The effective reduction of tourniquet application time after minor modification of the CLSI H03-A6 blood collection procedure

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Abstract

Introduction: The phlebotomists' procedures are a still source of laboratory variability. The aim of this study was to verify the efficacy of minor modification in procedure for collection of diagnostic blood specimens by venipuncture from CLSI H03-A6 document is able to reduce the tourniquet application time.

Materials and methods: Thirty phlebotomists were invited to participate. Each phlebotomist was trained individually to perform the new venipuncture procedure that shortens the time of tourniquet release and removal. The phlebotomy training program was delivered over 8h. After training, all phlebotomists were monitored for 20 working days, to guarantee the adoption of the correct new procedures for collection of diagnostic blood specimens. After this time frame the phlebotomists were evaluated to verify whether the new procedure for blood collection derived from CLSI H03-A6 document was effective to improve the quality process by decrease in tourniquet application time. We compared the tourniquet application time and qualitative difference of phlebotomy procedures between laboratories before and after phlebotomy training.

Results: The overall mean ± SD tourniquet application time before and after this intervention were 118 ± 1 s and 30 ± 1 s respectively. Minor modifications in procedure for blood collection were able to reduce significantly the tourniquet application time (-88 s, $P < 0.001$).

Conclusions: The minor modifications in procedure for collection of diagnostic blood specimens by venipuncture from CLSI H03-A6 document were able to reduce the tourniquet application time. Now the proposed new procedure for collection of diagnostic blood specimens by venipuncture could be considered usefulness and should be put into practice by all quality laboratory managers and/or phlebotomy coordinators to avoid preanalytical errors regard venous stasis and guarantee patient safety.

Key words: venous stasis; preanalytical phase; tourniquets; phlebotomy; practice guidelines

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Introduction

The Clinical Laboratory Standard Institute (CLSI) mission is to develop best practices in clinical and laboratory testing and to promote their broad implementation by means of a consensus-driven process that balances the perspectives of industry, government, and the health care professions(1). The phlebotomists' procedures in private- and public laboratories in South America are far from being standardized or even harmonized (2). We previously showed that the wide distribution and implementation of the CLSI H03-A6 (3) document is able to improve the laboratory quality process, although the steps for collecting diagnostic blood specimens by venipuncture cannot be considered a gold standard yet, since inherent errors are still possible (i.e. variability as regards venous stasis) (4). From a practical standpoint, the tourniquet-induced venous stasis promotes the efflux of water, diffusible ions and low molecular weight substances from the vessel, thereby increasing the concen-

tration of various blood analytes at the punctured site, thus influencing the correct interpretation of test results and increasing the likelihood of spurious variation (i.e., *in vitro* hemolysis) (5-7). The tourniquet application time and forearm clenching should be verified by every quality laboratory manager at work in the laboratory services, in order to both, eliminate this source of laboratory errors and safeguard the quality all through the total testing process. Based on our previous study, we had suggested changes on phlebotomy procedure steps from CLSI H03-A6 document as follows: i) to put on gloves, to cleanse the venipuncture site and to allow to dry before applying the tourniquet and selecting the venipuncture site and vein; ii) to release and remove the tourniquet immediately when the first tube starts to fill (4). The aim of this study was to verify the efficacy of minor modification in procedure for collection of diagnostic blood specimens by venipuncture from CLSI H03-A6 document (3) is able to reduce the tourniquet application time.

Materials and methods

Phlebotomy training program

The same thirty phlebotomists from São Paulo state (Brazil) previously appraised by us (2,4), were invited to take part to this study. These professionals had 5 (4.8-5.6) years of experience in diagnostic blood specimens collection by venipuncture. The above professionals were operational at institutions (5 public- and 5 private-laboratories) where about 200 blood collections by venipuncture are performed *per* working day. Each phlebotomist was trained individually to perform the new venipuncture procedure as previously proposed by our group (4) with recent updates regarding the preanalytical phase (8,9) (Table 1, proposed new procedure). The phlebotomy training program was delivered over 8 hours, during which the importance of each step of the updated procedure was clearly explained (Table 1, importance of each step from proposed new procedure). One external/expert auditor from DICQ® trained all phlebotomists in one month (from September to October 2012). DICQ® is a National System of Accreditation from Brazilian Society of Clinical Analyses (10). This accreditation system is based on ISO 15189 document (11). After training, all phlebotomists were monitored by the laboratory quality manager (these professionals had followed the training too) for twenty working days, to guarantee the adoption of the correct procedures for the collection of diagnostic blood specimens, in agreement with the procedure proposed by our group (4). This period is generally considered sufficient by the quality laboratory's managers for including new procedures. Then each participating phlebotomist was re-evaluated during one normal working day. We decided to retrain and re-evaluate the thirty phlebotomists twice after the first assessment (2,4), in order to minimize the inter-individual variability of performance since we were aware of the relevance of the workday routine of these professionals.

Phlebotomy evaluation

To assess the performance of the phlebotomists during the collection of diagnostic blood specimens the previously check list used was followed (2,4). We aimed at re-evaluating: i) time of tourniquet application; ii) inappropriate requests to patients to clench their fist repeatedly; iii) excessively aggressive disinfection of the forearm by the phlebotomist, which can produce venous stasis; iv) drawing order of vacuum tubes during specimen collection; v) adequacy of mixing blood in primary vacuum tubes that contain anticoagulant or clot activating additives (as recommended by manufactures). So, this check list allowed the re-evaluation of whether this procedure for blood collection by venipuncture - a minor modification in CLSI H03-A6 document (3) - was effective to improve the quality of the blood collection process by eliminating any possible source of errors, that we hypothesized was still current in this worldwide distributed and implemented document (CLSI H-03-A6).

To standardize the evaluation of tourniquet application time and to reduce bias, the performance of each phlebotomist was evaluated when blood was being collected from patients with the following characteristics: between the ages of 18 and 65 years, nonpregnant, nonobese (i.e., body mass index [BMI] < 30 kg/m^2), neither undergoing chemo-

Table 1. Comparisons of CLSI H03-A6 and the new procedure for collection of diagnostic blood specimens by venipuncture.

Step	Current procedure recommended by CLSI H03-A6 document	Step	Proposed new procedure	Importance of each step from proposed new procedure
i	prepare accession order	i	prepare accession order	to guarantee patient identity assurance
ii	approach and indentify the patient; sanitize hands	ii	approach and indentify the patient; sanitize hands	
iii	verify the patient's fasting status or diet restrictions, as appropriate, and inquire	iii	verify the patient's fasting status or diet restrictions, as appropriate, and inquire	fasting status is an important source of variability
	if the patient has a latex sensitivity; select appropriate gloves and tourniquet		if the patient has a latex sensitivity; select appropriate gloves and tourniquet	to prevent allergic reaction and/or anaphylactic shock attributed to latex allergy
iv	assemble necessary supplies and select appropriate tubes according to the requests	iv	assemble necessary supplies and select appropriate tubes according to the requests	to prevent errors in laboratory medicine induced by supplies and addictives such as anticoagulants and clot activators
v	position the patient	v	position the patient	to eliminate possible interferences of blood distribution due to different posture
vi	apply the tourniquet and select the venipuncture site and vein	**vi**	**put on gloves**	preventing phlebotomists' exposure to potentially infectious blood pathogens
vii	put on gloves	**vii**	**cleanse the venipuncture site**	cleaning prevents infection by skin microorganisms
viii	cleanse the venipuncture site and allow to dry	**viii**	**request the patient just close his/her hand (never request the patient to "pump")**	the clenching of the forearm before venipuncture modifies the concentration of several analytes (i.e. potassium)
ix	perform venipuncture; once blood flow begins, request the patient to open his/her hand	**ix**	**apply the tourniquet and select the venipuncture site and vein**	to prevent venous stasis and hemolysis
		x	**perform venipuncture; once blood flow begins, request the patient to open his/her hand**	
x	fill tubes using the correct order of draw		**also release and remove the tourniquet**	
xi	release and remove the tourniquet	**xi**	**fill tubes using the correct order of draw**	to prevent errors by cross contamination between additives
xii	place the gauze pad over the puncture site	xii	place the gauze pad over the puncture site	safe feature for preventing phlebotomists' exposure to potentially infections by bloodborne pathogens
xiii	remove the needle, activate any safety feature, and dispose of the device	xiii	remove the needle, activate any safety feature, and dispose of the device	
xiv	apply pressure to the site, making sure bleeding has stopped, and then bandage the arm	xiv	apply pressure to the site, making sure bleeding has stopped, and then bandage the arm	applying pressure to the site is an efficient prevention of bruise
xv	label the tubes and record the time of collection; some facilities also specify phlebotomist	xv	label the tubes and record the time of collection; some facilities also specify phlebotomist	to reduce missing identification and guarantee the traceability of the process
	identification on the tubes		identification on the tubes	
xvi	observe special handling requirements (if any required)	xvi	observe special handling requirements (if any required)	to guarantee diagnostic blood specimens stability
xvii	send properly labeled blood collection tubes to the appropriate laboratories	xvii	send properly labeled blood collection tubes to the appropriate laboratories	

Steps in bold text represent changes in current procedure recommended by CLSI H03-A6 document (3) suggested by Lima-Oliveira *et al.* (4).

therapy nor catheterization, and not afflicted with any apparent vascular disease. All these conditions were carefully excluded because they might be associated with difficulties during the collection of diagnostic blood specimens, which thereby might introduce bias into the evaluation. The performance of each phlebotomist was monitored in 5 different phlebotomies; the time of tourniquet application was measured with a calibrated chronometer. The time interval between tourniquet application and removal was recorded in seconds. This procedure is the same procedure published and used by us before to evaluate phlebotomists' performance (2,4).

Statistical analysis

The tourniquet application time showed normal distribution (Kolmogorov-Smirnov test; $P > 0.05$) and date were expressed as mean ± standard deviation (SD). Differences were tested by paired Student t-test. Fisher exact test (two-tailed) was used to compare the qualitative differences of the phlebotomy procedures among laboratories before and after phlebotomy training. McNemar Chi-square test for dependent samples was used to compare before-after laboratory training. The values with $P < 0.05$ were considered statistically significant. Statistical analyses were performed with Statistica for Windows, version 8.0 (StatSoft Inc., Tulsa, OK, USA).

Results

The results are shown in table 2 and table 3. The new phlebotomy instruction was able to eliminate several non-conformities, especially those related to prolonged tourniquet application time (Table 3); the overall mean ± SD was 30 ± 1 s. Private laboratories applied the tourniquet for statistically significant shorter times than public laboratories (28 ± 1 s *vs*. 32 ± 1 s; P = 0.002). None of the phlebotomist inappropriately requested that the patient clench their fist repeatedly (i.e., more than twice).

Discussion

Our results show that the procedure previously proposed (4) reduces drastically the tourniquet application time. The overall mean ± SD before and

Table 2. Improvement of phlebotomy error rates pre- and post- training of H03-A6 and proposed new procedure.

Error description	Laboratories before training #				Laboratories after training with CLSI H03-A6 document ##				Laboratories after training with new procedure			
	All (N = 30)	Public Lab (N = 15)	Private Lab (N = 15)	P	All (N = 30)	Public Lab (N = 15)	Private Lab (N = 15)	P	All (N = 30)	Public Lab (N = 15)	Private Lab (N = 15)	P
Inappropriate request to the patient to clench the fist repeatedly	25/30	14/15	11/15	0.329	29/30*	15/15	14/15	1.000	0/30***	0/15	0/15	---
Inadequate friction procedure during the cleaning of the venipuncture site	27/30	13/15	14/15	1.000	0/30**	0/15	0/15	---	0/30	0/15	0/15	----
Incorrect sequence of vacuum tubes	26/30	13/15	12/15	1.000	0/30**	0/15	0/15	---	0/30	0/15	0/15	----
Incorrect mixing of vacuum tubes	25/30	15/15	10/15	0.042	0/30**	0/15	0/15	---	0/30	0/15	0/15	----

Comparison of error rates between public and private laboratories before training (McNemar Chi-square test, *P = 0.113 and **P < 0.001), and after training to the proposed new procedure (Fisher exact test two-tailed test *** P < 0.001 **** P = 0.237).
---, not calculated;
data previously published (2);
data previously published (4).

after this intervention were 118 ± 1 s and 30 ± 1 s respectively (Table 3). Minor modifications in procedure for blood collection were able to reduce significantly the tourniquet application time (-88 s, P < 0.001). This significant reduction of the application time is able to eliminate the venous stasis impact, that is an important source of unpredictable laboratory variability (5-8,12-14). Private laborato-

Table 3. Evaluation of tourniquet application time after phlebotomy training program (CLSI H03-A6 document *vs. New procedure*).

Laboratories	Phlebotomists	Tourniquete time (s)			P-value
		Procedure from CLSI H03-A6 # (mean ± SD)	*New Procedure* (mean ± SD)	Difference	
1 Public	1	156 ± 3	31 ± 2	-125	<0.001
	2	154 ± 1	32 ± 2	-122	<0.001
	3	154 ± 2	31 ± 1	-123	<0.001
2 Public	4	144 ± 1	32 ± 1	-112	<0.001
	5	140 ± 1	33 ± 2	-107	<0.001
	6	141 ± 1	32 ± 1	-109	<0.001
3 Public	7	153 ± 2	31 ± 1	-122	<0.001
	8	150 ± 1	32 ± 1	-118	<0.001
	9	149 ± 1	31 ± 1	-118	<0.001
4 Public	10	145 ± 1	33 ± 2	-112	<0.001
	11	144 ± 2	31 ± 1	-113	<0.001
	12	146 ± 1	31 ± 2	-115	<0.001
5 Public	13	147 ± 1	32 ± 1	-115	<0.001
	14	146 ± 2	32 ± 3	-114	<0.001
	15	147 ± 1	32 ± 1	-115	<0.001
1 Private	16	97 ± 1	30 ± 1	-67	<0.001
	17	92 ± 1	29 ± 1	-63	<0.001
	18	90 ± 1	30 ± 1	-60	<0.001
2 Private	19	87 ± 2	25 ± 1	-62	<0.001
	20	84 ± 1	26 ± 1	-58	<0.001
	21	85 ± 1	26 ± 1	-59	<0.001
3 Private	22	83 ± 2	28 ± 2	-55	<0.001
	23	81 ± 1	27 ± 2	-54	<0.001
	24	80 ± 1	29 ± 2	-51	<0.001
4 Private	25	83 ± 2	26 ± 1	-57	<0.001
	26	85 ± 3	26 ± 1	-59	<0.001
	27	87 ± 1	27 ± 1	-60	<0.001
5 Private	28	95 ± 1	28 ± 1	-67	<0.001
	29	90 ± 2	27 ± 1	-63	<0.001
	30	93 ± 1	29 ± 1	-64	<0.001

Differences between CLSI H03-A6 and *New procedure* are shown in seconds and were tested by paired Student t-test (P-value). # date of tourniquet time from training with CLSI H03-A6 document were previously published (4).

ries exhibit a significantly lower time of tourniquet application than public laboratories after the training period (i.e., 31.7 ± 0.7 *vs.* 27.5 ± 1.6 s; P < 0.001). A reliable explanation for this is that private laboratories have more ergonomic furniture in blood collection rooms (4). Recently Bölenius *et al*. (15) used the hemolysis index (HI) to assess the efficiency of a large-scale 2 h educational intervention, concluding that the training had only minor effects on blood collection practices. This large-scale 2 h education intervention was supported by laboratory instructors from the Country Council of northern Sweden focusing on rehearsal and implementation of the national and local venous blood specimen collection guidelines that is similar to international standards (CLSI H03-A6 document). In our opinion, this kind of training program should instead be strongly recommended and performed worldwide. Moreover, previous investigations had shown that educational program and technological interventions for phlebotomists are relevant and promote decrease of sample errors consequently resulting in quality improvement (16-19). Maybe Bölenius *et al*. found only minor effects because the rules of the CLSI H03-A6 document increase the tourniquet application time and the Sweden guidelines recommend to reverse vacuum tubes 5-10 times using an automatic mixer by inversion without rest after filling of the tubes. Paternmark and Landberg convincingly demonstrated that: (a) mixing blood samples immediately after collection may not be mandatory for all types of tubes; and (b) instant mixing by automatic mixer may produce spurious hemolysis and thereby introduce a bias for those parameters that are most susceptible to RBC injury (9). Therefore, the quality indicator HI chosen by Bölenius *et al*. was fully influenced by the phlebotomy guidelines used. Continuous monitoring and management of preanalytical errors (i.e., by quality indicators) are crucial for improving the quality of laboratory performance, and are also necessary for all clinical laboratories accredited by International Organization for Standardization (ISO) document 15189 (11,20-22). Some recent updates regarding the preanalytical phase should be considered when performing a phlebotomy training program, such as: i) supply changes among different manufacturers of syringes for blood gas analyses, which can represent new sources of laboratory variability; and likewise for not in-laboratory validated vacuum tubes by the quality laboratory managers (as recommended by ISO 15189 document) (23-27); ii) transport boxes which do not guarantee the maintenance of the temperature during blood specimens transportation (28); iii) consolidated paradigms ranging from filling of vacuum tubes to mixing procedures which appear unsupported by accurate experimental verification; e.g., all blood specimens collected in vacuum tube systems by venipuncture apparently do not need to be mixed (9). Moreover apparently incorrect vigorous mixing of the primary blood vacuum tubes does not promote laboratory variability (29); more so, no clinical impact has been observed in routine and specialized coagulation laboratory testing when the vacuum tubes are incompletely filled (when filled to more than 90% but less than 100%) (8).

In conclusion, for a long time the preanalytical phase has been known as the "dark side of the moon" (30-35). The minor modifications in procedure for collection of diagnostic blood specimens by venipuncture from CLSI H03-A6 document were able to reduce the tourniquet application time. Now the proposed new procedure for collection of diagnostic blood specimens by venipuncture should be strongly suggested for use by all quality laboratory managers and/or phlebotomy coordinators in their services in order to avoid preanalytical errors regard venous stasis and guarantee patient safety.

Potential conflict of interest

None declared.

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