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Clinical chemistry reference database for Wistar rats and C57/BL6 mice

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Abstract

Clinical chemistry data are decisive for evaluating altered organ function or damage in experimental animals. Few publications provide reliable clinical chemistry reference intervals, and analytical methods are often not described. Here, we investigated common clinical chemistry values in adult male and female Wistar rats and C57/BL6 mice (n=30/group). Blood samples were taken and analysed for electrolytes, substrates, metabolites and enzymes. In addition, we investigated cystatin C, an important marker of glomerular dysfunction. All data were obtained using commercially available kits frequently employed in most clinical chemistry laboratories and compared with data from other studies, as well as with human data. Significant gender-specific differences were observed in rats (electrolytes, retention parameters and transaminases) and in mice (cholesterol, glucose). High variability was noted for sodium, potassium, glucose, creatine kinase, lactate dehydrogenase and transaminase levels. Both rodent species showed markedly higher α-amylase activity than humans. This report demonstrates significant differences between genders for many analytes in rats and for fewer parameters in mice. Some reference values displayed major discrepancies between rodents and humans.

Keywords: C57/BL6 mouse; clinical chemistry; reference data; reference interval; Wistar rat.

Introduction

Measurement of clinical chemistry analytes is a valuable tool in daily clinical practice and basic research. However, very few publications describe clinical chemistry reference values for laboratory rodents. Furthermore, these publications have a significant lack of sufficient numbers, and the methods used to determine clinical chemistry values are often not described (Frith et al., 1980). Other studies are either old (Burns and De Lannoy, 1966), provide data obtained under different conditions (25°C vs. 37°C) (Hirano and Takagaki, 1976; Harrison et al., 1978), the number of parameters investigated was low (Zhou and Hansson, 2004) or selection of the population was not valid (Liberati et al., 2004). Finally, many studies analyse pooled plasma samples, which can lead to unreliable reference values (Solberg, 1987).

Rats are classically used for basic medical research to allow many surgical procedures. Although genetic manipulations are very complex and hard to implement, the first knockout rat lines have recently been established (Zan et al., 2003). Rats have a short generation time and hence provide many established inbred lines.

Mice have become one of the most important laboratory animal species for basic medical research. As a result of the detailed characterisation of the mouse genome and the ability to alter the expression of specific genes, mice represent the ideal target to investigate changes in morphology and function. It is important to note that such alterations might be reflected not only in morphology, but also in clinical chemistry values. Another advantage is their short generation time, which facilitates the rapid creation of inbred lines. Animals from such lines are practically syngeneic (Festing, 1999).

The determination of clinical chemistry reference data in rats and mice is of particular importance to identify deviations observed in genetically altered or pharmacologically treated animals. Hence, blood sample analysis can be used as a readily accessible tool to reveal the effects of altering the function of genes or the pathophysiological mechanism of interest.

Clinical chemistry data vary between species. For example, α-amylase activity is much higher in rodents than in humans. Without detailed knowledge of these discrepancies, inter-species comparison of clinical chemistry analytes can lead to incorrect conclusions.

In view of the many different inbred lines available for rodents, it is reasonable to determine specific clinical chemistry reference intervals for each line. Although different reference data are provided for rats (Charles Rivers Laboratories, 1982) standardised methods are not specified (Taconic Technical Library, 2006a). Methodological differences and wide variance in values prevent direct comparison of results in most cases. It is still unclear whether there are significant differences in reference data in diverse mouse lines (Hough et al., 2002).

This study supplies a broad range of routine clinical chemistry reference data for Wistar rats and C57/BL6 mice, both of which are frequently used in basic medical research.

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Table 1 Median, reference interval (2.5–97.5th percentiles), maximum and minimum for 20 clinical chemistry analytes of Wistar rats (weight 200–300 g) and C57/BL6 mice (weight 20–30 g).

Analyte	Unit	Wistar rats						C57/BL6 mice						Human	
		Median		Reference interval		Max/Min		Median		Reference interval		Max/Min		Reference interval	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F
Sodium	mmol/l	137	144*	142 131	157 135	143 131	157 134	155	158	165 149	162 147	165 149	163 147	145 135	145 135
Potassium	mmol/l	5.2	4.8*	6.5 3.9	5.6 4.3	6.6 3.8	5.6 4.3	4.3	4.6	6.1 3.1	5.1 3.5	6.2 3	5.1 3.5	4.5 3.4	4.5 3.4
Calcium	mmol/l	2.3	2.40*	2.48 2.02	2.59 2.06	2.49 2.01	2.6 2.05	2.04	2.06	2.33 1.88	2.29 1.79	2.34 1.87	2.3 1.78	2.26 2	2.26 2
Creatinine	µmol/l	37	46*	48 31	53 37	48 30	53 37	17	15	28 11	28 11	29 10	28 11	97 44	80 44
Urea	mmol/l	7.0	9.3*	9.3 4.0	11.3 6.8	9.5 3.8	11.3 6.7	9.0	8.7	13.2 3.2	11.5 6.0	13.3 2.8	11.7 5.7	8.3 2.0	8.3 2.0
Uric acid	µmol/l	30	24	54 12	54 12	54 12	54 12	89	107	119 36	232 18	119 36	232 12	381 200	357 140
Bilirubin	µmol/l	<1.7	<1.7	1.7 1.7	1.7 1.7	1.7 1.7	1.7 1.7	5.1	6.8	8.6 <1.7	15.4 1.7	8.6 <1.7	15.4 1.7	<19	<19
Total protein	g/dl	5	6	6 4	8 4	6 4	8 4	4	4	6 2	5 3	6 2	5 3	83 66	83 66
Lactate	mmol/l	3.44	2.90*	5.61 1.9	5.42 1.15	5.73 1.8	5.53 1.04	0.22	0.28	1.09 0.04	0.8 0.04	1.12 0.01	0.82 0.02	2.44 0.63	2.44 0.63
Cholesterol	mmol/l	1.4	1.5	2.0 1.1	2.5 0.7	2.1 1.1	2.5 0.6	2.3	2.0*	3.0 1.5	2.6 1.1	3.1 1.4	2.7 1.1	5.7 3.6	6.2 3.9
Triglycerides	mmol/l	1.1	1.4*	2.1 0.4	3.4 0.4	2.1 0.3	3.5 0.4	1.1	1.1	1.8 0.4	1.9 0.7	1.9 0.4	2.0 0.6	2.5 0.5	2.5 0.5
Glucose	mmol/l	6.8	6.3	9.2 5.1	8.4 5.7	9.2 5.0	8.5 5.7	8.3	9.9*	15.9 2.9	16.7 5.9	16.3 2.6	16.9 5.7	6.1 3.9	6.1 3.9
Cystatin C	mg/l	0.12	0.09	0.05 0.01	0.17 0.06	0.14 0.09	0.17 0.06	0.06	0.06	0.07 0.05	0.07 0.05	0.07 0.05	0.07 0.05	1 0.5	1 0.5
CK	U/l	435	270*	811 139	809 95	829 121	828 76	139	102	377 33	267 30	386 24	273 24	80 10	80 10
ALT	U/l	33	46	49 24	67 23	50 23	68 22	21	22	38 14	43 13	39 13	44 12	23 5	19 5
AST	U/l	66	81*	96 50	153 61	98 49	156 59	45	51	78 28	89 12	79 26	91 10	19 5	15 5
GGT	U/l	–	–	0 0	8 0	0 0	8 0	–	–	–	–	–	–	28 6	18 4
α-Amylase	U/l	1996	1356*	3161 1416	2260 1132	3207 1371	2290 1103	1942	1940	3044 1541	2722 1197	3084 1501	2762 1157	<120	<120

Table 1 (Continued)

Analyte	Unit	Wistar rats						C57/BL6 mice						Human	
		Median		Reference interval		Max/Min		Median		Reference interval		Max/Min		Reference interval	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F
LDH	U/l	114	276*	298	789	304	807	348	293	471	701	480	717	240	240
				83	102	77	84			124	102	114	86	80	80
AP	U/l	132	27*	193	45	196	46	153	129	217	230	221	235	170	155
				65	16	62	15			67	49	63	44	62	50

For comparison, human clinical chemistry reference intervals are also shown (Greiling and Gressner, 1995). Rodents have much higher α-amylase activity and lower total plasma protein as compared to humans.
*Significant difference between male and female animals ($p<0.05$).

In addition, we provide for the first time reference data for cystatin C, a marker of glomerular function, in these rodents. Hitherto, cystatin C data have only been obtained for small populations of nephrectomised Sprague-Dawley rats (Bokenkamp et al., 2001) or female Sprague-Dawley rats treated with radiolabelled human cystatin C (Tenstad et al., 1996).

Results

Clinical chemistry reference intervals for Wistar rats and C57/BL6 mice

Twenty analytes, representing the most commonly examined clinical chemistry parameters, are listed in Table 1. The median, reference intervals and maximum and minimum were calculated for both genders of Wistar rats (n=30/group) and C57/BL6 mice (n=30/group). Differences in the median were analysed between genders and highlighted when $p<0.05$. γ-Glutamyl transferase (GGT; Wistar rats and C57/BL6 mice) and bilirubin (Wistar rats) levels did not reach the detection limit in most animals and were not subjected to further statistical analysis.

Significant gender differences were found for a large number of analytes in Wistar rats, with higher values for potassium, lactate, triglycerides, cystatin C, transaminases, CK, α-amylase and alkaline phosphatase (AP) in males. Female Wistar rats showed significantly higher levels of sodium, calcium, creatinine, urea, total protein, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH).

In C57/BL6 mice, fewer marked gender-specific differences were found. Higher levels of cholesterol were observed in males and higher levels of glucose in females.

Clinical chemistry reference intervals for Wistar rats and C57/BL6 mice in comparison to humans

To compare the data obtained with human clinical chemistry reference intervals, Table 1 also lists values from the literature (Greiling and Gressner, 1995). In contrast to rodents, human plasma shows a much higher total protein amount. However, many enzymes show higher activity in rodents (e.g., α-amylase CK, LDH, ALT) compared to humans. Furthermore, sodium and potassium levels in Wistar rats and C57/BL6 mice are slightly higher than in humans, while calcium levels are similar between the species.

Clinical chemistry reference intervals for Wistar rats and C57/BL6 mice in comparison to other databases

Mean clinical reference values from this study were compared to data from Charles Rivers Laboratories (1982), Taconic Technical Library (2006a) and data published in Experimental and Surgical Techniques in the Rat (Waynforth and Flacknell, 1992), as partially depicted in Table 2 (Wistar rats). For most analytes, there are no significant

differences between databases. However, GGT activity was markedly lower in Wistar rats in our study compared to the results provided by Waynforth and Flacknell (1992) and Taconic Technical Library (2006a). The Waynforth and Flacknell data are only for male animals. In addition, comparison is complicated by the fact that there are differences between the clinical chemistry equipment and analysis techniques used.

Discussion

Clinical chemistry analytes are used to monitor metabolism and organ function in laboratory animals. These analytes are not useful without reference values.

Generating clinical chemistry reference intervals for rodents

Preconditions for the generation of reference values are defined by the IFCC (Büttner et al., 1970; Dybkær and Solberg, 1987; PetitClerc and Solberg, 1987; Solberg, 1987, 2004; Solberg and PetitClerc, 1988; Solberg and Stamm, 1991) and have been widely adopted for clinical chemistry of human analytes. These recommendations involve strict regulations for the creation of reference populations, high quality of pre-analytical steps and suggest statistical methods. However, in the few reports of reference values in animals, the IFCC recommendations are only included partially or not at all (Hirano and Takagaki, 1976; Liberati et al., 2004; Zhou and Hansson, 2004).

The present study provides clinical chemistry reference intervals that have been generated according to the following guidelines: it includes a 'reference population' for Wistar rats and C57/BL6 mice, the pre-analytical step was maintained constant throughout the experiments, and statistical calculation of reference intervals was in line with IFCC recommendations.

Reference population

To define a Wistar rat or C57/BL6 mouse as 'normal' is complex because of the different and sometimes confusing definitions of the term 'normal' (Grasbeck, 2004). For this study, only healthy animals were included and held under the same conditions to avoid aberrations caused by different animal feeding and housing (Delanghe et al., 1989).

As laboratory results often depend on gender and age (Deschamps and Lahrichi, 1973), it was necessary to create separate reference intervals for the two sexes (Liberati et al., 2004). In addition, it has been shown that clinical chemistry values can be age-dependent (Charles Rivers Laboratories, 1982; Zhou and Hansson, 2004). However, in our study we used two commonly utilised species covering an age and weight chosen in many other experiments (Zacharowski et al., 2002; Bornstein et al., 2004; Zhou and Hansson, 2004; Petzelbauer et al., 2005). To fulfil the strict requirements for a reference population, high numbers of individuals should be included (Solberg and PetitClerc, 1988). Admittedly, for many animal models such high numbers of individuals may not be practical or even possible to investigate. In small animals such as mice, the extraction of a sufficient amount of blood leads to the death of the animal, and therefore the number of individuals is limited. Furthermore, in rodents, individuals often stem from inbred lines, providing a more similar genetic background than in a randomised population of humans. Hence, a lower number of animals might be sufficient as a reliable reference population.

Pre-analytical step

Clinical chemistry reference data depend on the device and method used and thus cannot easily be compared with data generated by other means (Solberg and Stamm, 1991; Sharp and Laregina, 1998; Liberati et al., 2004). All animal blood samples were analysed on systems used for routine clinical diagnostics (Cobas Mira S®, EFOX 5053®, Behring Nephelometer II®) that are subject to strict quality control. To avoid false results, e.g., due to haemolysis or contamination (Bermes and Forman, 1976; PetitClerc and Solberg, 1987; Solberg and PetitClerc, 1988; Schnell et al., 2002), all pre-analytical steps (blood withdrawal, storage) were strictly controlled. Errors in measurement could be ruled out by quality controls prior to analysis.

Sample size

To avoid plasma pooling due to small sample size, use of a device with a small dead-space is imperative. In the present study this was achieved using the Cobas Mira S system. Using the accurate principle of flame photometry on the EFOX 5053®, we were also able to obtain reliable and exact data for electrolytes in small samples (Eppendorf AG, 2000). Reference values for cystatin C in rats and mice were generated for the first time in this study. The Behring nephelometer (BN) II® was used for cystatin C analysis. However, in C57/BL6 mice, blood from three animals had to be pooled to achieve an adequate sample size for nephelometry.

Statistics

For the description of crude reference populations, the use of non-parametric statistics is recommended by some authors (Solberg, 2004), as a Gaussian distribution is often not observed. Hence, the use of mean and standard deviation as statistical tools becomes problematic and can lead to misinterpretation of data. A solution to this problem is calculation of the median, minimum and maximum, which allows detection of outlying parameters in larger data sets. The definition of a reference value means that 95% of the analyte values have to be found between the 2.5th and 97.5th percentiles (Solberg, 2004). These values then provide a powerful tool to compare data between different species or groups (Table 1).

Unfortunately, most authors do not follow the strict recommendations for the evaluation of reference intervals and only present mean reference values for analytes (Charles Rivers Laboratories, 1982, 1993; Waynforth and Flacknell, 1992; Taconic Technical Library, 2006a,b). To allow comparison of our results with data from other studies, we also calculated the mean analyte values,

Table 2 Mean reference values in male/female Wistar rats from the present study in comparison with the literature.

Analyte	Unit	Present study				Charles Rivers Laboratories (1982)				Taconic Technical Library (2006a)			
		n	Male	n	Female	n	Male[a]	n	Female[a]	n	Male	n	Female
Sodium	mmol/l	30	137	30	144	30	145	28	145	10	138	10	138
Potassium	mmol/l	30	5.3	29	4.9	29	6.5	29	6	10	5	10	5.2
Calcium	mmol/l	29	2.3	29	2.39	–	–	–	–	10	2.42	10	2.6
Creatinine	μmol/l	29	37	29	46	–	–	–	–	10	81	10	84
Urea	mmol/l	30	6.8	30	9.3	30	3.2	29	3.7	10	3.3	10	3.2
Uric acid	mmol/l	29	30	29	30	–	–	–	–	10	262	10	172
Bilirubin	μmol/l	31	<1.7	31	<1.7	–	–	–	–	10	6.8	10	1.7
Total protein	g/dl	30	5	30	6	29	7	29	7.5	10	6	10	6
Lactate	mmol/l	29	3.54	29	2.85	–	–	–	–	–	–	–	–
Cholesterol	mmol/l	30	1.5	30	1.5	–	–	–	–	10	2.1	10	2.4
Triglycerides	mmol/l	30	1.2	27	1.7	–	–	–	–	10	1.1	10	1.2
Glucose	mmol/l	29	6.9	29	6.4	28	6.4	29	6.1	10	11.3	10	9.2
Cystatin C	mg/l	30	0.12	30	0.09	–	–	–	–	–	–	–	–
CK	U/l	29	444	28	347	–	–	–	–	–	–	–	–
ALT	U/l	28	35	25	52	29	31[b]	28	33[b]	10	39[b]	10	163[b]
AST	U/l	28	70	26	113	30	62[b]	28	66[b]	10	155[b]	10	40[b]
GGT	U/l	29	<0.3	30	<0.3	–	–	–	–	10	6.9[b]	10	4.4[b]
α-Amylase	U/l	29	1942	30	1465	–	–	–	–	–	–	–	–
LDH	U/l	30	135	23	369	–	–	–	–	10	460[b]	10	568[b]
AP	U/l	30	130	30	27	29	137[b]	28	108[b]	10	40[b]	10	39[b]

The comparison is complicated due to differences in techniques and animal age ([a]19–21 weeks; [b]temperature during measurement unknown).

although not all data showed a Gaussian distribution (Table 2).

Comparison with the literature

When comparing clinical chemistry reference data for a single species, differences in the detection technique and the statistical test used need to be considered. If different species are compared, e.g., mouse and human, diverse metabolism and anatomy complicate matters even more. In such cases, reliability of the reference data/intervals compared is mandatory.

In rodents, electrolytes display higher plasma levels than in humans (Greiling and Gressner, 1995), which might be explained by haemoconcentration or haemolysis. However, we can exclude haemoconcentration due to fasting because all animals received water *ad libitum*, and serum protein levels in both rodent strains were even lower than in humans (Table 1). In addition, we observed only a few cases of haemolysis, which were excluded from the study. Therefore, higher baseline levels of sodium and potassium in Wistar rats and C57/BL6 mice are considered to be physiological and have been demonstrated by others (Charles Rivers Laboratories, 1982, 1993; Waynforth and Flacknell, 1992; Zhou and Hansson, 2004; Taconic Technical Library, 2006a,b).

Feeding of laboratory animals has an important influence on a broad range of clinical chemistry parameters. In contrast to human plasma, creatinine and uric acid levels in both rodents are low (Table 1). Lack of exercise and strict vegetarian feeding, which is low in purines, (National Research Council, 1995) might be a possible explanation (Delanghe et al., 1989). Both rats and mice also displayed high variability of plasma glucose levels, as they were not subjected to fasting (Table 1). Nevertheless, it is important to note that the heparinised plasma used in our study did not contain a glycolysis inhibitor. To avoid ongoing glycolysis and hence lower glucose levels, plasma was analysed immediately after defrosting.

Although there are some similarities between human and rodent clinical chemistry values, some marked differences stand out. The nourishment of Wistar rats and C57/BL6 mice is rich in crude fibre and cellulose, which is cleaved into smaller digestible fragments by α-amylase in the gastro-oesophageal vestibule. Hence, the activity of α-amylase in rodent saliva is much higher than in humans (MacKenzie and Messer, 1976; Schmidt-Nielsen, 1999).

On the other hand, bilirubin levels in rats did not reach the detection limit (Table 1), while bilirubin levels in mice were higher (Table 1), but still much lower than in humans (Greiling and Gressner, 1995). This observation is in contrast to mean bilirubin plasma values provided by others, who reported similar results to human plasma levels (Charles Rivers Laboratories, 1982, 1993; Taconic Technical Library, 2006a,b). These different findings might be due to the diverse clinical chemistry methods applied. However, a satisfying explanation cannot be given, as most authors do not specify the method or kit used, or bilirubin was not measured at all (Zhou and Hansson, 2004). Similar results were found for GGT, which also did not reach the detection limit in most animals in this study (Table 1).

Differences in anatomy, such as the lack of a gall bladder in rats (Sharp and Laregina, 1998), diverse protein synthesis and metabolic liver function might also explain differences in transaminase levels between rodents and humans, with distinctly higher AST activity in female rats (Table 1). As AST is not specific for the liver, the reason

for these higher values in females is difficult to explain. As they occur together with high ALT activity, it might be hypothesised that the AST measured might also be derived from the liver. Mice showed lower mean of AST plasma activity, which was still higher than in humans (Table 1). These results are similar to findings from other studies (Liberati et al., 2004; Zhou and Hansson, 2004).

We also found a discrepancy between high human and low rodent plasma total protein levels (Table 2), which has also been observed by others (Charles Rivers Laboratories, 1982, 1993; Liberati et al., 2004; Zhou and Hansson, 2004; Taconic Technical Library, 2006a,b). However, a satisfying explanation for this observation cannot be given because further differentiation and separation of plasma proteins have not been carried out in any study.

It has been shown that serum cystatin C levels correlate negatively with glomerular filtration rate if the rate of formation of endogenous cystatin C is constant. This was demonstrated in rats (Tenstad et al., 1996), adult humans and children (Bokenkamp et al., 1998; Schuck et al., 2002; Heilman and Mazur, 2005). However, to the best of our knowledge, this is the first time that reference intervals have been established for cystatin C in Wistar rats and C57/BL6 mice. In Wistar rats, cystatin C levels were much lower (Table 1) than in human plasma (Bokenkamp et al., 1998; Thomas, 1998; Heilman and Mazur, 2005) and similar results were observed in C57/BL6 mice (Table 1). However, the NCBI genome databank indicates that the protein sequences of cystatin C in human, rat and mouse vary (Bioinformatic Harvester©), although the molecular mass of the protein itself is similar in all three species. Hence, it might be speculated that the epitopes of the polyclonal antibodies used in this study (N Latex Cystatin C, Dade Behring), which have been developed especially for human cystatin C, might not be able to effectively bind to the rodent protein. Despite this assumed lack of specificity, we recently showed that the method applied can still be useful in evaluating renal function (data not published). A comparison between inulin clearance and the test used in this study has already been established, and good correlation was found between cystatin C elevation and the decrease in inulin and creatinine clearance (Bokenkamp et al., 1998; Taes et al., 2004; Heilman and Mazur, 2005).

To date, published reference intervals for clinical chemistry data in rodents are very rare. Most reports do not fulfil the criteria recommended by the IFCC (PetitClerc and Solberg, 1987; Solberg, 1987, 2004; Solberg and PetitClerc, 1988; Solberg and Stamm, 1991; van der Heiden et al., 1994) and therefore comparison between results is difficult, if not impossible. In this study we clearly demonstrate that reliable clinical chemistry reference intervals for commonly used rodent strains can be established. Further investigations, such as the determination of iso-enzymes (e.g., for AP and LDH), the use of suitable antibodies (cystatin C) and the separation of plasma proteins, would provide more detailed information in this field of research.

Materials and methods

All procedures were carried out in accordance with the AAALAC guidelines and Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services, National Institutes of Health, Publication No. 86-23). In addition, all experiments were approved by the local ethics committee and regional government on animal experimentation in Germany. All animals used were allowed access to a standard diet and water *ad libitum*.

Animals

Adult and healthy male and female Wistar rats (body weight 200–300 g, age 10–12 weeks) and male and female C57/BL6 mice (body weight 20–30 g, age 10–12 weeks) were used (n=30/group).

Samples

Blood samples were collected over a period of 6 months. Each animal was anaesthetised (thiopental 100 mg/kg i.p. for rats; pentobarbital 90 mg/kg i.p. for mice), and then blood was taken either from the aorta or the left cardiac ventricle. Blood was centrifuged at 3400 *g* for 10 min and stored at -20°C. After thawing, samples were analysed immediately at 37°C to facilitate the comparison of results.

Equipment

COBAS MIRA S® (filter absorption photometer) The Roche Cobas Mira S® was used for analysis of substrates, metabolites and enzymes. The system has a very small internal dead-space, which allows the evaluation of small-volume samples, such as those obtained from mice. Quality control was carried out using Precinorm U® (Roche Diagnostics, Mannheim, Germany) for high values and Precipath U® (Roche) prior to analysis.

Eppendorf EFOX 5053® (flame photometer) The Eppendorf EFOX 5053® flame photometer provides high accuracy for the analysis of electrolytes in small-volume samples. It can simultaneously determine sodium, potassium and calcium in urine, serum and plasma samples. Quality control was carried out using Lypocheck Level 1 Assayed Chemistry Control® (Bio-Rad, Inc., Hercules, USA) for the reference interval and Lypocheck Level 2 Assayed Chemistry Control® (Bio-Rad, Inc.) for outliers.

Behring Nephelometer II® Serum levels of cystatin C were measured with the BN II by nephelometry, which quantifies an antigen by analysing the increase in turbidity, as measured by an increase in the scattering of laser light. Most modern nephelometers, such as the BN II, compare the rate of formation of antigen-antibody complexes (determined by computer analysis of light scattering data) to that for known antigen standards to precisely measure protein antigens (some of which are immunoglobulins) present in moderate concentrations. Quality control was achieved using defined cystatin C controls (Dade Behring, Marburg, Germany).

Statistical analysis

Statistical analysis was performed with GraphPad Prism 4.0 (GraphPad Software, Inc., San Diego, USA) for Windows with a group size of 30 animals. According to the recommendations of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (Solberg, 2004) the inter-percentile interval bounded by the 2.5th and 97.5th percentiles, was used. Owing to a lack of Gaussian distribution, the median of each analyte was compared between genders using the Mann-Whitney test.

Values were considered significant at $p<0.05$ (Table 1). Finally, the maximum and minimum of each parameter were calculated.

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