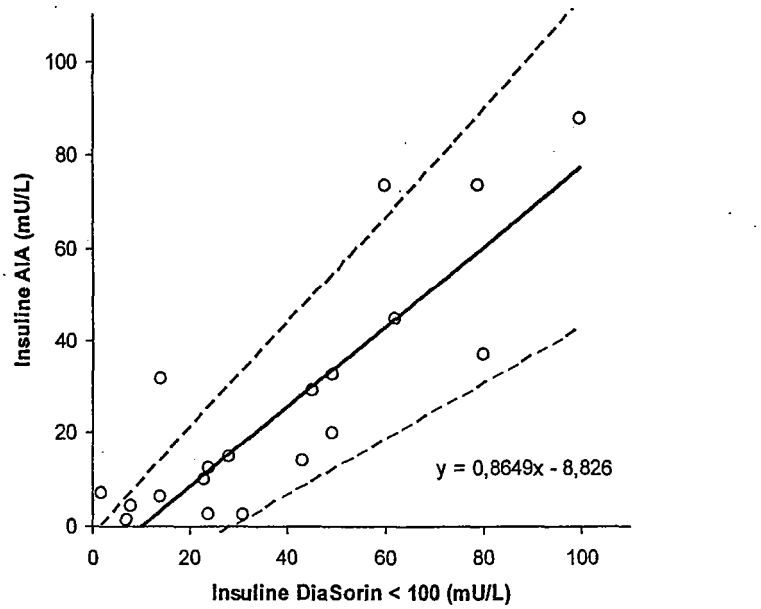

Figure 7: Comparison between the insulin results of less than 100 mU/L obtained from 19 plasmas of dogs with the DiaSorin kit and AIA-360 (TOSOH Bioscience) (Passing Bablock regression). ____ Trendline, ---------- confidence interval 95%.

If the specimens whose insulin measured by DiaSorin is higher than 100 mU/L are excluded, i.e. definitely supraphysiologic plasma concentrations, and for which it is possible to have specimens with exogenic insulin, the poor correlation between the results obtained with the DiaSorin kit and the AIA-360 is better (fig 7). The correlation coefficient (confidence interval 95% between brackets) is 0,86 [0,60-1,15], $p<0.001$

III.5.3. Clinical Interpretation

Insulin is measured in the plasma of dogs at the time of 2 endocrinopathies, diabetes type 1 and type 2, and generally always interpreted according to the value of the glycaemia measured at the same time, values we did not have here. It is thus difficult to give a clinical interpretation of these tests. This is why with the AIA-360 from TOSOH we carried out a follow-up of post prandial glycaemia in 3 dogs. The results obtained were consistent with the physiological data (Fig 8).

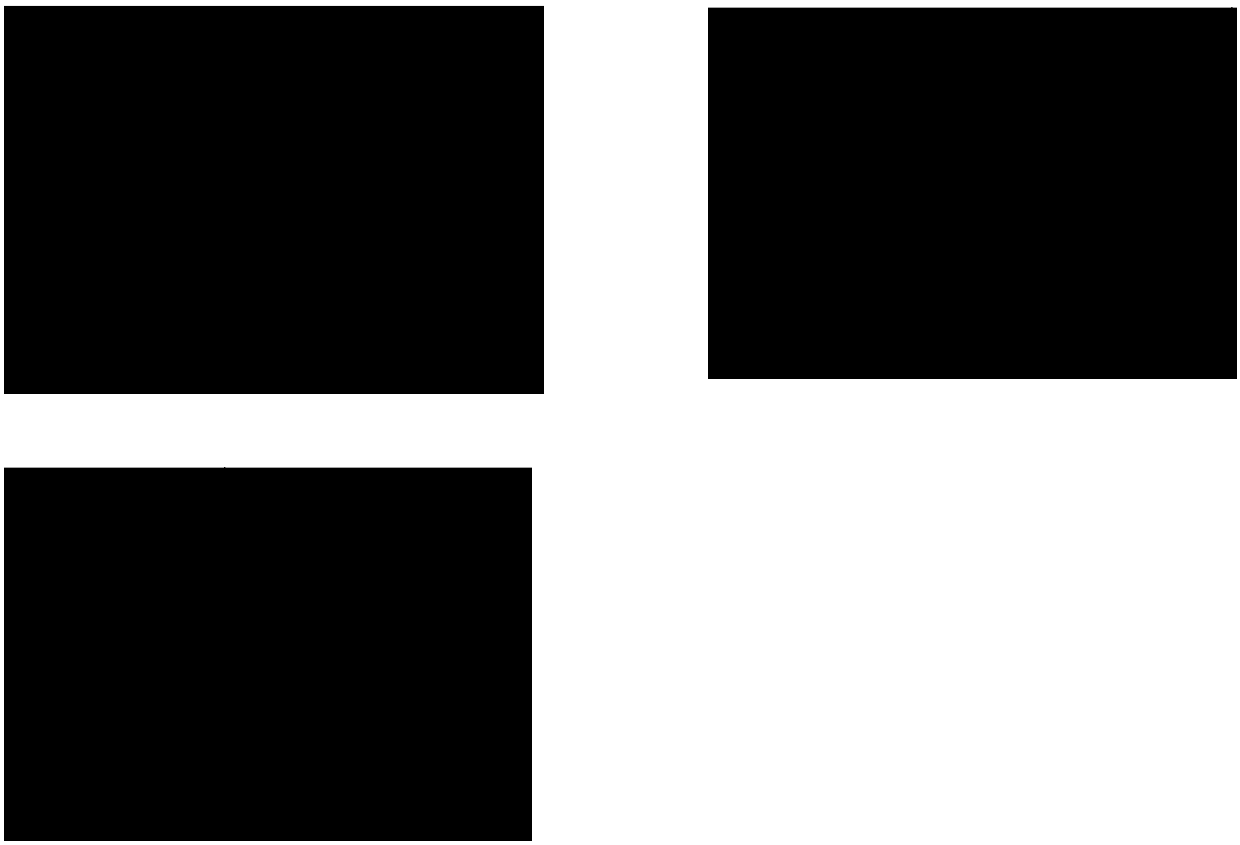

Figure 8: Curves of postprandiale insulin obtained in 3 healthy dogs with the AIA-360.

III.5.4. Conclusion

The AIA-360 from TOSOH gives insulin results completely consistent with the DiaSorin kit with the values most frequently recorded in the current veterinary surgeon practice. The post prandial follow-ups also show curves consistent with physiology.

III.6. TROPININ I RESULTS

III.6.1. Distribution of Concentration Values of Plasma Specimens

In 73 dogs, the plasmatic cTnI had been measured with the reference method, whose measurement limit of this method is 0,1 ng/mL. All the healthy dogs have an undetectable plasmatic cTnI. Among the 73 samples, 55 had a non-quantifiable plasmatic cTnI; the plasmatic cTnI measured in the other 18 samples varied from 0,17 to 32,12 µg/L.

The plasmatic concentrations interval of the tested samples thus corresponds with the interval of the physiological and pathological plasmatic concentrations, observed under the conditions of the current veterinary surgeon practice.

III.6.2. Method Comparison

All the samples whose plasmatic cTnI was undetectable with the Centaur kit gave also non-quantifiable results with the AIA. Only, one sample whose plasmatic cTnI was right at the top of the measurement limit with Centaur (0,17 ng/mL) gave a result of 0 ng/mL with the AIA (but not <; lower than the measurement limit).

The results show a very good correlation between those obtained with the Centaur kit and the AIA-360 (fig 9) when the cTnI is quantifiable. The correlation coefficient is 0,95 [0,88-0,98], $p<0.001$. The reference values range with the AIA goes from non-detectable (measurement limit evaluated at 0,02 ng/mL by TOSOH) to 10,08 ng/mL, whereas it varies from non-detectable to 32,12 ng/mL with Centaur. There is a net bias between the two methods (fig. 10).

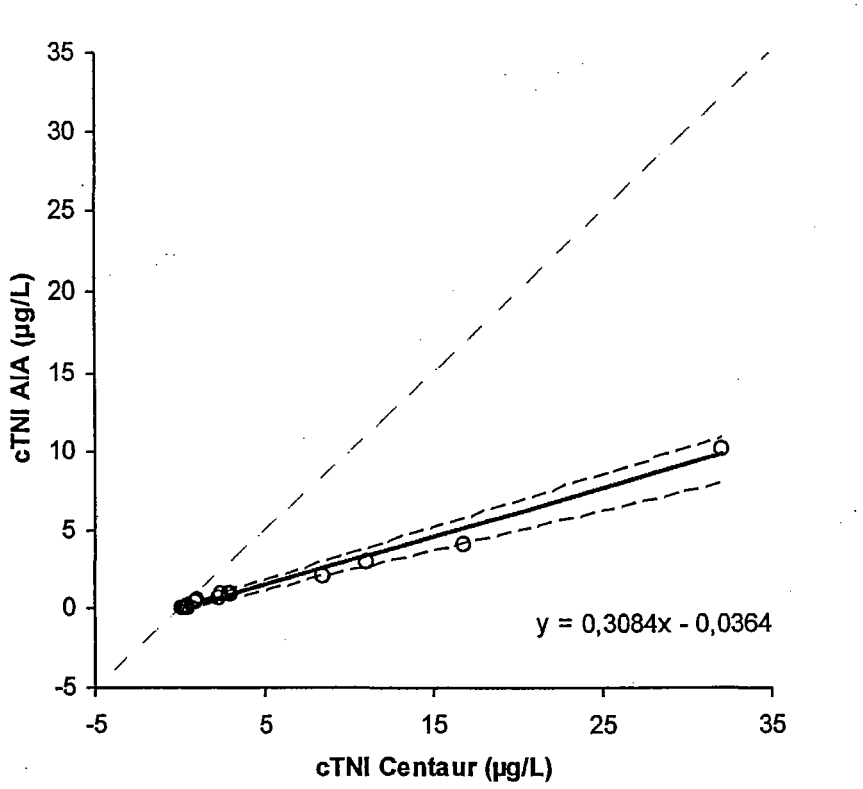

Figure 9: Comparison between the troponin results obtained in 18 plasmas of dogs in which the plasma cTnI was measurable with the DPC kit and AIA-360 (TOSOH Bioscience) (Passing Bablock regression).

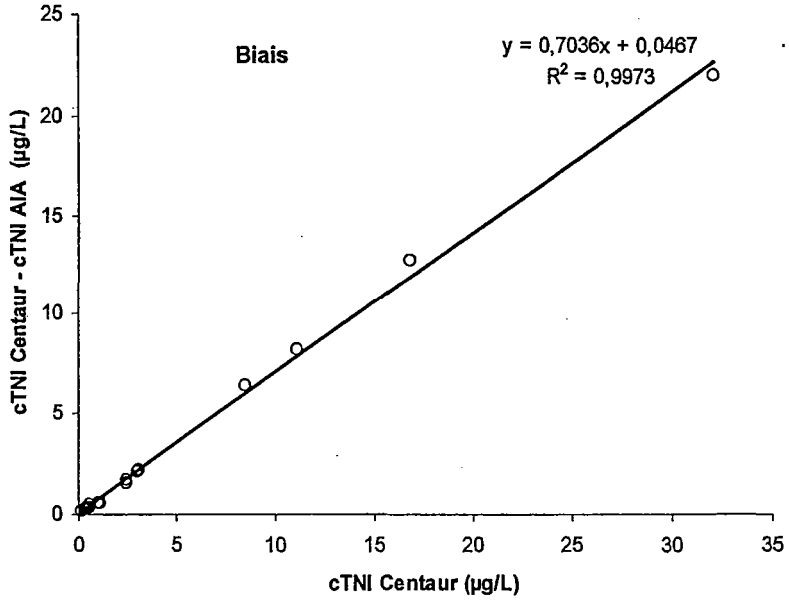

Figure 10: Bias between the plasmatic cTnI measured by Centaur and the AIA (simple linear regression).

III.6.3. Clinical Interpretation

The plasmatic cTnI is a specific marker of the myocardial suffered by humans but also in dogs. It was shown that it is not measurable in healthy dogs, but that its plasmatic concentration increases and becomes detectable in traumatic cases of myocardites or cases of advanced cardiopathies. If one considers that dogs can be placed into two categories (healthy dogs with undetectable cTnI and dogs with measurable cTnI), all the animals are well classed. In human medicine, plasmatic cTnI higher than 1,5 µg/L is regarded as a sign of a severe myocardial attack. Such limits are not yet well defined in veterinary medicine, and the evaluation of the importance of the myocardial affliction according to the plasmatic cTnI must be the subject of clinical studies.

	n	$cTnI_{AIA}$ <0,02 µg/L	$cTnI_{AIA}$>0,02 µg/L
cTNIcentaur <0,1 µg/L	55	55	0
cTNIcentaur >0,1 µg/L	18	0	18

Table 7: Comparison of the clinical classifications of the troponin values between Advia Centaur and AIA according to the detection limits. cTnICENTAUR = troponin measured on Advia Centaur; cTnIAIA = troponin measured on AIA

III.6.4. Conclusion

The AIA makes it possible to highlight an increase in the plasmatic cTnI in dogs suffering from myocardial infarction.

III.7. PROGESTERONE RESULTS

III.7.1. Distribution of Concentration Values of Plasma Specimens

The results of progesterone of the 33 specimens of dogs varied from 0,83 to 113 nmol/L with the reference method. The reference values go from <12 nmol/L in bitches in anoestrus to 32 nmol/L before ovulation. 9 specimens had a progesterone > 32 nmol/L. The interval of the plasmatic concentrations of the samples tested thus corresponds to the interval of the physiological and pathological plasmatic concentrations, observable under the conditions of the current veterinary surgeon practice.

III.7.2. Comparison of Results

The results show a good correlation between those obtained with the DPC kit and the AIA-360 (fig 11). The correlation coefficient (confidence interval 95% between brackets) is 0,92 [0,84-96], $p<0.001$. The interval of the reference values with the AIA varies from 0,6 nmol/L to higher than the upper limit.

Only one sample, of which the progesterone measured by the DPC was 113 nmol/L, gave with the AIA a result higher than the linearity limit (approximately 143 nmol/L). That is hardly problematic insofar as these two values are clinically regarded in the same manner as being extremely above the reference range.

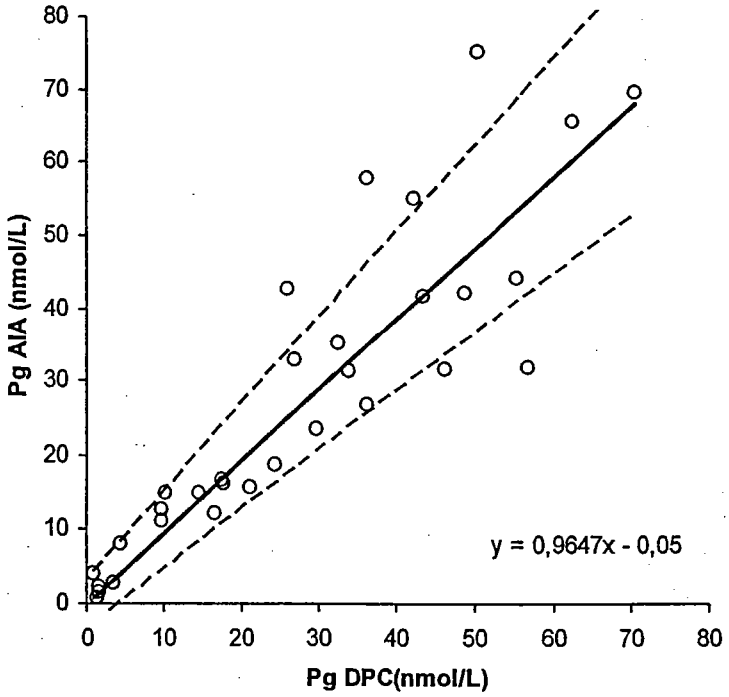

Figure 11: Comparison between the progesterone results obtained from 32 dogs plasmas with DPCkit and AIA-360 (TOSOH Bioscience) (Passing Bablock regression).
_____ Trendline, ---------- confidence interval 95%.

III 7.3. Clinical Interpretation

Progesterone is measured in the bitch within the framework of the gynaecological follow-up and optimisation of implantation. A value of 12 nmol/L is indicative of anoestrus, and implantation is recommended 24 to 48 hours afterwards, when progesterone has exceeded 32 nmol/L. We have watched the same value limits for the AIA and the DPC considering the regression curve.

Pg DPC (nmol/L)	n	Pg AIA (nmol/L) <12	12<-<32	>32
<12	9	8	1	0
12≤ - <32	10	0	7	3
>32	14	0	4	10

Tableau 8. Comparison of the clinical classifications of the progesterone values between DPC and AIA if the same value limits are applied. PgDPC = progesterone measured on DPC; PgAIA = progesterone measured on AIA

When comparing the results obtained in the low value interval between the DPC and the AIA, only 1 specimen, identified with a progesterone <12 nmol/l by the DPC is classed between 12 and 32 nmol/L by the AIA; however, the values obtained by the 2 methods are

very close and can even be regarded as analytically identical: PgDPC = 10,2 nmol/L versus PgAIA = 14,8 nmol/L. Three specimens, whose progesterone measured between 12 and 32 nmol/L by DPC kit, have classified values of >32 nmol/L by the AIA (PgAIA of 32,7 - analytically comparable with>32 nmol/L, 42,7 and 76,8 respectively). Lastly, on the 14 specimens whose Pg measured by the DPC is > 32 nmol/L, only one gave a slightly lower progesterone on the AIA (PgAIA 26,7 nmol/L versus PgDPC 36,4 nmol/L). Three other specimens have progesterone analytically comparable to 32 nmol/L (PgAIA 31,4, 31,6 and 31,8 nmol/).

III.7.4. Conclusion

The AIA-360 from TOSOH gives results for progesterone completely comparable with those obtained with DPC Immulite.

IV. GENERAL CONCLUSION

The AIA-360 is a very simple and practical instrument. The obtained results are completely comparable with references methods, particularly for canine cortisol, totalT4 feline and canine, progesterone and cardiac Troponin I canine. The obtained results for measuring insulin and free T4 in dogs are encouraging and completely consistent with the physiological and physiopathological data currently available.

APPENDIX 1: LIST OF ANALYSED SPECIMENS

Identification specimen	Reference results T4 nmol/L	TSH pmol/L	Cortisol µmol/L	Insulin mU/L	Progesterone nmol/L	cTnI µg/L	Space
1	34						CN
2	18						CN
29	37	0,05					CN
32	38	0,17					CN
33a	37	0,23	22				CN
33b			86				CN
36a			119				CN
36b			568				CN
38a			84				CN
38b			919				CN
39a			152				CN
39b			392				CN
44	22	0,09					CN
59	2	1,4					CN
60	13	1,1					CN
61a	13	0,37	25				CN
61b			123				CN
62a	69		65				CN
62b			6				CN
62c			35				CN
64a			16				CN
64b			273				CN
65a	31		82				CN
65b			598				CN
67a			144				CN
67b			300				CN
69	19						CN
70	24						CN
72				<2			CN
73				14			CN
87a	4	0,19	100				CN
87b			1138				CN
90	30	0,21	56				CN
91	12	0,19	35				CN
96				54			CN
113	11	0,73					CN
114	28	0,14					CN
121a	26	0,16	45				CN
121b			165				CN
122a	26	0,09	21				CN
122b			171				CN
123a	11		24				CN
123b			238				CN
132	5	0,11	72				CN
133a			114				CN
133b			682				CN
134a			99				CN
134b		-	334				CN
135a			21				CN

Identification specimen	Reference results						
	T4 nmol/L	TSH pmol/L	Cortisol µmol/L	Insulin mU/L	Progesterone nmol/L	cTnI µg/L	Space
135b			507				CN
143	60		201				CT
167	12	1,2					CN
168	68						CT
170a			122				CN
170b			502				CN
171a	55		108				CT
171b			296				CN
172a	3	0,03	81				CN
172b			631				CN
173a	46		71				CN
173b			263				CN
175a			117				CN
175b			252				CN
176	22	0,3	60				CN
178				14			CN
181				23			CN
187	88						CT
190	40						CN
191	66						CT
192	17						CN
193	27						CT
194	20						CN
195	27						CN
196	69						CT
197	28						CT
198	21	0,6					CN
199	18	0,1					CN
200	14	0,09					CN
202a			<5				CN.
202b			<5				CN
205a			117				CN
205b			216				CN
206a			374				CN
206b			1094				CN
207a			67				CN
207b							CN
207c							CN
223	13	0,83					CN
224	111						CN
225	13						CN
226	21						CT
229a	18		40				CN
229b			332				CN
230a	26		103				CN
230b			385				CN
231a	14	0,17	149				CN
231b			359				CN
232a			82				CN
232b			849				CN
233a			82				CN
233b			489				CN
248	32						
249	16	0,03					CN

Identification specimen	Reference results						
	T4 nmol/L	TSH pmol/L	Cortisol µmol/L	Insulin mU/L	Progesterone nmol/L	cTnI µg/L	Space
253	23						CN
254	16						
255	12						CN
256	24						
257	32		14				CN
259a	13		61				CN
259b			289				CN
264a				164			CN
264b				254			CN
264c				263			CN
264d				230			CN
266				46			CN
268				49			CN
283	19	1					CN
284	39						
285	52						CN
286	16						CN
287	29	0,26					CN
288	24	0,44					CN
289	24						CN
290	28						
291	39						
292	27						
293	23						CN
294	18	0,09					CN
295	21		49				CN
296a	26		26				CN
296b			378				CN
297a	34		69				CN
297bb			197				CN
298	24		225				CN
299	17						CN
300a			92				CN
300b			653				CN
301a			64				CN
301b			333				CN
322	25						CN
323	20						CN
324	143						CT
325	260						CT
326	26	0,4					CN
327	15	0,06					CN
328	28						
329a	21	0,17	85				CN
329b			304				CN
330a	30		196				CN
330b			460				CN
331a	26		182				CN
331b			336				CN
332a			13				CN
332b			19				CN
333a			50				CN
333b			56				CN
334a			77				CN

Identification specimen	Reference results						
	T4 nmol/L	TSH pmol/L	Cortisol µmol/L	Insulin mU/L	Progesterone nmol/L	cTnI µg/L	Space
334b			181				CN
335a			116				CN
335b			362				CN
345	25						CT
346	21						CN
347	19	0,03					CN
348	21						CN
349	19						CN
350	26	0,16					CN
351	17	0,79					CN
352	20						CN
353	24		46				CN
354a	23		149				CN
354b			1047				CN
355a	26	0,12	103				CN
355b			373				CN
356a	19		141				CN
356b			499				CN
358a			66				CN
358b			372				CN
377	21	0,61					CN
378	15	0,03					CN
379	6						CN
380	26						CN
381	28						CN
382	19						CT
383	31						CN
387a	15		64				CN
387b			197				CN
388a			185				CN
388b			406				CN
422				12			CN
499				45			CN
533				177			CN
559				6			CN
560/1				8			CN
588				28			CN
692				3			CN
730				7			CN
733				20			CN
883				18			CN
974				140			CN
1006				25			CN
1009				13			CN
1089				16			CN
1090/1				160			CN
1090/2				62			CN
1188				49			CN
1212/1				79			CN
1212/2				100			CN
1213				82			CN
1227				2			CN
1228				31			CN
1326				11			CN

Identification specimen	Reference results						
	T4 nmol/L	TSH pmol/L	Cortisol µmol/L	Insulin mU/L	Progesterone nmol/L	cTnI µg/L	Space
1337				43			CN
1437				60			CN
1506				23			CN
1630				80			CN
1631				31			CN
1634				21			CN
1755				30			CN
1757				44			CN
1758				24			CN
1824				10			CN
1826				24			CN
5020					1,6		CN
5021					46,3		CN
5022					56,8		CN
5023					0,83		CN
5024.					24,6		CN
5026					32,5		CN
5030					9,8		CN
5044					43,5		CN
5045					14,6		CN
5046					26,1		CN
5047					10,2		CN
5048					48,7		CN
5080					36,4		CN
5101					42,4		CN
5102					1,4		CN
5103					50,5		CN
5104					36,4		CN
5105					55,6		CN
5118					9,6		CN
5129					27		CN
5130					113		CN
5162					62,6		CN
5163					17,6		CN
5164					34		CN
5190					3,5		CN
5191					29,9		CN
5192					16,6		CN
5193					21,3		CN
5230					17,9		CN
5231					1,7		CN
5259					4,5		CN
5262					12,8		CN
5293					70,8		CN
T-26						<0,10	CN
T-102						<010	CN
T-123						<0,10	CIA
T-201						<0,1	CN
T-282						3,09	CN
T-292						<0,10	CN
T-327						0,33	CN
T-356						<0,1	CN
T-367						<0,10	CIA
T-412						<0,10	CN

Identification specimen	Reference results						
	T4 nmol/L	TSH pmol/L	Cortisol µmol/L	Insulin mU/L	Progesterone nmol/L	cTnI µg/L	Space
T-481					<0,10		CN
T-533					<0,10		CN
T-611					8,47		CN
T-730					<0,1		CN
T-1102					<0,1		CN
T-1111					<0,10		CN
T-1157					<0,10		CN
T-1219					0,52		CN
T-1248					0,29		CN
T-1277					3,01		CN
T-1394					<0,10		CN
T-1454					. <0,10		CN
T-1457					<0,10		CN
T-1510					<0,10		CN
T-1517					<0,10		CN
T-1519					11,11		CN
T-1619					<0,1		CN
T-1692					<0,10		CN
T-1701					<0,1		CN
T-1706					<0,1		CN
T-1740					0,37		CN
T-1761					<0,1		CN
T-1779					2,44		CN
T-1843					0,17		CN
T-1871					<0,10		CN
T-1891					<0,10		CN.
T-1948					<0,10		CN
T-1991					<0,10		CN
T-2038					32,12		CN
T-2041					<0,10		CN
T-2054					2,48		CN_
T-2073					<0,10		CN
T-2107					<0,10		CIA
T-2110					0,55		CN
T-2299					0,5		CN
T-2306					<0,10		CN
T-2363					<0,10		CN
T-2374					<0,10		CN
T-2417					<0,10		CN
T-2419					0,39		CN
T-2442					<0,10		CN
T-2649					<0,10		CN
T-2693					1,02		CN
T-2695					<010		CN
T-2710					<0,10		CN
T-2738					<0,1		CN
T-2751					<0,1		CN
T-2764					<0,10		CN
T-2810					<0,10		CN
T-2816					<0,1		CN
T-2851					<0,1		CN
T-2855					<0,1		CN
T-2859					<0,10		CN
T-2865					<0,10		CN

Identification specimen	Reference results						
	T4 nmol/L	TSH pmol/L	Cortisol µmol/L	Insulin mU/L	Progesterone nmol/L	cTnI µg/L	Space
T-2883					1,12		CN
T-2886					0,55		CN
T-2922					<0,10		CN
T-2976					<0,10		CN
T-3236					0,11		CN
T-3328					16,82		CN
T-3341					<0,10		CN
T-3439					<0,10		CN
T-3450					<0110		CN

APPENDIX 2 : RESULTS FOR DAILY CONTROLS

Date: 16/05/2005

Daily check	Substrate replacement	4MU background	Lamp Intensity	BG smp	BG ref	Subst smp	Subst ref
Result	OK	OK	OK	472	1078	5213	49209

Calibrations T4, FT4, Cortisol, cTnI, Insulin.

Date: 26/05/2005

Daily check	Substrate replacement	4MU background	Lamp Intensity	BG smp	BG ref	Subst smp	Subst ref
Result	OK	OK	OK	446	993	3210	51670

Control	Result cortisol	ranges	Result T4	ranges	Result Insulin	ranges	Result FT4	ranges
1	4,26	4,03-5,57	1,6	1,4-2,2	7,1	6,1-9,2	0,49	0,39-0,64

Date: 27/05/2005

Daily check	Substrate replacement	4MU background	Lamp Intensity	BG smp	BG ref	Subst smp	Subst ref
Result	OK	OK	OK	419	934	3174	51305

Control	Result cortisol	ranges	Result T4	ranges	Result Insulin	ranges	Result FT4	ranges
3	37.31	30.74-42.44	15.1	11.3-16.9	171	146.2-201.9	3.91	3.59-4.96

Date: 30/05/2005

Daily check	Substrate replacement	4MU background	Lamp Intensity	BG smp	BG ref	Subst smp	Subst ref
Result	OK	OK	OK	403	827	3361	50250

Control	Result cortisol	ranges	Result T4	ranges	Result Insulin	ranges
3	34.67	30.74- 42.44	10.4	11.3-16.9	176.1	146.2-201.9

Date: 31/05/2005

Daily check	Substrate replacement	4MU background	Lamp Intensity	BG smp	BG ref	Subst smp	Subst ref
Result	OK	OK	OK	403	899	3728	52229

Con trol	Result cortisol	ranges	Result T4	ranges	Result Insulin	ranges	Result FT4	ranges
3	41,32	30,74 - 42,44	19,8	11,3-16,9	162	146,2-201;9	4,36	3,59-4,96

Date: 02/06/2005

Daily check	Substrate replacement	4MU background	Lamp Intensity	BG smp	BG ref	Subst smp	Subst ref
Result	OK	OK	OK	422	958	3496	50153

Control	Result T4	Result T4	Result T4	Result T4	Result T4	ranges
1	1,7	2,3	2,1	1,9	2,3	1,4-2,2
2	6,1	6,7	7,3	6,6	5,9	5,2-7,8
3	18,4	14,5	17,4	17	17,8	11,3-16,9

Decontamination

Date: 03/06/2005

Daily check	Substrate replacement	4MU background	Lamp Intensity	BG smp	BG ref	Subst smp	Subst ref
Result	OK	OK	OK	444	991	3274	49402

Control	Result T4	ranges	Result insulin	ranges	Result cortisol	ranges
1	2,0	1,4-2,2				
2	5,4	5,2-7,8	72,6	62,9-86,8	20,68	15,96-22,04
3	14 19,9 18,5	11,3-16,9				

Recalibration T4

Date: 04/07/2005

Daily check	Substrate replacement	4MU background	Lamp Intensity	BG smp	BG ref	Subst smp	Subst ref
Result	OK	OK	OK	418	882	2964	50904

Control	Result cTnI	ranges
1	0,75	0,61-1,13
2	2,62	2,34-3,82
3	26,80	25,38-38,08

Date: 05/07/2005

Daily check	Substrate replacement	4MU background	Lamp Intensity	BG smp	BG ref	Subst smp	Subst ref
Result	OK	OK	OK	420	950	3030	51486

Control	Result cTnI	ranges
1	0,85	0,61-1,13
2	2,89	2,34-3,82
3	26,88	25,38-38,08

Date: 08/07/2005

Daily check	Substrate replacement	4MU background	Lamp Intensity	BG smp	BG ref	Subst smp	Subst ref
Result	OK	OK	OK	420	985	3103	92922

Calibrations T4 nouveau lot + ccTnI new lot

Date: 11/07/2005

Daily check	Substrate replacement	4MU background	Lamp Intensity	BG smp	BG ref	Subst smp	Subst ref
Result	OK	OK	OK	461	947	3054	48674

Control	Result cTnI	ranges
1	0,77	0,61-1,13
2	2,48	2,34-3,82

Date: 12/07/2005

Daily check	Substrate replacement	4MU background	Lamp Intensity	BG smp	BG ref	Subst smp	Subst ref
Result	OK	OK	OK	398	894	3246	51620

Control	Result T4	ranges	Result Insulin	ranges
1	1,7	1,4-2,2	6,4	6,1-9,2
2	5,7	5,2-7,8	76,0	62,9-86,8
3	14,4	11,3-16,9	173,2	146,2-201,9

Date : 28/07/2005

Daily check	Substrate replacement	4MU background	Lamp Intensity	BG smp	BG ref	Subst smp	Subst ref
Result	OK	OK	OK	441	919	3156	47190

Control	Result Insulin	ranges
2	63,3	62,9-86,8

Date: 20/10/2005

Daily check	Substrate replacement	4MU background	Lamp Intensity	BG smp	BG ref	Subst smp	Subst ref
Result	OK	OK	OK	369	834	2057	48107

Calibration progesterone.

APPENDIX 3: DECONTAMINATION PROCEDURE

DECONTAMINATION OF THE WASH/DILUENT LINE

Introduction :

This section describes the recommended procedure for the decontamination of the Wash/Diluent line on the TOSOH AIA systems.

Required material

Decontamination solution:

Prepare a solution of Hypocloride at 1,2 ° Chloride

OR

Dissolve one tablet TE Elgalite CT-1 (cat nr T001904) in 5L of water

Water (Grade type I, NCCLS)

Procedure

The decontamination of the Wash/Diluent line is done on the request of the the Tosoh Bioscience service.

1 - Fill the container with the decontamination solution.

2 –Remove the filter at the end of the tube of the container for the Wash solution (or Diluent solution).

3 –Put the tube in to the decontamination solution.

4 - Click on “Maintenance” then “Mechanical Functions”

5 –Click on “Prime Wash” (or “Prime Diluent”) and press “Start”.

6.- When you receive the message “ Priming complete” press “OK”

7.- Repeat “Prime Wash” (or “Prime Diluent”) 5 times.

Wait at least 30 minutes, if possible leave the decontamination overnight.

8.-Replace the container with decontamination solution with water.

9 –Click on “Prime Wash” (or “Prime Diluent”) and press “Start”.

10 - When you receive the message “ Priming complete” press “OK”

11 - Repeat “Prime Wash” (or “Prime Diluent”) 10 times.

12 -Replace the container with water with freshly prepared Wash (or Diluent)

13 – Put a new filter at the end of the tube.

14 – Click on “Prime Wash” (or “Prime Diluent”) and press “Start”.

15 - When you receive the message “ Priming complete” press “OK”

16 - Repeat “Prime Wash” (or “Prime Diluent”) 10 times.

Run the controls samples and when necessary recalibrated the instrument.

DECONTAMINATION OF THE SUBSTRATE LINE

Introduction :

This section describes the recommended procedure for the decontamination of the substrate line on the TOSOH AIA systems.

Required material

0.2 N HCl

70 ° ethanol

Procedure

The decontamination of the substrate line is done on the request of the the Tosoh Bioscience servive.

1- Fill the bottle used for ethanol with 0.2 N HCl and put this vial in the position of the substrate.

2 –Click on “Maintenance” then “Mechanical Functions”

3 –Click on “Substrate replacement” and press “Start”.

4.- When you receive the message “ Substrate complete” press “OK”

5.- Repeat “Substrate replacement” three times.

6.-Wait 30 minutes and prepare a fresh vial of substrate.

7.-Rince a empty substrate vial with ethanol and fill it with ethanol. Place the vial on the analyser in the position of the substrate.

8.-Click on “Substrate replacement” and press “START”.

9 –Wait for the message “Substrate replacement complete”

10-Click “OK”

11-Repeat “Substrate replacement” 3 times

12- Replace the vial on the analyser with the fresh substrate.

13 – Shut down and restart the system including the daily maintenance.