

Effects of anticoagulants and duration of storage on blood lead levels

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Objectives: To explore the influence of different blood sample collecting tubes and the length of storage times on the measurement of blood lead levels (BLLs). **Methods:** A total of 57 blood specimens were collected from 47 lead-exposed workers and 10 workers not exposed to lead. For each subject, 8 blood samples were drawn with tubes containing different anticoagulants. All specimens were kept in a freezer at a temperature of 4°C until analyzed. By treating tubes containing lead-free sodium heparin as the reference group, the effects of container types and storage times were analyzed by the generalized estimating equation (GEE) model. **Results:** For subjects with high BLLs, the blood lead measurements obtained from the blood sample tubes containing the other 7 types of anticoagulants were lower than those of the reference group. Among blood samples from both the high BLL and low BLL groups, higher BLLs existed in tubes containing the anticoagulants, Na₂ EDTA and K₃ EDTA. On average, the BLLs from tubes using the anticoagulants, buffered Cit, Na 9:1, and Cit, and Na-0.129M Silic were lower than those in the reference group by 1.616 μ g/dL and 3.182 μ g/dL, respectively. No significant differences existed in the blood lead concentrations obtained from the blood samples stored in a 4°C environment and in different types of blood containers for 12 months, whether from the high BLL or the low BLL groups. **Conclusions:** When collecting blood samples for high BLL analyses, the two anticoagulants (i.e, buffered Cit, Na 9:1, and Cit, and Na-0.129M Silic) are not recommended. However, blood lead concentrations remain stable, even when blood samples have been stored for a period of 12 months. (*Taiwan J Public Health*. 2007;26(4):254-260)

Key Words: blood lead levels, container type, repeating analysis, stability

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INTRODUCTION

For many centuries, lead has been widely used in daily life and is still important today, in spite of the health hazards it presents in the workplace[1]. The health risks associated with exposure to lead are well-known and measurement of blood lead levels (BLLs) is a common practice in monitoring environmental and occupational exposure to lead[2-4].

In Taiwan, The Blood Lead Surveillance System was established in 1992. Health care

providers have been frequently requested to measure BLLs. Furthermore, since analysis of blood BLLs is reliable, they are routinely used as biomarkers. However, variations in blood lead measurements still exist within and between laboratories[2-14]. The variations in BLL measurements may result from random and systematic errors[10]. The variations could result from laboratory operational procedures, personnel factors, and the instruments used[9]. Currently, little research has been done on factors influencing blood lead analysis, specifically the types of blood tubes (vacutainers) used and the effects of long-term storage. Accordingly, the present study was undertaken to determine the effects of different types of vacutainers and length of storage on BLL measurement.

MATERIALS AND METHODS

Blood specimens were collected from 57 subjects, including 47 lead-exposed workers and 10 workers without known lead exposure. A history of lead exposure was closely correlated with the subject's BLLs. Therefore, based on their exposure status, the 57 subjects were divided into high and low BLL groups in the subsequent analyses. After obtaining informed consent from each subject, a 3-mL venous blood was drawn from each participant using 8 vacutainers containing different anticoagulants (Table1). In order to maintain consistency in the blood collection procedures, each participant's elbow was washed with soap, then wiped with 75% alcohol. Venipuncture was performed using a Monoject 216 blood collection needle. All blood collections were conducted in the administrative areas, far away from worksites, so as to avoid possible contamination from the environment. The collected blood samples were kept in a container with ice, shipped back to our laboratory within 4 hours, and were kept in a

freezer with a temperature of 4°C until analysis.

For each of the subjects, the blood samples were kept in eight different vacutainers (Becton Dickinson Vacutainer System). In order to achieve consistency, the 8 tubes from the same subject were analyzed at the same time. Among the 8 tubes containing blood samples drawn from the same subject, the number 2 tube was treated as the reference and the remaining 7 tubes were the different types of vacutainers used for comparison to test whether the blood lead concentrations varied with the different vacutainers.

The BLLs were measured using a graphite furnace atomic absorption spectrophotometer (GFAAS, AA906, System 3000) using a D2 lamp background correction and connected to a data processor. During the analysis, temperature settings were as follows: drying (80°C~140°C), ashing (500°C), atomization (1500°C), and cleansing (2000°C). Nitrogen was the inert carrier gas and the pressure was set at 15 PSI.

All instruments were soaked in a 20% nitric acid solution for 48 hours in order to remove potential lead contamination. Certified whole blood controls (Nycomed Pharma Co., Oslo, Norway) were included for the accuracy test. The control samples included three target lead levels: low (3.4 µg/dL; No. 404107), medium (38.5 µg/dL; No.404108), and high (66.0 µg/dL; No.404109). All specimens were analyzed three times and the average concentration of the three readings were taken if the relative standard deviations were <7%. To avoid the blood specimen "matrix effect" during analysis, the standard addition method was used to build up the calibration curve. The detection limit for the analysis was 0.3 µg/dL. To ensure the quality of BLL testing, our laboratory has a consistent intra-laboratory quality control and has participated in the blood lead proficiency test program of the United States Centers for Disease Control and Prevention since 1992.

The generalized estimating equation (GEE) [15] was used to model the influence of the various vacutainers and length of storage upon the BLLs. As suggested by Liang and Zeger (1986), the GEE model provides a method of analyzing correlated data. This method has been applied to analyze longitudinal data in which subjects are measured at different points in time or clustering.

RESULTS

During the one-year study period, each of the subject's 8 specimens was analyzed at least 5 times. Table 1 lists the 8 designated vacutainers used in this study. Each of vacutainers had its own specific function and was arranged in order (i.e., tube 1-). Tubes 1-3 were blood tubes that underwent special treatment and were suited to test for trace elements. Tube 2 served as the standard for the comparisons.

As shown in Table 2, the BLLs in the high BLL group were significantly different from the BLLs of tubes 1, 3, 4, 7, and 8. The BLLs of Tubes 5 and 6 did not show statistical differences from the standard. Most of the

BLLs were lower than tube 2. Tubes 7 and 8 had the most obvious decrease in BLLs (1.616 $\mu\text{g/dL}$ and 3.182 $\mu\text{g/dL}$, respectively).

In Table 2, we showed similar results for the low BLL group. BLLs of most of the tubes were lower than tube 2; however, the magnitude of differences was not as sizable as that observed in the high BLL group. In terms of the changes in BLLs that may occur over long-term storage with the 8 different designated types of collection tubes, the analysis indicated that the blood lead concentration of tubes 2 and 4 deviated from the other tubes significantly, while variations observed from the other tubes were trivial (Table 3). In other words, the BLLs in the high or low BLL groups remained stable, even though the blood specimens had been stored for 12 months at 4°C.

DISCUSSION

Since the 1980's, accuracy in blood lead analysis has improved significantly. One of the reasons is that the use of atomic absorption spectrophotometer analysis has greatly reduced the discrepancies due to analyzing instruments. In Taiwan, following the advancement of

Table 1. Eight Vacutainers Containing Different Anticoagulants Used in the Study

Chemistry Tube	Content	Function
Tube 1	Sodium heparin	Heavy metal tube (Both the cap and the whole tube have been immersed with acid solution, suitable for trace element and nutrient analysis)
Tube 2	Sodium heparin	Lead tube (heavy metal screen has been conducted to exclude potential lead contamination, only suitable for detecting metal lead)
Tube 3	Na ₂ EDTA	Heavy metal tube (Both the cap and the whole tube have been immersed with acid solution, suitable for trace element and nutrient analysis)
Tube 4	K ₃ EDTA	Blood routine examination
Tube 5	Sodium heparin	General emergency biochemistry analyzing
Tube 6	Lithium heparin	General emergency biochemistry analyzing
Tube 7	Buffered Cit, Na 9:1	Prothrombin time, Activated partial thromboplastin time (PT, aPTT)
Tube 8	Cit, Na-0.129M Silic	Erythrocyte Sedimentation Rate(ESR)

Table 2. Generalized Estimating Equation Analysis of the Effect of Types of Containers on Blood Lead Levels

High BLL group Vacuum tubes [#]	Regression coefficient	Standard Error	t statistics	p value
Tube 1	-0.483	0.164	-2.940	0.003
Tube 3	0.348	0.158	2.200	0.028
Tube 4	0.508	0.172	2.950	0.003
Tube 5	-0.242	0.207	-1.170	0.242
Tube 6	-0.040	0.188	-0.210	0.834
Tube 7	-1.616	0.199	-8.130	<0.001
Tube 8	-3.182	0.185	-17.250	<0.001
Low BLL group Vacuum tubes [#]				
Tube 1	-0.178	0.048	-3.700	<0.001
Tube 3	0.226	0.066	3.430	<0.001
Tube 4	0.202	0.064	3.170	0.002
Tube 5	-0.052	0.050	-1.030	0.302
Tube 6	-0.120	0.047	-2.570	0.010
Tube 7	-0.094	0.042	-2.230	0.026
Tube 8	-0.168	0.071	-2.380	0.018

[#] Tube 2 was the reference tube.

Table 3. Effects of Blood Samples Stored in a 4°C Environment and in Different Types of Blood Containers on Blood Lead Levels

High BLL group Vacuum tubes	Regression coefficient [*]	Standard error	t statistics	p value
Tube 1	0.046	0.053	0.880	0.380
Tube 2	0.139	0.061	2.280	0.023
Tube 3	-0.052	0.082	-0.630	0.529
Tube 4	-0.269	0.068	-3.970	<0.001
Tube 5	-0.061	0.070	-0.870	0.384
Tube 6	0.090	0.067	1.350	0.179
Tube 7	-0.099	0.073	-1.360	0.174
Tube 8	-0.156	0.077	-2.030	0.042
Low BLL group Vacuum tubes				
Tube 1	0.014	0.028	0.490	0.625
Tube 2	0.057	0.028	2.030	0.043
Tube 3	0.047	0.047	1.020	0.309
Tube 4	0.009	0.038	0.230	0.819
Tube 5	-0.035	0.051	-0.680	0.499
Tube 6	-0.019	0.017	-1.080	0.279
Tube 7	-0.025	0.030	-0.840	0.404
Tube 8	0.001	0.041	0.030	0.979

^{*} Duration of blood sample storage in month. Blood samples have been stored for a period of 12 months.

occupational health and the improvement of environment engineering, the government established The Blood Lead Surveillance System in 1992. Since then, the numbers of lead-poisoning cases among lead-exposed workers has dramatically decreased. Currently, blood lead surveys, which constitute a part of the surveillance system, are mandatory for workers exposed to lead.

There are medical institutes that have been commissioned to perform blood lead analyses in Taiwan; however, they often use varying types of collection tubes in their surveys. Cost seems to play a role in selecting a specific type of collection tube. However, few studies have examined the potential influence of types of collection tubes on the measurement of blood lead concentrations. There is a need to understand the effects of different types of containers on BLLs. In addition, the effect of long-term storage of blood samples at 4°C should be evaluated. Previous reports have indicated that whole blood samples stored in polycarbonate containers at -10°C did not show significant changes in the concentration of blood lead for up to 60 days, while other studies shown that BLLs remain unchanged for two weeks when the samples are stored in polyethylene and polypropylene vessels at 4°C [16,17].

Indeed, over the past two decades, the technology has improved. Better needles and vacutainers are also available now. Specifically, there are special collection tubes designed for specific purposes, such as the detection of trace elements. In order to make a comprehensive comparison of the effects of different types of tubes on BLLs, we have selected the most widely used tubes in Taiwan, including the more expensive tubes specifically designed for lead and trace element analysis (tubes 1, 2, and 3). In addition, we explored the effect of the length of storage (up to 12 months) on BLLs.

A test tube specifically designed for blood lead analysis (tube 2) was used as our comparison reference with which all other test tubes were checked for discrepancies. The present study revealed that in the high BLL group, tubes 1, 3, 4, 7, and 8 all showed differences in comparison with tube 2. When considering the regression coefficients (tube 1, -0.483; tube 3, 0.348; tube 4, 0.508; tube 7, -1.616; and tube 8, -3.182), it is apparent that the BLLs increased in some cases and decreased in others. The largest differences occurred in tubes 7 and 8. Clearly, these two containers were not suitable for use in blood lead monitoring. A similar result occurred in the low BLL group (Table 2). Although most of the tubes had lower BLLs than tube 2, the differences were negligible.

Because of the observed variations in BLLs, we need to determine whether the variations in BLLs are caused by contamination from other equipment or the collection tubes. We speculate that if the samples are contaminated during the process of blood collection (i.e., the needles are contaminated), the first collection tube should be the most significantly contaminated. However, in a previous study[18], three blood tubes of the same type were used consecutively to draw blood samples from the same source. The first tube did not show a higher level of blood lead. Based on this observation, it is unlikely that the collection tubes used were contaminated. Thus, we were confident that the differences found in this study came from the actual materials and contents of the containers. As shown Table 3, for both the high BLL and the low BLL groups, only minor fluctuations were observed in the BLLs measured from samples stored in different vacutainers over the one-year period. It seems that the BLLs from tube 2 tended to increase over time. However, as judged by the regression coefficients, the increase from 0.139 to 0.057 $\mu\text{g/dL}$ per month was negligible from a

practical standpoint. Most types of vacutainers did not have a significant influence on BLLs; however, the BLLs were underestimated in the last two types of vacutainers (tubes 7 and 8; Table 2). As a result, more care should be exercised when selecting vacutainers.

The most commonly used tube for blood lead surveys in Taiwan is the K₃ EDTA anticoagulant vacutainer (Tube 4). Results regarding this blood tube when compared with tube 2 showed slightly higher BLLs for both the high BLL and the low BLL groups (Table 2). However, the difference was negligible, which indicated that the results of tube 4 were quite accurate. In contrast, among the high BLL group, the results of tube 4 showed a slight decrease in BLLs. In terms of the regression coefficients, the decrease was, on average, 0.269 $\mu\text{g}/\text{dL}$ per month. The variations among the studied samples were negligible from a practical standpoint. Thus, we conclude that the most commonly used collection tube for blood lead surveys in Taiwan is an economical and suitable tool for this purpose. These findings may be helpful for future studies in analyzing BLLs.

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血液樣本於不同抗凝固劑與儲存時間 對血鉛濃度的影響

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目標：本研究目的是要瞭解血液樣本存放於不同採血管以及儲存時間，對血中鉛濃度之影響。**方法：**總計有57個血液樣本納入本研究。包括47名鉛作業人員與10名非鉛作業人員。每位研究對象均採用8種不同抗凝固劑之採血管收集血液檢體，並以4°C冷藏直至分析。血鉛濃度比較是以建議血鉛分析使用之採血管，內含Sodium Heparin抗凝固劑之無鉛真空採血管為參考比較組。不同採血管與儲存時間對血鉛濃度的影響採用廣義線性模式GEE(generalized estimating equation)方法來分析。**結果：**不論在高血鉛值或低血鉛值兩組之血液樣本中，不同採血管血鉛濃度多低於參考比較組，且達顯著差異。其中內含Na₂ EDTA、K₃ EDTA抗凝固劑之採血管不論在高血鉛值或低血鉛值兩組中，均較參考比較組血鉛濃度為高。而內含Buffered Cit, Na 9:1與Cit, Na-0.129M Silic兩種抗凝固劑之採血管，其血鉛濃度與參考組比較，顯著下降1.616μg/dL and 3.182μg/dL。在各種採血管血液樣本以4°C儲存時間達12個月，血鉛濃度並無太大變化。**結論：**不同採血管與建議血鉛分析採血管比較，其中內含Buffered Cit, Na 9:1與Cit, Na-0.129M Silic兩種抗凝固劑之採血管，在高血鉛值組，建議不宜做為血鉛分析之採血管，其他採血管對血鉛之影響及長達12個月的儲存時間，雖然血鉛濃度會有所差異，但此種差異在實質上影響甚微。(台灣衛誌 2007；26(4)：254-260)

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