Available online at www.sciencedirect.com
SCIENCE DIRECT®
Theriogenology 66 (2006) 592–598
Theriogenology
www.journals.elsevierhealth.com/periodicals/the

A review of sample handling considerations for reproductive and thyroid hormone measurement in serum or plasma

Rebecca L. Hegstad-Davies *

Veterinary Diagnostic Laboratory, University of Minnesota, 1333 Gortner Avenue, St. Paul, MN 55108, USA

Abstract

Inappropriate sample handling of blood, serum or plasma collected for the determination of hormone concentration may lead to inaccurate endocrine data and diagnoses. Many useful studies have been conducted to determine the potential effects of sample handling on measured hormone concentration. Unfortunately, because reported results frequently differ according to assay method, species studied and parameters measured, it is not always possible to predict the effect of specific sample handling scenarios as they are presented in practice. The objective of this review is to provide a summary of what has been reported in the literature regarding sample-handling procedures that may affect the measurement of hormone concentration in plasma or sera from animals being evaluated for reproductive function.

Keywords: Endocrinology; Blood sample; Sample handling; Hormone; Stability

Inappropriate sample handling of blood, serum or plasma collected for the determination of hormone concentration may lead to inaccurate endocrine data and diagnoses. Veterinary diagnostic laboratories share a mission to provide accurate and quantitative analyses of hormone concentration in samples originating from various species and localities. Laboratories meet specific standards for laboratory service and participate in internal and external proficiency testing programs. Each endocrinology laboratory is expected to establish quality assurance and quality control procedures that provide guidelines for assuring that the data generated are accurate and reproducible**.** In addition to ''minding the store'' in these ways, laboratory diagnosticians depend upon the participation and knowledge of veterinarians with respect to appropriate sample handling in order to deliver meaningful diagnostic data.

Referral laboratories provide sample collection directions, but ultimately, they have limited control over the actual collection procedures used. As a result, the integrity of incoming samples is highly variable. As a general rule, sample integrity and analyte stability will be preserved if samples are collected into the correct type of tube to yield plasma or serum, allowed to fully clot if serum (60 min), or centrifuged as soon as possible, if plasma. Generally, serum separator tubes are to be avoided. Serum separator tubes can be problematic because of the potential for the gel layer to be disrupted during shipping. Furthermore, when chemiluminescent assay is used, some hormones are measured inaccurately when these certain types of these tubes are used [32,44]. The plasma or serum should be placed into a plastic tube and kept cool or frozen until analyzed. Typically this means sending the sample in an insulated container to arrive at the laboratory within a short interval. Laboratory sample handling recommendations are provided to optimize success, and are predictably more restrictive than they may need to be based on published studies.

* Tel.: +1 612 626 0168; fax: +1 612 624 8707.
E-mail address: rdavies@umn.edu.

0093-691X/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2006.05.004

Frequently, questions arise about the suitability of samples that were collected under less than these ideal conditions. Occasionally it is difficult to centrifuge a sample within 40 min and more than one clinician (or technician or endocrinologist!) has had the experience of realizing that a sample has been left at room temperature for longer than recommended. The most common questionable scenarios relate to anticoagulant choice, use of serum separator tubes, time interval prior to centrifugation, and the effects of storage temperature and time on whole blood, plasma or serum. In recent years, assay methodology has become a procedural feature that may also affect the accuracy of endocrine data interpretation. Many laboratories now utilize more than one type of assay system. It is not uncommon for laboratory personnel to use radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA) or chemiluminescent assay systems to measure hormone concentration in samples. Different methodologies may require different handling procedures.

Many useful studies have been carried out to determine the potential effects of sample handling on measured hormone concentration. Unfortunately, because reported results frequently differ according to assay method, species studied and parameters measured, it is not always possible to predict the effect of specific sample handling scenarios as they are presented in practice. In this review, sampling handling features that may affect the measurement of hormone concentration in sera from animals being evaluated for reproductive function are summarized.

Most commonly, reproductive hormone testing will include the measurement of steroids (progesterone and testosterone); pituitary hormones (luteinizing hormone (LH) and follicle stimulating hormone (FSH)), and thyroid hormones (thyroxine (T4 and Free T4), triiodothyronine (T3), thyrotropin (TSH), and thyroglobulin autoantibodies (TgAA)). The objective of this review is to provide a summary of what has been reported in the literature regarding sample handling procedures for these hormones in several species. The information summarized in Table 1 is offered as a resource for those collecting samples, especially if a particular sample was handled differently than what is recommended by the referral laboratory. Data and references are provided which will provide

Table 1
Features of blood collection, processing and storage for measurement of commonly requested reproductive and thyroid hormones

Hormone	Handling feature	Summary	Species	Reference
Follicle stimulating hormone (FSH)	Sample type	FSH concentration was the same when measured in serum or plasma (H, EDTA, fluoride)	Human	[1]
	Pre-centrifugation storage	FSH was stable in heparinized blood stored for 48 h at 22–26 °C and for 1 wk at 4 °C	Human	[2]
		FSH concentration was stable in EDTA-treated blood stored for 24 h at 4 or 24 °C	Human	[3]
	Time of day	FSH secretion is episodic. The pulse pattern varies depending on estrous cycle phase	Canine	[4]
	Post-centrifugation storage	FSH concentration was stable in plasma (EDTA, H and fluoride) and serum for 24–120 h when stored at 4 and 30 °C	Human	[1]
		FSH concentration was stable in plasma (EDTA) for 8 d when stored at 4 and 20 °C	Human	[5]
	Repeat freeze–thaw cycles	Several (4–10) freeze–thaw cycles did not affect FSH measured in plasma (EDTA)	Human	[5,6]
Luteinizing hormone (LH)	Sample type	LH concentration was the same in serum and plasma (H)	Human	[7]
		LH concentration was the same when measured in serum or plasma (EDTA, H, fluoride)	Human	[1]
		LH concentration was the same in serum, H or EDTA plasma	Bovine	[8]
	Time of day	LH secretion is episodic. Pulse pattern varies with estrous cycle	Canine, Bovine	[4,9–11,12]
	Pre-centrifugation storage	LH concentration in heparinized and untreated blood stored at 4 and 22 °C was stable for 24 and 4 h, respectively	Human	[7]
		LH concentration in blood collected into EDTA tubes was stable at 4 or 24 °C for 24 h	Human	[3]
		LH concentration in blood without anticoagulant and in heparinized and EDTA-treated blood was stable when stored at 4 °C or room temperature for 72 h	Bovine	[8]

Table 1 (*Continued*)

Hormone	Handling feature	Summary	Species	Reference
	Post-centrifugation storage	LH concentration in heparinized plasma was stable at 22 °C for 24 h	Human	[7]
		LH concentration in plasma (EDTA, H and fluoride) and serum was stable at 4 °C for 5 d	Human	[1]
		LH concentration in plasma (EDTA) was stable at 4 and 20 °C (but not 37 °C) for 8 d	Human	[5]
		LH concentration in serum and plasma (H, fluoride) decreased when stored at 30 °C for 5 d	Human	[1,5]
	Repeat freeze–thaw cycles	LH concentration in plasma (EDTA) and serum did not change after 4–10 freeze–thaw cycles	Human	[5,6]
	Effect of long-term storage	LH concentration in plasma (H) was unchanged after 9 mo of storage at −70 °C	Human	[7]
Progesterone	Sample type	EDTA may affect chemiluminescent assays		
		Progesterone concentration measured by qualitative ELISA was higher in whole blood than in plasma (H)	Canine	[13,19]
		Progesterone concentration measured by ELISA were higher in serum than in plasma (EDTA, H and fluoride)		
		Serum and plasma (H, EDTA, fluoride) concentration did not differ	Llama	[14]
		Progesterone concentration was higher in serum when Becton Dickinson Vacutainer SST were used to collect blood for use with a chemiluminescent assay	Human	[44]
		EDTA may affect chemiluminescent assays		
		Progesterone concentration in heparinized blood was higher than in serum	Bovine	[15]
	Assay method	Accuracy was variable depending upon method	Canine, Feline	[16,17]
		Progesterone concentration measured in serum and plasma using RIA was higher than concentration measured using a chemiluminescent method	Canine	[18]
		Progesterone concentration measured in serum using ELISA was higher than that measured in plasma (H, EDTA, sodium fluoride oxalate potassium)	Canine	[19]
		Progesterone concentration measured in plasma (H) using ELISA was higher than concentration measured using RIA	Equine	[48]
	Time of day	Progesterone concentration in serum was higher at 0800 h than at 1500 h in pregnant animals	Canine	[20]
		Progesterone concentration in serum was higher at 1800–2000 h than at 0800–0900 h in proestrous and estrous bitches. This pattern reversed after ovulation	Canine	[19]
	Pre-centrifugation storage	Progesterone concentration declined rapidly (within 4 h) in untreated clot and SST and in heparinized and EDTA-treated whole blood stored at 4, 20–22 or 37–40 °C	Bovine	[8,15,21,22, 23,25]
		Progesterone concentration was stable in serum and heparin-treated whole blood stored for 6 h at 4 or 20 °C	Porcine, Canine, Equine	[15,21]
		Progesterone concentration was stable in heparinized whole blood and whole blood collected without anticoagulant when stored for 48 h at 4 or 22 °C		
		Progesterone concentration declined within 2 h when whole blood without anticoagulant was refrigerated after collection. Progesterone was stable if blood was held at room temperature for the first 2 h prior to centrifugation	Canine	[18]
		Progesterone concentration was stable in whole blood collected without anticoagulant, but not in heparinized blood when stored at 22 °C for 6 h	Ovine	[15]
		Progesterone concentration decreased blood treated with heparin, EDTA or fluroide and stored for 24 h at 22 °C	Caprine	[24]
		Progesterone concentration was stable in untreated whole blood and in heparinized and fluoride-treated blood stored for 24 h at 4 and 22 °C	Llama	[14]

Table 1 (*Continued*)

Hormone	Handling feature	Summary	Species	Reference
	Post-centrifugation storage	Progesterone concentration decreased in plasma (EDTA) and serum when stored for 6 h at 40 °C, and in plasma (EDTA) and serum when stored for 24 h at 4 °C	Bovine	[22]
		Progesterone concentration was stable in serum when stored for 8 d at −20 or 4 °C	Bovine	[8]
		Progesterone concentration was stable in serum stored for 18–22 h at 4 or 22 °C	Canine, equine	[25]
		Progesterone concentration decreased in whole blood stored at room temperature for 18 h	Equine	[25]
	Lipemia	Did not affect serum or plasma (H, EDT) progesterone measurement	Canine	[38]
	Hemolysis	Did not affect measured concentration of progesterone in plasma (EDTA)	Bovine, Equine, Canine	[25]
	Repeat freeze–thaw cycles	Progesterone concentration measured in serum decreased by 1% for each (up to 10) cycle	Human	[6]
Testosterone	Pre-centrifugation storage	Stable in plasma from heparinized blood stored at 4 or 22 °C for 4 d	Human	[26–28]
	Time of day	Secretion is episodic. Diurnal and seasonal rhythms have been reported in some species	Human, Canine	[29,30]
	Post-centrifugation storage	Stable in serum or plasma (H) when stored at −28, 4 or 22 °C for 4 d	Human	[26]
	Lipemia	Did not affect serum or plasma (H, EDT) testosterone measurement	Canine	[38]
	Repeat freeze–thaw cycles	Plasma concentration (H) did not change after 10 freeze–thaw cycles	Human	[26]
		Concentration was stable in plasma (H) stored at −25 °C for 3 y	Human	[27]
Total T4, thyroxine	Sample type	T4 was lower in heparinized plasma than in EDTA plasma or serum	Canine	[31]
		T4 concentration in serum and plasma (EDTA and H) did not differ	Bovine	[8]
		T4 concentration may be 20–30% overestimated when Becton Dickenson Vacutainer SST are used to collect blood for use with chemiluminescent assay EDTA may affect chemiluminescent assay results	Human	[32]
	Assay method	Accuracy of ELISA methods for T4 concentration measurement was variable	Canine, Feline	[33]
	Time of day	Fluctuations in baseline values will occur, but diurnal rhythms have not been documented	Canine, Feline	[34,35,49]
	Pre-centrifugation storage	T4 concentration did not change in heparinized or EDTA-treated whole blood stored for 72 h at 4 °C or at room temperature	Canine, Bovine	[31,36,8]
		T4 concentration collected in whole blood was stable after storage for 24 h at room temperature	Equine	[37]
	Post-centrifugation storage	T4 concentration was stable in serum stored for 8 d at −20 °C, 4 °C or room temperature	Canine	[25,31,36]
	Lipemia	Did not affect serum or plasma (H, EDTA) T4 measurement	Canine	[38]
	Hemolysis	Did not affect serum or plasma (EDTA) T4 measured by RIA (does affect measurement by chemiluminescence)	Equine	[25]
	Repeat freeze–thaw cycles	T4 was stable after 8 freeze–thaw cycles	Canine	[31]
	Fasting	Serum T4 concentration was not affected by 12, 18, 24, or 36 h fast	Canine	[39]
T3, triiodothyronine	Type of sample	T3 can be 20–30% elevated when Becton Dickenson Vacutainer SST are used to collect serum for use with chemiluminescent assay	Human	[32,44]
		T3 concentration in serum and plasma (EDTA and H) did not differ EDTA may affect chemiluminescent assays	Bovine	[8]

Table 1 (*Continued*)

Hormone	Handling feature	Summary	Species	Reference
	Time of day	T3 secretion is episodic, but diurnal rhythms have not been reported	Canine	[34,35,49]
	Pre-centrifugation storage	T3 concentration was stable in whole blood collected in EDTA-treated tubes and stored for 72 h at 4 °C or at room temperature	Canine, Bovine	[25]
	Post-centrifugation storage	T3 concentration was stable in serum stored for 8 d at 4 °C or 22–26 °C T3 concentration declined in serum stored for 7 d at 22 or 37 °C	Canine	[8,25,40]
		T3 concentration was stable in serum stored for 19–22 mo at −20 °C	Equine	[37]
	Hemolysis	T3 was not affected by plasma (EDTA) hemolysis when measured by RIA. T3 may be affected by hemolysis when measured with chemiluminescence	Canine, Equine	[25]
	Fasting	T3 concentration did not change after a 12, 18, 24 or 36 h fast	Canine	[39]
Free T4 by dialysis (fT4D)	Sample type	Plasma (EDTA) fT4D concentration was greater than serum concentration after 5 d storage at −20 °C	Canine	[36]
	Time of day	See total T4 comments		
	Assay method	Free T4 concentration can be measured by standard equilibrium dialysis (SED), radioimmunoassay (RIA) or modified equilibrium dialysis (MED). Serum fT4 concentrations were comparable when SED and MED were used. There were significant differences between RIA's and between RIA and dialysis assays. Results were lower when measured by RIA. The MED assay was determined to be the best choice	Canine	[41]
	Post-centrifugation	fT4D concentration (serum, EDTA) did not change after storage for 1 or 5 d at −20 °C. However, concentration increased significantly after storage at 37 °C	Canine	[36,40]
	Storage tube	FT4D concentration increased after storage of plasma (EDTA) or serum at 37 °C in glass compared with plastic. These differences were not apparent if samples were assayed immediately or stored at −20, 4 or 22 °C	Canine	[36]
	Hemolysis	Hemolysis affected (reduced) serum fT4 concentration measurement when ELISA method was used	Canine	[42]
TSH, thyrotropin	Sample type	TSH concentration measured in EDTA plasma was lower than that measured in serum	Canine	[45]
		Avoid EDTA, SST and hemolysis if chemiluminescent assay is used	Human	[32]
	Assay method	Correlation between methods varies	Canine	[43]
TSH, thyrotropin	Time of day	Fluctuations in baseline values will occur, but diurnal rhythms have not been documented	Canine	[45,46,50]
	Post-centrifugation	TSH was stable in serum stored at 4 and 20 °C for 4 d, and for 2 months when stored at −20 °C	Canine	[40,45,46,47]
	Hemolysis	Avoid hemolysis if chemiluminescent or ELISA assay is used	Human	
TgAA, thryoglobulin autoantibody	Sample type	Serum is preferred. Avoid lipemia, hemolysis, hyperbilirubinaemia	Canine	[40]
	Storage	Avoid freeze–thaw cycles	Canine	[40]

H = heparin; SST = serum separator tubes; EDTA = ethylenediaminetetraacetic acid; ELISA = enzyme linked immunosorbent assay; RIA = radioimmunoassay.

insight regarding potential consequences of handling a sample in a 'unique' way. Some inadvertent sample-handling scenarios may not require re-collection. Alternatively, if the sample is truly inappropriate for delivering accurate diagnostic information, interpretive confusion will be avoided by promptly repeating the sample collection according to recommended procedures.

Endocrine diagnostic testing is affected by many factors (medication, stress, concurrent illness, and individual animal characteristics such as age, breed, gender and reproductive state), and interpretation of endocrine data is sufficiently complex. Minimizing the effects of sample handling on hormone concentration measurements will remove at least some of the variability that can be a feature of endocrine diagnostics.

References

[1] Evans MJ, Livesey JH, et al. Effects of anticoagulants and storage temperatures on stability of plasma and serum hormones. Clin Biochem 2001;34:107–12.

[2] Diver MJ, Hughes JG, et al. The long-term stability in whole blood of 14 commonly-requested hormone analytes. Ann Clin Biochem 1994;31:561–5.

[3] Ellis MJ, Livesey JH, Evans M. Hormone stability in human whole blood. Clin Biochem 2003;36:109–12.

[4] Olson PN, Bowen RA, et al. Concentrations of reproductive hormones in canine serum throughout late anestrus, proestrus and estrus. Biol Reprod 1982;27:1196.

[5] Livesey JH, Hodgkinson SC, et al. Effect of time, temperature and freezing on the stability of immunoreactive LH, FSH, TSH, growth hormone, prolactin and insulin in plasma. Clin Biochem 1980;13:151–5.

[6] Reyna R, Traynor KD, et al. Repeated freezing and thawing does not generally alter assay results for several commonly studied reproductive hormones. Fertil Steril 2001;76:823–5.

[7] Tsatsoulis A, Mavroudis K, et al. The stability in vitro of bioactive and immunoreactive LH in human blood and plasma. J Endocrinol 1988;117:139–45.

[8] Reimers TJ, McCann JP, et al. Effects of storage times and temperatures on T3, T4, LH, prolactin, insulin, cortisol and progesterone concentrations in blood samples from cows. J Anim Sci 1983;57:683–91.

[9] Concannon PW, Hansel W, et al. The ovarian cycle of the bitch: plasma estrogen. LH and progesterone. Biol Reprod 1986;34(Supplement):119.

[10] Jeffcoate IA. Concentrations of luteinizing hormone and oestradiol in plasma and response to injection of gonadotophin-releasing hormone analogue at selected stages of anoestrus in domestic bitches. J Reprod Fertil 1992;94:423–9.

[11] Hegstad RL, Johnston SD, et al. Concentrations and pulse analyses of adrenocorticotrophin and luteinizing hormone in plasma from dogs in pro-oestrus, oestrus, dioestrus or anoestrus. J Reprod Fert Suppl 1993;47:77–84.

[12] Hegstad RL, Johnston SD, Pasternak DM, et al. Plasma concentrations of luteinizing hormone and adrenocorticotropic hormone in blood collected during the luteal and follicular phases of the estrous cycle in cows. Am J Vet Res 1992;53:909–15.

[13] England GCW. ELISA determination of whole blood and plasma progestogen concentrations in bitches. Vet Rec 1991;7:221–2.

[14] Abal MA, Ghezzi M, et al. Concentrations of progesterone during storage of whole blood from Llama (Lama glama): effects of anticoagulants, storage time and temperature. Acta Vet Scand 1996;37:123–5.

[15] Wiseman BS, Vincent DL, et al. Changes in porcine, ovine, bovine and equine blood progesterone concentrations between collection and centrifugation. Anim Reprod Sci 1982–1983;5:157–65.

[16] Manothaiudom K, Johnston SD, et al. Evaluation of the ICA-GEN-Target canine ovulation timing diagnostic test in detecting canine plasma progesterone concentrations. J Am Anim Hosp Assoc 1995;31:57–64.

[17] Hegstad RL, Johnston SD, et al. Use of a rapid, qualitative enzyme-linked immunosorbent assay to measure serum progesterone concentration in cats. J Reprod Fertil Suppl 1993;47:535.

[18] Volkmann DH. The effects of storage time and temperature and anticoagulant on laboratory measurements of canine blood progesterone concentrations. Theriogenology, 2006 [Epub ahead of print].

[19] Thuroczy J, Wolfling A, et al. Effect of anticoagulants and sampling time on results of progesterone determination in canine blood samples. Reprod Dom Anim 2003;38:386–9.

[20] Steinetz BG, Goldsmith LT, et al. Diurnal variation of serum progesterone, but not relaxin, prolactin, or estradiol-17B in the pregnant bitch. Endocrinology 1990;127:1057–63.

[21] Oltner R, Edqvist LE. Changes in plasma progesterone concentration during storage of heparinized whole blood from cow, horse, dog and pig. Acta Vet Scand 1982;23:1–8.

[22] Vahdat F, Hurtgen JP, et al. Effect of time and temperature on bovine serum and plasma progesterone concentration. Theriogenology 1979;12:371–4.

[23] Vahdat F, Hurtgen JP, Whitmore H, et al. Decline in assayable progesterone in bovine plasma, effect of time, temperature, anticoagulant, and presence of blood cells. Am J Vet Res 1981;42:521–2.

[24] Fahmi HA, Williamson NB, et al. The influence of some sample handling factors on progesterone and testosterone analysis in goats. Theriogenology 1985;24:227–33.

[25] Reimers TJ, Lamb SV, et al. Effects of hemolysis and storage on quantification of hormones in blood samples from dogs, cattle, and horses. Am J Vet Res 1991;52:1075–80.

[26] Kley HK, Rick W. The effect of storage and temperature on the analysis of steroids in plasma and blood. J Clin Chem Clin Biochem 1984;22:371–8.

[27] Kley HK, Schlaghecke R, et al. Stability of steroids in plasma over a 10-year period. J Clin Chem Clin Biochem 1985;23: 875–8.

[28] Wickings EJ, Nieschlag E. Stability of testosterone and androstenedione in blood and plasma samples. Clin Chim Acta 1976; 71:439–43.

[29] Siklosi G, Hintalan A, et al. Episodic secretion of hormones and the diagnostic value of single blood estimates. III. Testosterone, androstenedione, dehydroepiandrosterone, dehydroepiandrosterone sulphate, cortisol. Acta Med Hung 1984;41:213–22.

[30] Falvo RE, DePalatis LR, et al. Annual variations in plasma concentration of testosterone and luteinizing hormone in the laboratory male mongrel dog. J Endocrinol 1980;86:425.

[31] Reimers TJ, McCann JP, et al. Effects of storage, hemolysis, and freezing and thawing on concentrations of thyroxine, cortisol and insulin in blood samples. Proc Soc Exp Bio Med 1982;170: 509–16.

[32] Interference in use of BD Vacutainer SST™ Blood collection tubes. Immulite Technical Bulletin 1215. Diagnostic Products Corporation, Los Angeles, CA, USA, August 9, 2004; 1–2.

[33] Lurye JC, Behrend EN, et al. Evaluation of an in-house enzyme-linked immunosorbent assay for quantitative measurement of serum total thyroxine concentration in dogs and cats. J Am Vet Med Assoc 2002;221:243–9.

[34] Miller, Nelson RW, et al. Serial thyroid hormone concentrations in healthy euthyroid dogs, dogs with hypothyroidism, and euthyroid dogs with atopic dermatitis. Br Vet J 1992;148:451–8.
[35] Kemppainen RJ, Sartin JL. Evidence for episodic but not circadian activity in plasma concentrations of adrenocorticotrophin, cortisol and thyroxine in dogs. J Endocr 1984;103:219–26.
[36] Behrend EN, Kemppainen RJ, et al. Effect of storage conditions on cortisol, total thyroxine, and free thyroxine concentrations in serum and plasma of dogs. J Am Vet Med Assoc 1998;212: 1564–8.
[37] Allen AL, Scott WM, et al. Effects of delayed serum separation and long-term storage on the measurement of thyroid hormones in equine blood samples. Vet Clin Path 1997;26:10–2.
[38] Lee DE, Lamb SV, et al. Effects of hyperlipemia on radioimmunoassay for progesterone, testosterone, thyroxine, and cortisol in serum and plasma samples from dogs. Am J Vet Res 1991;52:1489–91.
[39] Reimers TJ, McGarrity MS, et al. Effect of fasting on thyroxine, 3,5,3′-triiodothyronine, and cortisol concentrations in serum of dogs. Am J Vet Res 1986;47:2485–90.
[40] Nachreiner RF, Refsal KR. Chapter 1: collection, storage and transport of samples. In: Mooney CT, Peterson ME, editors. British Small Animal Veterinary Association Manual of canine and feline endocrinology. Gloucester, England: Brit Small Animal Veterinary Association; 2003. p. 1–5.
[41] Schachter S, Nelson RW, et al. Comparison of serum-free thyroxine concentrations determined by standard equilibrium dialysis, modified equilibrium dialysis, and 5 radioimmunoassays in dogs. J Vet Intern Med 2004;18:259–64.
[42] Lucena R, Moreno P, et al. Effects of haemolysis, lipaemia and bilirubinaemia on an enzyme-linked immunosorbent assay for cortisol and free thyroxine in serum samples from dogs. Br Vet J 1998;156:127–31.
[43] Marca MC, Loste A, et al. Evaluation of canine serum thyrotropin (TSH) concentration, comparison of three analytical procedures. J Vet Diag Invest 2001;13:106–10.
[44] Bowen RA, Chan Y, Rehak NN, et al. Effect of blood collection tubes on total triiodothyronine and other laboratory assays. Clin Chem 2005;51:424–33.
[45] Nishi I, Okada Y, Hayashi J, et al. Effect of various sampling condition on the measurement of circulating TSH and free thyroxine. Rinsho Byori 1992;40:417–22.
[46] Bruner JM, Scott-Moncrieff JC, et al. Effect of time of sample collection on serum thyroid-stimulating hormone concentrations in euthyroid and hypothyroid dogs. J Am Vet Med Assoc 1998; 212:1572–5.
[47] Williams DA, Scott-Moncrieff C, et al. Validation of an immunoassay for canine thyroid-stimulating hormone and changes in serum concentration following induction of hypothyroidism in dogs. J Am Vet Med Assoc 1996;209:1730–2.
[48] Nagy P, Solti L, et al. Progesterone determination in equine plasma using different immunoassays. Acta Veterinaria Hungarica 1998;46:501–13.
[49] Peterson ME, Graves TK, et al. Serum thyroid hormone concentrations fluctuate in cats with hyperthyroidism. J Vet Intern Med 1987;1:142–6.
[50] Iverson L, Jensen A, et al. Biological variation of canine serum thyrotropin (TSH) concentration. Vet Clin Path 1999;28:16–9.