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-
elements 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 CdCl₂ 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).

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 no 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.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>0.9418</td>
<td>9</td>
<td>0.1046</td>
<td>0.9277</td>
<td>0.5157</td>
<td>2.2107</td>
</tr>
<tr>
<td>Within groups</td>
<td>3.3842</td>
<td>30</td>
<td>0.1128</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4.3260</td>
<td>39</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Method</th>
<th>Cadmium concentration (µg/l), mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICP-MS</td>
<td>3.220 ± 0.237</td>
</tr>
<tr>
<td>GF-AAS</td>
<td>3.364 ± 0.162</td>
</tr>
</tbody>
</table>

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.

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

Cd = Cadmium

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

**ACKNOWLEDGEMENTS**

We would like to acknowledge the assistance of the National Institute of Health (NIH), Department of Medical Sciences, Ministry of Public Health, for giving permission to carry out this study, perform the research work and for financial support. We would like to express our gratitude to all the veterinarians and animal husbandry workers at SK Pattaya Ranch, Chon Buri, Thailand.
REFERENCES


