PREPARATION OF *IN VIVO* COW CONTROL BLOOD SAMPLES FOR CADMIUM ANALYSIS

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Abstract. Quality control is essential for any analysis in the laboratory. The objective of this study was to prepare *in vivo* cow control blood samples. The experiment was performed by feeding cows with a single dose of cadmium in the form of cadmium chloride, withdrawing the blood at an appropriate time to get the highest level of cadmium and detecting the level of cadmium in the blood. It was found that feeding the cow a single dose of 0.06 mg cadmium per kg body weight resulted in the highest cadmium level of 3.622 µg/l 30-60 minutes after feeding. The samples were homogeneous because feeding the cows with single dose of cadmium let the cadmium be absorbed and distributed naturally. In addition, the samples were stable during transport. Therefore, they may be used as quality control samples to detect cadmium levels without using a lyophilized process. They could be used for proficiency testing and to evaluate whole blood analysis in the laboratory.

INTRODUCTION

In the laboratory, acceptable performance is maintained by measuring variability in the analytical process, and evaluating the results of the measurement according to certain specifications, the correcting the variability when necessary. Proficiency testing is necessary to establish confidence in the testing laboratories. Quality control (QC) of the sample is essential to assure the accuracy of any test. There are few QC sample alternatives and demand for reference materials exceeds supply (VTT Process, 2002). Currently, Thailand has to import such samples from foreign countries. To solve this problem, technology development is urgently needed for preparing these materials, especially QC blood samples for heavy metal, such as cadmium.

Cadmium (Cd) is a toxic metal. It has no essential biological function and is extremely toxic to humans. Cadmium is used widely in many industries and is an important source of environmental pollution. When cadmium is absorbed into the body, it usually accumulates in kidney for long periods of time (Goyer, 1996). When, it reaches a critical threshold it leads to serious kidney failure. The results of blood cadmium levels are used to diagnose toxicity. Whole blood cadmium levels have been used to evaluate occupational exposure (Nordberg and Nordberg, 1988).

In this study, QC blood samples containing cadmium were prepared from live cows fed with water containing cadmium. Blood samples were taken and prepared for QC, testing homogeneity and stability, using graphite furnace atomic absorption spectrophotometry. Assigned values were also determined by inductively coupled plasma-mass spectroscopy. The aim of this study was to obtain QC blood samples containing cadmium from live cows, which may be used as quality control samples and provide information for potential providers who are interested in producing QC samples.

MATERIALS AND METHODS

Reagents and equipment

The reagents and equipment used were: cadmium chloride (Merck), Seronorm® Trace El-

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ements Whole Blood, Triton X-100 (Merck), ammonium dihydrogen phosphate (Fluka), magnesium nitrate (Merck), a 21 G x 11/2" multi draw needle and holder, a 2 ml vacuum tube (VACUETTE® EDTA) (Greiner bio-one); a 20 G x 11/4" needle with lock plug (Terumo); a 450 ml blood bag with CDPA-1; BB*SCD456E (Terumo); a Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS); a Varian SpectrAA 640Z and Inductively Coupled Plasma-Mass Spectrometer (ICP-MS); and a Varian UltraMass.

Animals

Ethical clearance was obtained from the Faculty of Tropical Medicine-Animal Care and Use Committee, Mahidol University, Bangkok, Thailand.

The cows used were *Bos indicus*, Brahman stock. The subjects were males, age 1-2 years with a weight of 300 kg. There were 6 subjects with baseline cadmium blood levels of less than 0.5 µg/l that were reared at SK Pattaya Ranch, Chon Buri, Thailand.

Determination of optimal dose study

The cows were studied to determine the optimal dose of cadmium and timing of blood draw to obtain a level of 5 µg/l. Two milliliters of blood were drawn from an ear vein of each cow using a 20 G x 11/4" needle and placed in an EDTA vacuum tube.

Each of the 6 cows was fed a single dose of 500 ml of water containing cadmium (as $CdCl_2$ solution) at doses of 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 mg/kg body weight for the 6 cows, respectively. Two milliliters of blood were taken 30 minutes after feeding using the same needle and placed into a vacuum tube containing EDTA. The blood was drawn every 30 minutes for 4 hours. Each sample of cow blood was analyzed for cadmium concentration using GFAAS. The dose showing the highest concentration of cadmium was used for the *in vivo* cow quality control blood sample.

Testing for homogeneity

Homogeneity was assessed using the vials containing cow blood with cadmium. This was randomly selected to assess homogeneity in accordance with acceptable statistical designs (ISO, 1996; Thomas *et al*, 2001).

Testing for assigned value

The assigned value is a value attributed to a particular quantity and accepted, sometimes by convention, as having an uncertainty appropriate for a given purpose (ISO, 1996). This was the average of cadmium levels based on the GFAAS and ICP-MS results.

Testing for stability

The assigned value for the quality control material and matrix must be stable at elevated temperature to elucidate whether any degradation can be expected during transport. The stability was analyzed statistically using ANOVA to compare the level of cadmium at preparation time with the results on testing.

Data analysis

Homogeneity testing was expressed with the F-test (ISO, 1989; Thomas *et al*, 2001). The assigned value was expressed as a mean. Stability testing was expressed as a standard deviation (ISO, 1996, 1989).

RESULTS

Determination of optimal dose

The peak concentration of 3.62 µg/l occurred 30-60 minutes after a single oral dose of cadmium 0.06 mg/kg. Ninety minutes after the oral cadmium, there was a sharp decline in the blood cadmium concentration, which then declined more gradually between 90 and 240 minutes (Fig 1).

Homogeneity assessment

Between- and within-tube homogeneity were verified by determining blood cadmium levels in 10 randomly chosen tubes in duplicate. Blood cadmium concentration testing was performed in duplicate for each tube. The ANOVA analysis is shown in Table 1.

An F-test with a significance level of 0.05 revealed not significant differences in the between- and within-tube values. The p-values and the one-way ANOVA express the probability that the cadmium concentration was the same throughout the samples.

Assigning the value for cadmium in the blood

The assigned value for the cadmium level of the blood was based on 10 randomly chosen

Table 1
ANOVA of homogeneity assessment for *in vivo* cow blood QC samples.

Source of variation	SS	df	MS	F	p-value	F crit
Between groups	0.9418	9	0.1046	0.9277	0.5157	2.2107
Within groups	3.3842	30	0.1128			
Total	4.3260	39				

SS = sum of square; df = degree of freedom; MS= mean square; F = F-ratio for ANOVA; QC = quality control

Table 2
Assigned value for *in vivo* cow blood quality control samples.

Method	Cadmium concentration (µg/l), mean ± SD
ICP-MS	3.220 ± 0.237
GF-AAS	3.364 ± 0.162

ICP-MS = Inductive Couple Plasma-Mass Spectrometer
GF-AAS = Graphite Furnace Atomic Absorption Spectrophotometer

Table 3
Results of stability assessment for *in vivo* cow blood QC samples.

Duration of transport (round trip)		Cd concentration (µg/l),mean	% Recovery
Lab ID 1	6 days	3.450	102.56
Lab ID 2	6 days	3.361	99.91
Lab ID 3	7 days	3.446	102.42
Lab ID 4	10 days	3.420	101.66
(Assigned value: 3.364 mg/l)			

Cd = Cadmium

tubes using the GF-AAS and ICP-MS methods. Table 2 gives a summary of the means with standard deviations for the sample.

Stability testing

The stability of the samples was tested to determine the suitability of the material. It was evaluated for short-term stability during transport, not longer than 4 weeks. The ANOVA analysis is shown in Tables 3 and 4. The calibrator stability was determined using the recovery method. The acceptance criteria was 100 ± 10%. No differences in cadmium concentrations were detected during investigation period.

DISCUSSION

For *in vivo* cow blood quality control sample preparation, the optimal dose and appropriate time for blood draw to get a cadmium level of 5 µg/l was studied because the absorption rate was not clear. The National Academy of Sciences (2001) reported an absorption rate of cadmium in cows of less than 1%. Using this value, a cow must be given cadmium at 0.03 mg/kg body weight to get blood cadmium concentration of 5 µg/l. However, the results of our study show a cow blood cadmium concentration of 0.46 µg/l after dosing with cadmium at 0.03 mg/kg body weight. The rate of cadmium absorption was lower than estimated. This may be due to variation in species, environment, or nutrition.

After a single oral dose of cadmium at 0.06 mg/kg, the highest dose in our study, the highest level of cadmium in the blood did not reach 5 µg/l. However, absorption was quite fast, which may be a result of using water as the dosing medium. Absorption was at its highest 30-60 minutes after dosing giving a cadmium level of 3.62 µg/l.

Homogeneity was assessed using GF-AAS in two sub-samples taken from a number of bottles chosen at random from the production run. Between- and within-bottle variability was assessed using ANOVA, at a 95% confidence level (Table 2). The results indicate the samples were homogeneous.

For a particular analyte, the performance of the reference material was deemed acceptable for purpose of this study if the laboratory

Table 4
ANOVA of stability assessment for *in vivo* cow blood QC sample.

Source of variation	SS	df	MS	F	p-value	F crit
Between groups	0.0201	3	0.0067	0.3211	0.8101	3.4903
Within groups	0.2499	12	0.0208			
Total	0.2700	15				

SS = sum of square; df = degree of freedom; MS = mean square; F = F-ratio for ANOVA; QC = quality control

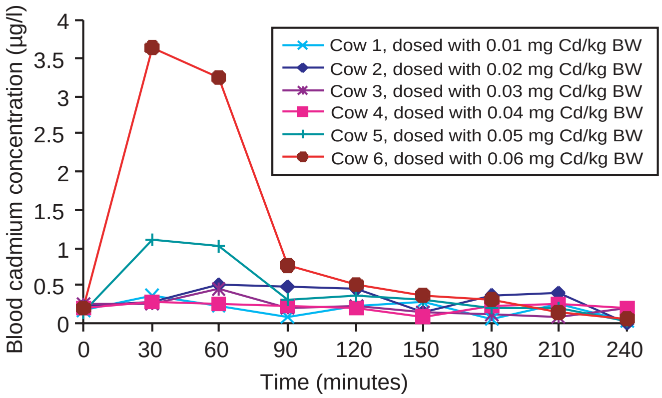

Fig 1–Cadmium concentration of cow blood after a single oral dose of cadmium.

result was within ±2SD for the analyte listed in the certificate of analysis for Certified Reference Materials. In our study, laboratory demonstrated acceptable performance on a particular analyte in reference to Seronorm® MR9067 from SERO, one of Europe's leading independent suppliers of control sera and reagents (SERO, 2003). The laboratory's results for the analyte were then used to calculate the assigned value. Table 2 gives a summary of the 2 analytical methods and the mean of blood cadmium concentrations with standard deviation. The F-test at a significance level of 0.05 did not reveal a significant difference between the 2 methods.

Two types of stability tests were performed. One was performed at storage temperature to obtain information about stability during storage. The other was performed at an elevated temperature to elucidate whether any degradation can be expected during transport. This study was usually of short duration, not longer than 4 weeks. The duration of transport in this study was 6, 7 and 10 days. Calibrator stability was determined by the recovery method for each QC sample after transport, compared with the value assigned at preparation time. The percent recovery was calculated by dividing result in conventional units (µg/l) of QC sample by the assigned value (µg/l) and multiplying the result by 100. The accepted criteria was 100 ± 10% (Sentinel, 2005). The data are shown in Tables 3 and 4 which support stability during transport.

The results of this study are consistent with a study by Cox *et al* (1989) which found the quality control sample prepared from cow blood was homogeneous because it was similar in composition to the real sample. It was more stable than the lyophilized QC sample because it did not have to be reconstituted before use, eliminating errors in measurement and mixing. Using cow blood provides many samples at one time and a safer sample since cows are not infected with hepatitis or HIV viruses.

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REFERENCES

Cox DH, Duncan CE, Wyatt GP, Meadow JS, Robinson JB. Bovine blood lead reference material. *J Anal Toxicol* 1989; 13: 204-7.

Goyer RA. Toxic effects of metals. In: Klaassen CD, ed. Casarett and Doull's toxicology. The basic science of poisons. 5th ed. New York: McGraw-Hill, 1996: 669-702.

International Organization for Standardization. ISO Guide 35. Certification of reference materials-General and statistical principles. 2nd ed. 1989.

International Organization for Standardization. ISO Guide 34. Quality system guidelines for the production of reference materials. 1st ed. 1996.

National Academy of Sciences. Nutrient requirements of dairy cattle: Seventh revised edition [document on the Internet]. National Academies Press Home Page, 2001. [Cited 2005 Jul 27]. Available from: URL: http://www.nap.edu/openbook/0309069971/html

Nordberg GF, Nordberg M. Biological monitoring of cadmium. In: Clarkson TW, Friberg l, Nordberg GF, Sager PR, eds. Biological monitoring of toxic metals. New York: Plenum Press, 1988: 151-68.

Sentinel CH. Srl. 510(k) Summary [document on the Internet]. Food and Drug Administration Home Page; 2005 May 28. [Cited 2005 Jul 1]. Available from: URL: http://www.fda.gov/cdrh/pdf5/K051457.pdf

SERO. Product description [document on the Internet]. SERO AS - Control sera, calibrators and reagents; 2003 Aug 11. [Cited 2005 Aug 10]. Available from: URL: http://www.sero.no

Thomas PJ, Pauwels J, Adriaan MH, Schimmel H, Lamberty A. Homogeneity and stability of reference materials. *Accred Qual Assur* 2001; 6: 20-5.

VTT Process. The selection and use of reference materials [document on the Internet]. VTT; 2002 Oct. [Cited 2005 Aug 10]. Available from: URL: http://www.vtt.fi