ORIGINAL ARTICLE: Under-filled blood collection tubes containing K2EDTA as anticoagulant are acceptable for automated complete blood counts, white blood cell differential, and reti...

Article *in* International journal of laboratory hematology · October 2010

DOI: 10.1111/j.1751-553X.2009.01211.x · Source: PubMed

International Journal of Laboratory Hematology
The Official journal of the International Society for Laboratory Hematology

ORIGINAL ARTICLE INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

Under-filled blood collection tubes containing K_2EDTA as anticoagulant are acceptable for automated complete blood counts, white blood cell differential, and reticulocyte count

M. XU*,†, V. A. ROBBE*, R. M. JACK*,†, J. C. RUTLEDGE*,†

*Department of Laboratories, Seattle Children's Hospital, Seattle, WA, USA
†Department of Laboratory Medicine, University of Washington, Seattle, WA, USA

Correspondence:
Min Xu, Department of Laboratories, A6901, Seattle Children's Hospital, 4800 Sand Point Way NE, Seattle, WA 98105, USA.
Tel.: (206) 987 2103;
Fax: (206) 987 3840;
E-mail: min.xu@seattlechildrens.org

doi:10.1111/j.1751-553X.2009.01211.x

Received 20 May 2009; accepted for publication 16 November 2009

Keywords
Blood collection volume, CBC, WBC differential

SUMMARY

Current laboratory standards from Clinical Laboratory Standards Institute (CLSI) and manufacturer's (Becton Dickinson) data indicate that under-filling K_2EDTA blood collection tubes can result in erroneous hematology values. To accommodate under-filled tubes and reduce collection volumes while optimizing our automation, we explored the acceptable limit of under-filled tubes for hematology values. We collected 8.0 ml of blood from 30 normal adult volunteers. Each donation was aliquoted in the following volumes: 4.0, 2.0, 1.0, 0.5 ml × 2. These samples were analyzed within 1 h of blood collection on Sysmex XE-2100 (Sysmex America Inc., Mundelein, IL, USA) for complete blood count, reticulocyte, and white blood cell differentials. Results of the under-filled tubes were compared to those of the standard volume. The Deming regression analysis show excellent correlation for all parameters between each under-filled blood collection volume compared to a standard 4 ml volume. The Bland and Altman analysis shows good agreement between both 1.0 and 2.0 ml compared to a 4.0 ml volume. The 0.5 ml compared to a 4.0 ml volume, however, shows increased variation on many parameters. In addition all three collection volumes show negative bias compared to the standard volume for platelet count, but the difference is considered insignificant with a percent difference of 5.5%, 3.2%, and 1.5% for 0.5, 1.0, and 2.0 ml collection volume respectively. Finally for 0.5 ml collection volume we noticed a low level of false positive flagging rate for white blood cell. Acceptable complete blood count values of under-filled powdered K_2EDTA tubes can be obtained with as little as 1.0 ml of blood.

INTRODUCTION

According to the Clinical and Laboratory Standards Institute (CLSI, 2004) document, *Procedures for the Handling and Processing of Blood Specimens*, 'The amount of additive placed into a tube is intended for a certain volume of blood. If less blood than required is drawn,

the excess amount of additive has the potential to adversely affect the accuracy of test results.' Another Clinical and Laboratory Standards Institute (CLSI, 2003) document, *Tubes and Additives for Venous Blood Specimen Collection*, also states that the draw volume shall be no more than 10% below the stated draw volume of the manufacturer. Both standards are applied to all tubes with different anticoagulants including EDTA.

Complete blood count (CBC) and white blood cell (WBC) differential counts are performed on whole blood collected in tubes containing K_2EDTA as an anticoagulant. Prior to early 1990, blood samples were collected in glass tubes containing liquid K_3EDTA for CBC and differentials. In the past decade, K_2EDTA as an anticoagulant has gained popularity. In 1993, The International Council for Standardization in Hematology recommended the use of K_2EDTA as the anticoagulant of choice in specimen collection for blood cell counting and sizing. In addition, due to the safety concern, the plastic K_2EDTA tubes are gradually replacing glass tubes as preferred blood collection tubes. In the early 2000s, Becton Dickinson (BD, Franklin Lakes, NJ, USA) began to manufacture plastic blood collection tubes with spray-dried K_2EDTA as the anticoagulant. One study compared the newly produced plastic tubes containing dried K_2EDTA with the liquid K_3EDTA glass tubes (Van Cott *et al.*, 2003). This study found no clinically significant differences between these two types of tubes for CBC including WBC, RBC, platelet (Plt), hemoglobin (Hb), hematocrit (Hct), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and red cell distribution width (RDW), reticulocyte counts (retic), WBC differentials including neutrophil (neu), lymphocyte (lym), monocyte (mono), eosinophil (eo) and basophil (baso), as well as instrument flagging rate. Selected parameters (WBC, RBC, Plt, retic, hemoglobin, hematocrit, MCV, MCHC, and RDW) had statistically significant differences, however, they were small. The slight differences were primarily due to the dilutional effect of the liquid anticoagulant in glass tubes.

We raise the question whether spray-dried K_2EDTA tubes are still required to be completely filled. The possibility exists that solid rather than liquid EDTA tubes have reduced the importance of the ratio of anticoagulant to blood with regard to the effect on hematology parameters. There is no published data on the effects of under-filling the dried K_2EDTA tubes on CBC results. Since the manufacturer (BD) has not provided new studies concerning blood volume requirements, the CLSI guideline regarding collection volume was based on the older studies on collection tubes with liquid anticoagulant (Lampasso, 1965; Sacker, 1975; Lewis & Stoddart, 1977). We have tried unsuccessfully to obtain updated information from BD regarding collection requirements using plastic tubes with spray-dried anticoagulant. We hypothesize that the blood volume requirement might not be applicable for the new generation of blood collection tubes.

Although the impact for blood collection volume requirements is significant for hospitals with predominantly pediatric patients, this has become more of a concern for adult hospitals with frequent phlebotomy in geriatric patients (Sanchez-Giron & Alvarez-Mora, 2008). To minimize blood collection in pediatric hospitals, which can be critical in small infants, microtainer collection tubes (0.5 ml) are often used. The drawbacks of microtainer tube collection have become more apparent with advances in automation that are not optimized for microtainers. During the past two decades, the automation of blood testing significantly changed the practice of laboratory medicine making the microtainers an exception to routine use. In addition, there have been incidents raising safety concerns with microtainers in our hospital. Microtainers do not have pierceable caps like the larger collection tubes; nursing staff have sometimes tried to pierce through the hard plastic tops when filling the tubes. Furthermore, the price of a microtainer is much higher than standard 4.0 ml tubes. In this study, different volumes of blood were collected in standard 4.0 ml lavender top tubes with dried K_2EDTA as anticoagulant. The CBC parameters, WBC differential, and reticulocyte counts were compared between different volumes of blood collected and standard 4.0 ml blood collection.

MATERIALS AND METHODS

Sample collection

The study was approved by Seattle Children's Hospital Institutional Review Board. After obtaining informed

consent, 30 normal adult volunteers, mainly female, (laboratory staff) donated a total of 8.0 ml blood each for the study. The whole blood samples were collected into a non-anticoagulated syringe and dispensed into five 4.0 ml blood collection tubes containing powdered K_2 EDTA as anticoagulant. The collection volumes for each tube were 4.0, 2.0, 1.0, and 0.5 ml (×2). The blood collection was completed over several weeks.

Testing

The tubes with different volumes of blood were analyzed for CBC, reticulocytes, and WBC differentials (neutrophils, lymphocytes, monocytes, eosinophils, and basophils) on a Sysmex XE-2100 within 1 h of blood collection integrating research samples with clinical samples during routine work. The samples were run with either auto-mode or manual mode on one of the two instruments. Results of the under-filled tubes, (0.5, 1.0, or 2.0 ml) were compared to the standard 4.0 ml volume for clinically significant differences. The samples were assayed randomly on two hematology analyzers to more accurately reflect routine patient testing. The correlation between the two instruments was excellent with slopes ranging from 0.954 to 1.034 and R values from 0.9304 to 0.9992 for all the parameters included in the study.

Statistics study

For each of the CBC and WBC differential parameters, as well as the reticulocyte count, the results from under-filled tubes were compared to the standard 4.0 ml volume using Deming regression analysis. The mean values for each parameter were compared between under-filled volumes and the 4.0 ml standard volume. The percent difference of the mean was calculated for each parameter by first subtracting the mean for 4.0 ml volume from that of the under-filled volume, then divided by the mean of 4.0 ml volume. Finally, the bias of each point compared to the mean was calculated using Bland and Altman bias plot from EP Evaluator 8 (David G Rhoads Associates, Inc., Kennett Square, PA, USA). The allowable total error (ATE) in percent is defined as 3× median CV. Median CV is derived from CAP proficiency testing (FH9-B and RT4-B, 2008) for Sysmex XE 2100.

RESULTS

The range for each CBC and WBC differential parameter, and reticulocyte count from 30 normal volunteers for 4.0 ml collection volume are as follow: WBC 4.16–10.69 × 10^9/l; RBC 3.94–5.70 × 10^{12}/l; platelet 179–360 × 10^9/l; Hb 10.8–15.4 g/dl; Hct 34.1–45.5%; MCV 63.6–95.9 fl; MCH 19.8–33.4 pg; MCHC 30.5–35.7 g/dl; RDW 12.0–16.3%; reticulocytes 0.68–2.70%; neutrophils 47.4–74.2%; lymphocytes 17.2–45.6%; monocytes 2.5–11.7%, eosinophils 0.1–9.0%; basophils 0.1–1.2%.

The mean values of all 30 samples for each parameter were summarized in Table 1. For all the parameters except basophils, the percent differences between under-filled volumes compared to the standard volume were insignificant. Although the mean value of the basophil count is similar for all collection volumes, the percent difference compared to standard collection volume was high which was caused by its small absolute value. Due to the low value and narrow ranges of basophils and eosinophils for samples from 30 normal volunteers, both parameters were omitted from the subsequent analysis.

To compare whether there was any significant difference between the paired samples, correlation-regression, as well as Bland and Altman bias plot were used in analysis. As shown in Table 2, all parameters with the exception of monocytes showed excellent correlation between different under-filled volumes compared to the standard volume. Both slope and R^2 were between 0.9 and 1.0 for all except 0.5 ml volume for Hct (slope, 0.960; R^2, 0.805), MCHC (slope, 0.999; R^2 0.785), and reticulocytes (slope, 0.8934; R^2, 0.8447). The correlation of monocytes in all three under-filled volumes was sub-optimal (R^2 between 0.616 and 0.778), most likely due to imprecision in small numbers compared to other parameters.

To further determine the agreement for each point between low blood collection volume and standard 4 ml collection volume, we used Bland and Altman plot (or bias plot) provided in software EP Evaluator 8. The ATE in percent for each parameter is listed in Table 3. ATE is determined by finding CV for Sysmex XE-2100 from CAP proficiency test (PT) survey data and multiplying by 3. As MCH and MCHC are calculated parameters, there are no statistics in CAP PT. Since the numbers of eosinophils and basophils

Table 1. Mean of all parameters from 30 samples for each collection volume and percentage difference of mean between three under-filled volumes and standard volume. For example, the percent difference for 0.5 ml volume equals (mean of 0.5 ml−mean of 4.0 ml)/mean of 4.0 ml

Collection volume (ml)	Mean				Percentage difference of mean (%)		
	0.5	1	2	4	0.5	1	2
WBC ($\times 10^9$/l)	6.7	6.85	6.79	6.82	−1.8	0.4	−0.4
RBC ($\times 10^{12}$/l)	4.62	4.63	4.64	4.62	0.0	0.2	0.4
Platelet ($\times 10^9$/l)	238.90	244.70	248.97	252.73	−5.5	−3.2	−1.5
Hb (g/dl)	13.57	13.48	13.49	13.45	0.9	0.2	0.3
Hct (%)	40.69	40.50	40.48	40.35	0.9	0.4	0.3
MCV (fl)	88.56	87.98	87.82	87.81	0.9	0.2	0.0
MCH (pg)	29.50	29.30	29.30	29.30	0.7	0.0	0.0
MCHC (g/dl)	33.30	33.30	33.30	33.30	0.0	0.0	0.0
RDW (%)	13.40	13.30	13.30	13.30	0.8	0.0	0.0
Neutrophil (%)	59.66	59.98	59.90	59.61	0.1	0.6	0.5
Lymphocyte (%)	29.86	29.78	30.06	30.07	−0.7	−1.0	0.0
Monocyte (%)	6.85	6.73	6.60	6.88	−0.5	−2.2	−4.1
Eosinophil (%)	2.72	2.68	2.66	2.72	−0.2	−1.7	−2.3
Basophil (%)	0.64	0.62	0.55	0.51	26.1	21.6	8.5
Reticulocyte (%)	1.57	1.60	1.59	1.62	−2.7	−1.0	−2.0

Hb, hemoglobin; Hct, hematocrit; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RDW, red cell distribution width.

Table 2. Correlation and regression analysis comparing CBC, reticulocytes, and WBC differentials between different volumes of blood collection and 4.0 ml standard volume

Volume	Slope/R^2	WBC	RBC	Plt	Hb	Hct	MCV	MCH	MCHC	RDW	Neu	Lym	Mono	Eo	Retic
0.5 ml	Slope	0.926	1.026	0.939	1.021	0.960	1.027	1.031	0.999	1.012	1.017	0.973	0.905	0.912	0.893
	R^2	0.961	0.922	0.936	0.913	0.805	0.990	0.972	0.785	0.986	0.974	0.948	0.616	0.959	0.845
1.0 ml	Slope	0.979	0.995	0.968	1.000	0.985	1.002	0.999	1.000	0.987	1.030	1.020	0.903	0.983	0.938
	R^2	0.978	0.972	0.955	0.953	0.933	0.998	0.991	0.936	0.993	0.973	0.954	0.647	0.974	0.921
2.0 ml	Slope	0.948	1.000	1.022	1.013	1.005	0.998	1.001	1.025	1.009	1.022	0.988	0.979	0.987	1.008
	R^2	0.989	0.994	0.967	0.991	0.988	0.999	0.993	0.959	0.993	0.984	0.973	0.631	0.958	0.931

Hb, hemoglobin; Hct, hematocrit; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RDW, red cell distribution width; neu, neutrophil; lym, lymphocyte; mono, monocyte; Retic, reticulocyte; eo, eosinophil.

are very low in normal volunteers, they are not included in the bias study. The objective is to determine whether the two collection volumes are clinically equivalent. The experiment passes if the difference between the two is less than the ATE for at least 95% of the specimens. For all the parameters tested, results of 2.0 ml collection volume passed the experiment, confirming agreement with the 4.0 ml collection volume. For the 1.0 ml collection volume, all parameters passed with the exception of the platelet count, percent lymphocyte and reticulocyte count. For the above three parameters, there were two or three outliers very close to or on both sides of the limits in Bland and Altman bias plot (Figure 1). However,

Table 3. The allowable total error (ATE) in percentage for CBC, reticulocytes, and WBC differentials on Sysmex XE-2100

	WBC	RBC	Plt	Hb	Hct	MCV	RDW	Neu	Lym	Mono	Retic
ATE (%)	8.4	3.3	8.4	3.6	5.1	3.0	4.2	6.3	11.1	42.9	17.7

Hb, hemoglobin; Hct, hematocrit; MCV, mean cell volume; RDW, red cell distribution width; neu, neutrophil; lym, lymphocyte; mono, monocyte; Retic, reticulocyte.

for the 0.5 ml collection volume, only the MCV, RDW, and percent neutrophil passed Bland and Altman bias analysis, seven parameters failed (data not shown). There were 2–8 outliers for those parameters. Although the mean values for all seven parameters with the exception of the platelet count, were not significantly changed for 0.5 ml collection volume compared to the standard, the imprecision was relatively high. The platelet count appeared to have a negative bias for all three under-filled collection volumes although the 2.0 ml collection volume passed the Bland and Altman bias analysis. We consider the differences as clinically insignificant since the average percentage difference was only 5.5%, 3.2%, and 1.5% for 0.5, 1.0, and 2.0 ml blood collection volume respectively. The underlyning mechanism for the negative bias was not clear.

Finally, we analyzed the difference for flagged messages generated by the automated instruments. As the samples were collected from apparently normal volunteers, most analysis did not have any flagged messages. Three out of 30 donors had some flagged messages. The first donor had an 'anemia' flag for all tubes regardless of the collected blood volumes. This person had a hemoglobin level of approximately 11 g/dl. The second donor had 'microcytosis and anemia' and 'PLT abnormal distribution' for all four tubes regardless of the collected blood volumes. This donor also had low MCV of approximately 63 fl which suggests possible iron deficiency or thalassemia trait. In addition, 'blasts?' flag was found on the same person only for the tube with 0.5 ml blood volume. Similarly, the third donor had 'Abn Lympho/L_Blasts?' flag only on 0.5 ml blood volume. This sample had no other abnormal values for any of the parameters tested. These data suggested that the flag messages were not significantly affected by the under-filled blood collection volume of 1.0 and 2.0 ml. For 0.5 ml collection volume, there was a low level of false positive flagging rate (2 out of 30 samples; 6%) for white blood cells. One of the possible reasons could be due to relatively high concentration of EDTA affecting cell morphology. Whether this effect was donor specific was not known. In any case, a slightly higher false positive flagging for 0.5 ml blood volume had no clinical significance.

There was no significant difference for CBC, reticulocytes, and WBC differentials when blood was collected with 1.0 or 2.0 ml volume in a 4.0 ml spray-dried EDTA collection tube compared to a completely filled tube. For 0.5 ml of collection volume, the imprecision was slightly higher. Thus, we are reluctant to recommend collecting 0.5 ml of blood in a 4.0 ml tube for analysis on Sysmex XE 2100.

DISCUSSION

We currently use microtainer tubes for approximately one-third of our total blood collection for CBC and WBC differentials. In practice, we found the following disadvantages to using microtainers which significantly affect patient and staff safety, and testing efficiency. First, we need to reject samples received in incompletely filled tubes, necessitating another blood draw for the patient. Secondly, the microtainers require small labels that prevent automated barcode reading and require manual sampling instead of complete automation. Efficiency is significantly reduced using microtainers compared to the standard blood collection tubes and the opportunity for misidentification of the sample is increased because the bar code cannot be read directly from the tube by the analyzer. The laboratory has also been asked to minimize the number of blood draw tubes on the nursing units. If smaller volumes of blood can be drawn into a standard 4.0 ml tube, only one size tube needs to be stocked on most units. In addition, the price of microtainers is 6–7 times more expensive than standard 4.0 ml tubes.

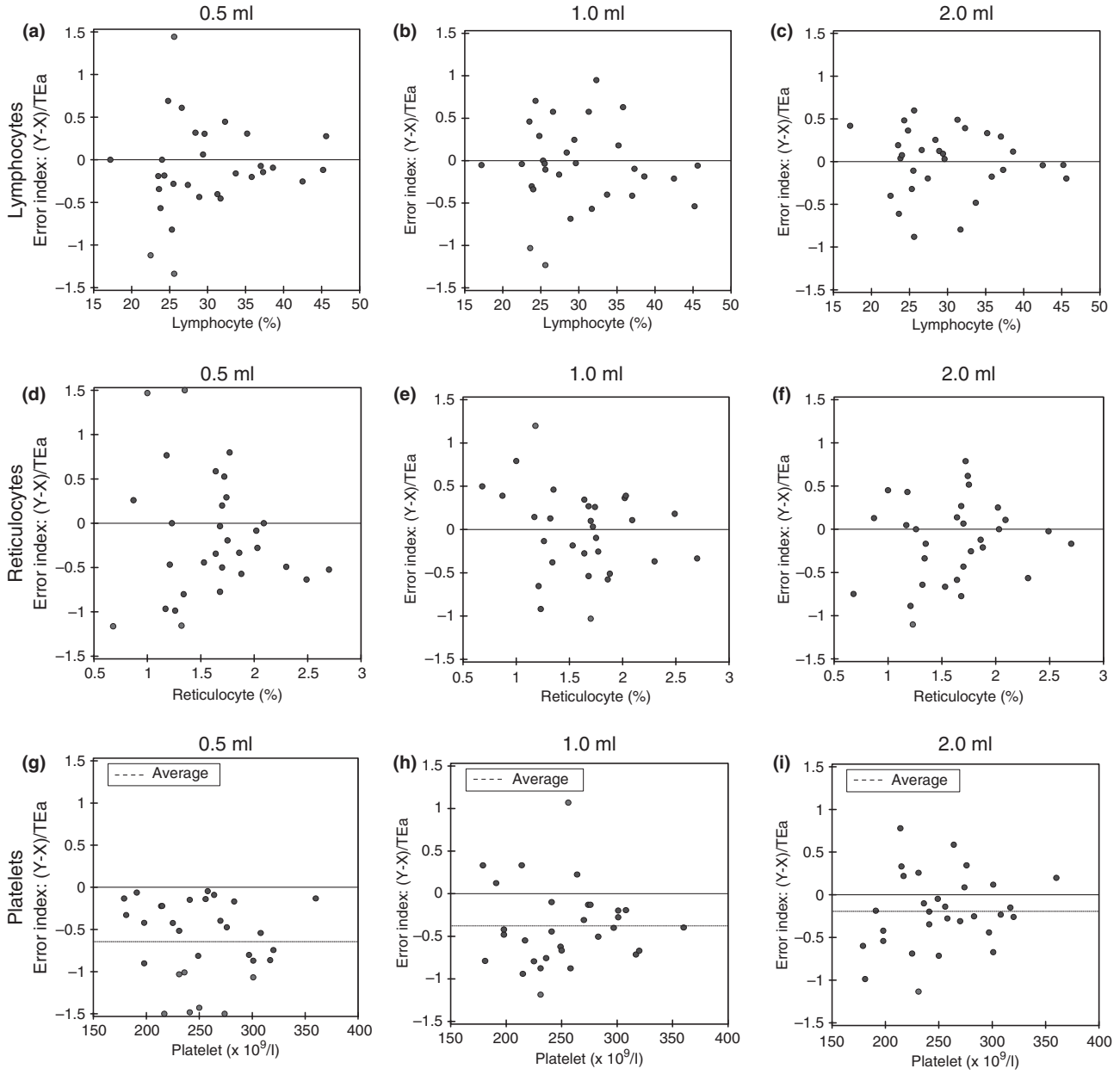

Figure 1. The Bland and Altman bias plot of lymphocyte, reticulocyte, and platelet count between different blood collection volumes compared to standard completely filled tube. The *x*-axis represents the lymphocyte, reticulocyte, and platelet count of standard 4.0 ml blood collection volume. The *y*-axis represents error index. (a–c) Lymphocyte count (%); (d–f) reticulocyte count (%); (g–i) platelet count ($\times 10^9$/l); a, d, g, 0.5 ml collection volume; b, e, h, 1.0 ml collection volume; c, f, i, 2.0 ml collection volume.

Our study clearly shows that there is no clinical difference although some samples are statistically different for some CBC parameters, reticulocytes, and WBC differentials. The instrument flagging increases when 0.5 ml blood was collected in the 4.0 ml blood collection tube. The Sysmex XE-2100 requires 200 µl of whole blood for automated sampling mode, plus approximately 100–200 µl of dead volume; 0.5 ml will

be insufficient if a repeat is necessary. Therefore, collecting 0.5 ml of blood into a 4.0 ml tube is not recommended as a standard practice. If <1.0 ml of blood needs to be collected for neonates or infants, microtainer would be recommended.

Our study is limited by using normal adults and a single type of analyzer. We could not, however, justify a study in neonates or hematology/oncology patients to test the extremes of the pediatric CBC population. While it is possible sample volume could affect parameters in these patients, we cannot discern a reason why that would be the case. When we have accepted under-filled samples, we had not observed unexpected CBC results for those patients.

CONCLUSION

In our experience collecting a minimum of 1.0 ml of whole blood in a 4.0 ml lavender top tube has no significant effect on routine CBC, reticulocytes, and WBC differential. Standardizing to one 4.0 ml lavender top tube for the vast majority of patients would reduce re-collection of samples, prevent misidentification of samples, simplify the testing process, decrease turn-around time, improve staff safety, and reduce inventory and supply costs. Reducing the amount of blood collected benefits both pediatric and geriatric patients most, as these patients often require frequent testing, and sample access can be challenging.

ACKNOWLEDGEMENT

This work is supported by Department of Laboratories, Seattle Children's Hospital. We would like to thank Ms. Judith A. Johanson for manuscript review and our Core Laboratory staff for running the tests.

REFERENCES

Clinical and Laboratory Standards Institute (CLSI). (2003) Tubes and additives for venous blood specimen collection. Approved Guideline – Fifth Edition. H1–A5, 16 (13), 5.

Clinical and Laboratory Standards Institute (CLSI). (2004) Procedures for the handling and processing of blood specimens. Approved Guideline – Third Edition. H18-A3, 24 (21).

Lampasso J.A. (1965) Error in hematocrit value procedure by excessive ethylenediamine tetraacetic acid. American Journal of Clinical Pathology 44, 109–110.

Lewis S.M. & Stoddart C.T.H. (1977) Effects of anticoagulants and containers (glass and plastic) on the blood count. Lab Practice 20, 787–792.

Sacker L.S. (1975) Specimen collection. In: Quality Control in Haematology (eds S.M. Lewis & J.F. Coster), pp. 224–227. Academic Press, New York.

Sanchez-Giron F. & Alvarez-Mora F. (2008) Reduction of blood loss from laboratory testing in hospitalized adult patients using small-volume (pediatric) tubes. Archives of Pathology and Laboratory Medicine 132, 1916–1919.

Van Cott E.M., Lewandrowski K.B., Patel S., Grzybek D.Y., Patel H.S., , Fletcher S.R., Kratz A. (2003) Comparison of glass K_3EDTA versus plastic K_2EDTA blood-drawing tubes for complete blood counts, reticulocyte counts, and white blood cell differentials. Laboratory Hematology 9, 10–14.