A systematic approach towards the prevention of the Laboratory errors and the optimization of the preanalytical phase management

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GABRIEL DE SOUZA LIMA OLIVEIRA

Título: **"A systematic approach towards the prevention of the Laboratory errors and the optimization of the preanalytical phase management"**

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Curitiba, 31 de agosto de 2015.

To
Maria Tereza de Souza Lima
"my friend, mother, and grandmother"

“Always improving: today better than yesterday,
then tomorrow better than today”

Unknown

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Resumo

A fase (pré-) pré-analítica engloba todos os procedimentos antes do início dos testes laboratoriais. Esta etapa do processo é responsável pela maioria dos erros laboratoriais, uma vez que os procedimentos relacionados envolvem vários tipos de profissionais que trabalham fora do laboratório, portanto sem supervisão direta pelo gestor do laboratório. Portanto, é fundamental organizar e gerenciar corretamente o pessoal e os procedimentos relacionados à fase (pré-) pré-analítica. A presente tese está focada na prevenção dos erros laboratoriais e na otimização do gerenciamento da fase pré-análtica. A atividade de pesquisa e os dados sobre os quais a presente tese se baseia, envolveu vinte estudos (Artigos Originais) e uma revisão não-sistemática. Com base nos principais argumentos tratados, esta tese será descrita em três capítulos principais. O primeiro refere-se à preparação do paciente para análises laboratoriais clínicas no que diz respeito ao tempo de jejum. O segundo envolve o procedimento para a coleta de espécime diagnóstico por punção venosa de acordo com: desempenho de flebotomista, impacto de estase venosa na função de plaquetas, contaminação por EDTA e mistura de tubos primários. Finalmente, o terceiro capítulo enfoca a verificação de dispositivos de diagnóstico in vitro usados diariamente na fase (pré-) pré-analítica. Esta tese pode ser uma ferramenta de gerenciamento da fase (pré-) pré-analítica para evitar erros de laboratório, com o conceito de que resultados laboratoriais de espécimes diagnostico inapropriados são inconsistentes e não permitem o tratamento adequado nem o monitoramento do paciente.

Palavras-chave: Coleta de amostras de sangue; Técnicas de laboratório clínico; Segurança do paciente; Controle de qualidade; Gestão de qualidade total

Abstract

The (pre)pre-analytical phase encompasses all the procedures before the start of laboratory testing. This step of the whole testing process is responsible for the majority of the laboratory errors, since the related procedures involve many sorts of non-laboratory prfessionals working outside the laboratory setting, thus without direct supervision by the laboratory staff. Therefore, it is fundamental to correctly organize and manage both personnel and procedures regarding (pre)pre-analytical phase. The present thesis is focused on the prevention of the Laboratory errors and the optimization of the pre-analitycal phase management. The research activity and data on which the present thesis is based, has concerned twenty studies (Original Papers), and one non-systematic review. Based on the main arguments dealt with, this thesis will be described in three main chapters. The first one concerns the patient preparation for clinical laboratory analyses as regards fasting time. The second involves the procedure for diagnostic blood specimen collection by venipuncture according to: phlebotomist performace, impact of venous stasis on laboratory platelets, EDTA contamination, and mix of primary blood tubes. Finally, the third chapter focuses on the verification of *in vitro* diagnostic devices daily used on (pre-)preanalitycal phase. This thesis could be a (pre)pre-analytical management-tool to prevent laboratory errors, with the concept that laboratory results from inappropriate blood specimens are inconsistent and do not allow proper treatment nor monitoring of the patient.

Words-keys: Blood specimen collection; Clinical laboratory techniques; Patient safety; Quality control; Total quality management.

Abbreviations Used

ACU	automatic centrifuge unit
ADP	adenosine diphosphate
ADPtest	IVD test for evaluation of platelet aggregation from Roche
ADP HStest	IVD test for evaluation of platelet aggregation from Roche
ALB	albumin
ALP	alkaline phosphatase
ALT	alanine aminotranspherase
AMYL	Amylase
AMY-P	pancreatic amylase
aPTT	activated partial thromboplastin time
ASPI	IVD test for evaluation of platelet aggregation from Roche
AST	Aspartate aminotransferase
AT	antithrombin III
BASO	Basophils
BD	Becton, Dickinson and Company
BMI	body mass index
BUN	blood urea nitrogen
Ca	Calcium
CI	confidence interval
CK	creatine kinase
Cl	Chloride
CLSI	Clinical and Laboratory Standard Institute
COL	total cholesterol
COLtest	IVD test for evaluation of platelet aggregation from Roche
CRE	Creatinine
CRP	C-reactive protein
CVa	analytical coefficient of variability
DBil	direct bilirubin
DICQ	National System of Accreditation from the Brazilian Society of Clinical Analyses
DSI	desirable specification for imprecision
EDTA	Ethylenediaminetetraacetic acid
EFLM	European Federation of Clinical Chemistry and Laboratory Medicine
Eos	Eosinophils
ESR	erythrocyte sedimentation rate
Fe	Iron
FIB	Fibrinogen
GA	Georgia
GBO	Greiner Bio One
GGT	g-glutamyltransferase

h	hour (s)
Hb	haemoglobin
HCT	haematocrit
HDL cholesterol	high-density lipoprotein cholesterol
HI	the hemolysis index
ISO	International Organization for Standardization
IVD	*in vitro* diagnostic
K	potassium
K_2EDTA	dipotassium ethylenediaminetetraacetic acid
LDH	lactate dehydrogenase
LEDS	light-emitting diodes
LIP	Lipase
LUC	large unstained cells
LYMP	lymphocytes
MA	Massachusetts
MCV	mean corpuscular volume
MEA	multiple electrode aggregometry
Mg	magnesium
MONO	monocytes
MPA	MODULAR PREANALYTICALS EVO-MPA system
MPV	mean platelet volume
Na	Sodium
NEU	neutrophils
NJ	New Jersey
NY	New York
OPB	output buffer
P	phosphate
PC	protein C
PDW	platelet distribution width
PLT	platelets
PS	protein S
PT	prothrombin time
PTH	parathormon
PTS	pneumatic tube transport system
RBC	red blood cells
RDW	RBC distribution width
RETIC	reticulocytes
RISTO H	IVD test for evaluation of platelet aggregation from Roche
RISTO L	IVD test for evaluation of platelet aggregation from Roche
SD	standard deviation
TG	triglycerides
TBil	total bilirrubin
TnT	Troponin T

TP	total protein
TRAP	IVD test for evaluation of platelet aggregation from Roche
TSH	thyroid-stimulating hormone
UA	uric acid
USA	United States of America
VWF	von Willebrand's factor
WBC	white blood cells
WG-PRE	Working Group on Preanalytical Phase
WHO	World Health Organization

Preface

During my PhD program I have dealt with the (pre)pre-analytical issues both at University of Verona (Italy), and at Federal University of Parana (Brazil). The work group consists of Prof. Gian Cesare Guidi, and Prof. Geraldo Picheth, as advisors; and Prof. Giuseppe Lippi, Prof. Gian Luca Salvagno, Prof. Martina Montagnana, and Prof. Fabiane Rego, as collaborators. They helped me to identify the sources of error to guarantee the patient safety. I am very lucky because I am a young scientist and this very important work group has opened the doors to me. With this work group I learned, worked, and helped to improve the patient safety. If laboratories are vital, young scientist are essential to study new sources of laboratory errors thus ensuring the patient safety in order to improve the outcomes of the clinical laboratory.

General introduction

With the objective to guarantee patient safety, clinical laboratories are continuously improving their quality management system through certification and accreditation. Laboratory certification (i.e., by the ISO 9001:2008 standard) aims to assess conformity to a specific documented system without verifying the technical competence, whereas accreditation (i.e., by ISO 15189:2012 standard) is a process aimed at providing an independent appraisal and recognition — by expert laboratory professionals — of the specific competence of testing [1, 2]. Only a small number of laboratories worldwide are accredited [3], and some clinical directors prefer certification to the accreditation process when the laboratories are located inside a hospital. Nevertheless, the laboratory accreditation process is the best way to guarantee patient safety in laboratory diagnostics [4].

All laboratory tests are performed in a process – meaning a sequence of activities that each laboratory orderly and correctly performs in order to transform a given input into the desired output – that starts outside the laboratory with physician prescription and ends with delivery of laboratory report [5]. The laboratory activity is virtually partitioned into three phases, collectively referred as the notable "brain-to-brain" loop concept: (pre-)pre-analytical, analytical, and (post-)post-analytical phases [6].

The (pre-)pre-analytical phase encompasses all the procedures before the start of the laboratory analysis, and is responsible for the majority of the laboratory errors [7-9]. Presently, (pre-)pre-analytical phase is formally known as the dark side of the moon [10] because so many best practices that exist in this field are mostly ignored or neglected by healthcare players. Since, almost 70% of medical decisions depend on the results of laboratory analysis [11, 12], best laboratory practices should be followed in order to both prevent errors and guarantee patient safety.

Medical errors that jeopardize patient safety have attracted a great deal of public attention, especially when the situation demands the review of a large numbers of patient records, or a recall of patient laboratory test results, as an Italian case that recalled ~ 3,500 patients for parathormon (PTH) laboratory test [4]. It is already known that 40% of diagnostic error are linked with results from diagnostic services such as laboratory assays, or imaging (radiology) [8, 13].

The present thesis is focused on the prevention of the Laboratory errors and the optimization of the pre-analitycal phase management. A systematic approach is a process used to determine the viability of a procedure based on the experiential application of clearly defined and repeatable steps with an evaluation of the outcomes. Moreover, the goal of a systematic approach is to identify the most efficient means to generate consistent, optimum results. The research activity and data on which the present thesis is based involved twenty studies (Original Papers), and one non-systematic review referred by their Roman numerals in the text. For exposition purposes, this thesis will be described in three main chapters. The first one concerns the patient preparation for clinical laboratory analyses as regards fasting time.

The second involves the procedure for diagnostic blood specimen collection by venipuncture according to: phlebotomist performace, impact of venous stasis on laboratory platelets, EDTA contamination, and mix of primary blood tubes. Finally, the third chapter focuses on the verification of *in vitro* diagnostic devices daily used on (pre-)preanalitycal phase (Figure 1).

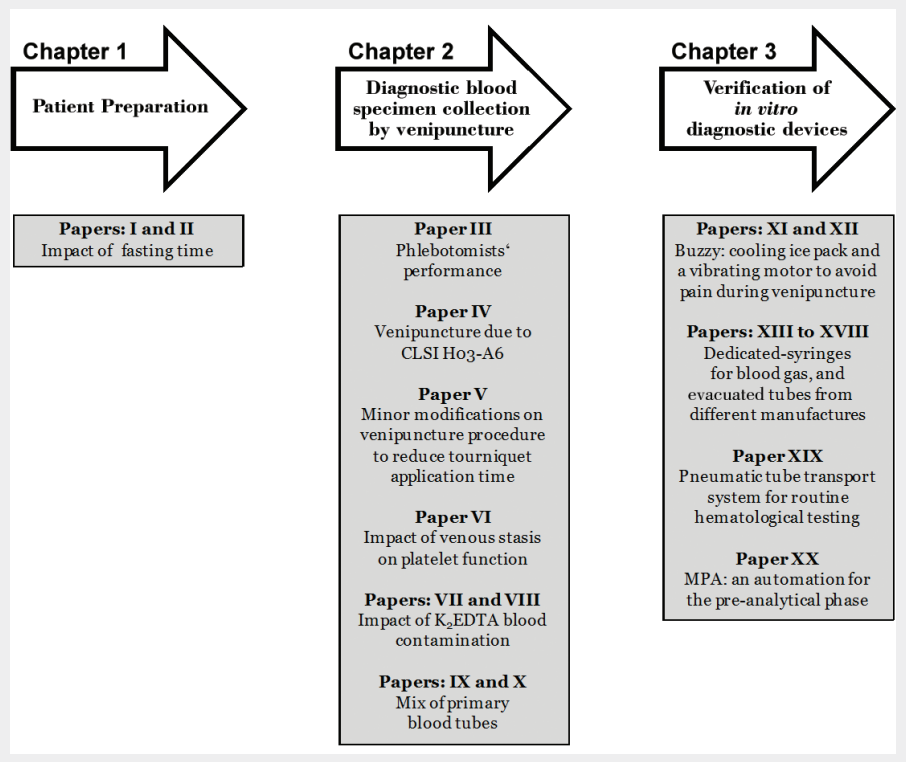

Figure 1. Prevention of the Laboratory errors, and the optimization of the pre-analitycal phase management

The twenty studies above were summarized in a non-systematic review (Paper XXI).

List of Original Papers

I. Lima-Oliveira G, Salvagno GL, Lippi G, Gelati M, Montagnana M, Danese E, Picheth G, Guidi GC. Influence of a regular, standardized meal on clinical chemistry analytes. Ann Lab Med. 2012;32(4):250-6.

II. Lima-Oliveira G, Salvagno GL, Lippi G, Danese E, Gelati M, Montagnana M, Picheth G, Guidi GC. Could light meal jeopardize laboratory coagulation tests? Biochem Med. 2014;24(3):343-9.

III. Lima-Oliveira G, Guidi GC, Salvagno GL, Montagnana M, Rego FGM, Lippi G, Picheth G. Is phlebotomy part of the dark side in the clinical laboratory struggle for quality? Lab Medicine 2012;43(5):172-176.

IV. Lima-Oliveira G, Lippi G, Salvagno GL, Montagnana M, Picheth G, Guidi GC. Impact of the phlebotomy training based on CLSI/NCCLS H03-a6 - procedures for the collection of diagnostic blood specimens by venipuncture. Biochem Med. 2012;22(3):342-51.

V. Lima-Oliveira G, Lippi G, Salvagno GL, Montagnana M, Picheth G, Guidi GC. The effective reduction of tourniquet application time after minor modification of the CLSI H03-A6 blood collection procedure. Biochem Med. 2013;23(3):308-15.

VI. Lima-Oliveira G, Lippi G, Salvagno GL, Gaino S, Poli G, Gelati M, Picheth G, Guidi GC. Venous stasis and whole blood platelet aggregometry: a question of data reliability and patient safety. Blood Coagul Fibrinolysis. 2015;26(6):665-8.

VII. Lima-Oliveira G, Salvagno GL, Danese E, Favaloro EJ, Guidi GC, Lippi G. Sodium citrate blood contamination by K_2-ethylenediaminetetraacetic acid (EDTA): impact on routine coagulation testing. Int J Lab Hematol. 2015;37(3):403-9.

VIII. Lima-Oliveira G, Salvagno GL, Danese E, Brocco G, Guidi GC, Lippi G. Contamination of lithium heparin blood by K_2-ethylenediaminetetraacetic acid (EDTA): an experimental evaluation. Biochem Med. 2014;24(3):359-67.

IX. Lima-Oliveira G, Lippi G, Salvagno GL, Montagnana M, Gelati M, Volanski W, Boritiza KC, Picheth G, Guidi GC. Effects of vigorous mixing of blood vacuum tubes on laboratory test results. Clin Biochem. 2013;46(3):250-4.

X. Lima-Oliveira G, Lippi G, Salvagno GL, Brocco G, Gaino S, Dima F, Volaski W, Rego FG, Picheth G, Guidi GC. Processing of diagnostic blood specimens: is it really necessary to mix primary blood tubes after collection with evacuated tube system? Biopreserv Biobank. 2014;12(1):53-9.

XI. Lima-Oliveira G, Lippi G, Salvagno GL, Montagnana M, Picheth G, Guidi GC. Quality impact on diagnostic blood specimen collection using a new device to relieve venipuncture pain. Indian J Clin Biochem. 2013;28(3):235-41.

XII. Lima-Oliveira G, Lippi G, Salvagno GL, Campelo MD, Tajra KS, dos Santos Gomes F, Valentim CD, Romano SJ, Picheth G, Guidi GC. A new device to relieve venipuncture pain can affect haematology test results. Blood Transfus. 2014;12 Suppl 1:s6-10.

XIII. Lima-Oliveira G, Salvagno GL, Lippi G, Brocco G, Voi M, Montagnana M, Picheth G, Guidi GC. Quality management of preanalytical phase: impact of lithium heparin vacuum tubes changes on clinical chemistry tests. Accred Qual Assur 2013;18(5):429-434.

XIV. Lima-Oliveira G, Lippi G, Salvagno GL, Montagnana M, Picheth G, Guidi GC. Preanalytical management: serum vacuum tubes validation for routine clinical chemistry. Biochem Med. 2012;22(2):180-6.

XV. Lima-Oliveira G, Lippi G, Salvagno GL, Montagnana M, Picheth G, Guidi GC. Sodium citrate vacuum tubes validation: preventing preanalytical variability in routine coagulation testing. Blood Coagul Fibrinolysis. 2013;24(3):252-5.

XVI. Lima-Oliveira G, Lippi G, Salvagno GL, Montagnana M, Poli G, Solero GP, Picheth G, Guidi GC. Brand of dipotassium EDTA vacuum tube as a new source of pre-analytical variability in routine haematology testing. Br J Biomed Sci. 2013;70(1):6-9.

XVII. Lima-Oliveira G, Lippi G, Salvagno GL, Montagnana M, Poli G, Solero GP, Picheth G, Guidi GC. K(3)EDTA Vacuum Tubes Validation for Routine Hematological Testing. ISRN Hematol. 2012;2012:875357.

XVIII. Lima-Oliveira G, Lippi G, Salvagno GL, Montagnana M, Picheth G, Guidi GC. Different manufacturers of syringes: a new source of variability in blood gas, acid-base balance and related laboratory test? Clin Biochem. 2012;45(9):683-7.

XIX. Lima-Oliveira G, Lippi G, Salvagno GL, Dima F, Brocco G, Picheth G, Guidi GC. Management of preanalytical phase for routine hematological testing: is the pneumatic tube system a source of laboratory variability or an important facility tool? Int J Lab Hematol. 2014;36(4):e37-40.

XX. Lima-Oliveira G, Lippi G, Salvagno GL, Danese E, Montagnana M, Brocco G, Voi M, Picheth G, Guidi GC. Does laboratory automation for the preanalytical phase improve data quality? J Lab Autom. 2013;18(5):375-81.

XXI. Lima-Oliveira G, Volanski W, Lippi G, Picheth G, Guidi GC. Pre-analytical phase management: a review of the procedures from patient preparation to laboratory analysis. Scand J Clin Lab Invest. 2017;77(3):153-163.

General aspects due to methods

Diagnostic blood samples collected were processed following both the Clinical and Laboratory Standard Institute (CLSI) H18-A4 and the CLSI H21-A5 standards (Papers I, II, VI-XIX). Briefly, serum tubes were left upright at room temperature (20 °C, more than 30 min, and less than 2 h; standardized by study) to allow complete blood clotting before centrifugation; whereas plasma samples (sodium citrate, or lithium heparin) were promptly centrifuged. Details regarding evacuated tubes used, centrifuge force, and time of centrifugation are shown on Table 1.

Table 1. Information regarding evacuated tubes used, centrifuge force, and time of centrifugation due to brand specifications'

Tubes			Centrifugation		Papers
Brand	Volume (mL)	Additive	*g* force	Time (min)	
Terumo	3.5	52.5 USP U of lithium heparin and gel separator	1200	10	I, X, XX
	3.6	0.4 buffered sodium citrate (9NC) 0.109 mol/L: 3.2 W/V%	1500	15	II, IX, X
	3.5	clot activator and gel separator	1500	10	IX
BD	2.7	0.109 M buffered sodium citrate	1500	10	VII
	6.0	102 I.U. of lithium-heparin	1300	10	VIII
	5.0	clot activator and acrylic gel separator (SST II)	1500	10	XI
GBO	10.0	plan tube without additive	2000	10	II

After centrifugation, serum-samples were separated in aliquots. Because shortening of activated partial thromboplastin time (aPTT) in thawed plasma-samples may ensue, due to residual platelet debris in the thawed plasma-samples, a re-centrifugation was done and the platelet poor plasma (PPP) samples were then separed in aliquots. All aliquots from each study (Original Paper) were stored and kept frozen at -70 °C until measurement. To assay the biomarkers in a single analytical run, all aliquots frozen at -70 °C from each study (Original Paper), were thawed in water bath at 37 °C at the same time; and incubated at room temperature for 1 h. Afterwards the samples were handled as fresh ones.

Information from laboratory instrumentations used, and tests performed by each study (Original Paper) are shown Table 2. Moreover, the statistical tests to assess differences between either samples or procedures are shown in Table 3.

Table 2. Information of laboratory instrumentations used, and tests performed by each study

Instruments	Original Paper	Painel of Tests
		Coagulation
ACL TOP 700	VII, IX, and XV	aPTT, PT, and FIB
	II	aPTT, PT, FIB, AT, PC, and PS
	X	aPTT, PT, FIB, AT, PC, PS, and HI
Multiplate	VI	ADP, ADP HS, ASPI, COL, RISTO H, RISTO L, and TRAP
		Immunochemistry
Cobas 6000	XIII	GLU, COL, HDL, TG, TP, ALB, CRE, UREA, UA, ALP, AMYL, AST, ALT, GGT, LDH, CK, TBil, DBil, P, CA, MG, FE, NA, K, and TRANSFERRIN
	XIV	GLU, COL, HDL, TG, TP, ALB, CRE, UREA, UA, ALP, AMYL, AST, ALT, GGT, LDH, CK, TBil, DBil, P, CA, MG, FE, NA, and K
	XX	COL, HDL, TG, TP, ALB, CRE, UREA, UA, ALP, AMYL, AMY-P, LIP, AST, ALT, GGT, LDH, CRP, CK, TBil, DBil, P, CA, MG, FE, NA, K , CL, and HI
	IX	GLU, COL, HDL, TG, TP, ALB, CRE, UREA, UA, ALP, AMYL, LIP, AST, ALT, GGT, LDH, CK, TBil, DBil, P, CA, MG, FE, NA, K, CL, CORTISOL, INSULIN, TSH, T3, T4, PTH, and HI
	XI	GLU, COL, HDL, TG, TP, ALB, CRE, UREA, UA, ALP, AMYL, LIP, AST, ALT, GGT, LDH, CRP, CK, TBil, P, CA, MG, FE, NA, K, CL, TRANSFERRIN, CORTISOL, INSULIN, TSH, T3, FT3, T4, FT4, and HI
Cobas b 221	XVIII	pH, pO2, sO2, pCO2, cHCO3, ctCO2, ctO2, BE, tHb, CA, NA, K, GLU, LAC, O2Hb, and p50
		Hematology
XE-2100D	IX	RBC, HCT, HGB, MCV, MCHC, RDW, WBC, LYMP, EOS, MONO, NEU, BASO, LUC, PLT, and MPV
	X	RBC, HCT, HGB, MCV, RDW, WBC, LYMP, EOS, MONO, NEU, BASO, LUC,PLT, MPV, and RETIC
	XII	RBC, HCT, HGB, MCV, MCHC, RDW, WBC, LYMP, EOS, MONO, NEU, BASO, PLT, and MPV
Advia 2120i	XVI and XVII	RBC, HCT, HGB, MCV, MCHC, RDW, WBC, LYMP, EOS, MONO, NEU, BASO, LUC, PLT, MPV, and PDW
	XIX	RBC, HCT, HGB, MCV, MCHC, RDW, WBC, LYMP, EOS, MONO, NEU, BASO, LUC, PLT, MPV, PDW, and RETIC
TEST 1 YDL	X and XIX	ESR

ACL TOP 700 (Instrumentation Laboratory, Milan, Italy); Multiplate, cobas 6000, and cobas b 221 (Roche Diagnostics GmbH, Penzberg, Germany); XE-2100D (Sysmex Corporation®, Kobe, Japan); Advia 2120i (Siemens Healthcare Diagnostics, Deerfield IL, USA); TEST 1 YDL (Alifax, Padova, Italy).

Table 3. Statistical analyses performed on each study

Original Paper	Statistical Description	Significance was set at
I, IX, XI, XVII	D'Agostino-Pearson omnibus test, Student paired t-test, Wilcoxon ranked-pairs test, and mean % difference were assayed. Biases were compared with the current desirable quality specifications for bias, derived from biological variation.	P <0.05
II	Wilcoxon ranked-pairs test, reference change value (RCV), and mean % difference were assayed. Only mean % differences higher than RCV were considered clinically significant. Desirable specification for imprecision (DSI) derived from biologic variation was used as criteria of acceptance in analytical interference testing.	P <0.05
III	Fisher exact two-tailed test, and Student paired t-test were applied.	P <0.05
IV, V	Kolmogorov-Smirnov test, Student paired t-test, Fisher exact two-tailed test, and McNemar Chi-square test were assayed.	P <0.05
VI	Wilcoxon ranked-pairs test, Bland and Altman plot, and mean % difference were assayed.	P <0.05
VII	Kolmogorov–Smirnov test, Student paired t-test, Bland and Altman plots were applied. Bland and Altman results were compared with the current desirable quality specifications for bias derived from biological variation.	P <0.05
VIII	Wilcoxon ranked-pairs test, and Bland and Altman plots were applied. Bland and Altman results were compared with the current desirable quality specifications for bias derived from biological variation.	P <0.05
X	D'Agostino-Pearson omnibus test, Student paired t-test, Wilcoxon ranked-pairs test, Bonferroni correction for multiple comparisons, and mean % difference were assayed. Biases were compared with the current desirable quality specifications for bias, derived from biological variation.	P <0.017
XII	D'Agostino-Pearson omnibus test, Student paired t-test, Wilcoxon ranked-pairs test and, mean % difference were assayed. Biases were compared with the current desirable quality specifications for bias, derived from biological variation.	P <0.001
XIII, XIV, XV	D'Agostino-Pearson omnibus test, RM ANOVA, Student paired t-test, Friedman test, Wilcoxon ranked-pairs test, Bonferroni correction for multiple comparisons, and mean % difference were assayed. Biases were compared with the current desirable quality specifications for bias, derived from biological variation.	P <0.005
XVI, XVIII	D'Agostino-Pearson omnibus test, RM ANOVA, Student paired t-test, Friedman test, Wilcoxon ranked-pairs test, and mean % difference were assayed. Biases were compared with the current desirable quality specifications for bias, derived from biological variation.	P <0.05
XIX	D'Agostino-Pearson omnibus test, Student paired t-test, Wilcoxon ranked-pairs test, and mean % difference were assayed. Biases were compared with the current desirable quality specifications for bias, derived from biological variation.	P <0.01
XX	D'Agostino-Pearson omnibus test, Student paired t-test, Wilcoxon ranked-pairs test, Bland and Altman, and mean % difference were assayed. Biases were compared with the current desirable quality specifications for bias, derived from biological variation.	P <0.05

Chapter 1

Patient preparation: fasting time

Overview

The CLSI H3-A6 standard (presently renamed to CLSI GP41-A6) contains specific information regarding patient posture during blood collection by venipuncture, stating that "specimens should be drawn with the patient seated comfortably in an appropriate chair or lying down" [14]. The World Health Organization (WHO) guidelines on drawing blood contain a different indication, as follows: "make the patient comfortable in a supine position (if possible)" [15]. Consequently, healthcare professionals often assume that these two postural positions – either supine or sitting – could be interchangeable and that the shift from one posture to the other may not generate significant bias on laboratory testing. It is noteworthy that inpatients' blood samples can be drawn at different moments and postures, e.g., in sitting position during the day, and in supine position during the night. On the other hand, outpatients (i.e., patients attending for blood collection sites) usually do not maintain a definite and/or stable position before venipuncture. Consequently, samples are frequently drawn from subjects who indifferently walked or remained standing for long time, or remained seated for short time – e.g., seated less than three minutes at phlebotomy room – before blood collection.

Lippi *et al.* demonstrated that postural change during venous blood collection impacts on coagulation tests, and is a major source of bias in routine clinical chemistry determinations [16, 17]. Briefly, the venous pressure in the lower parts of the body increases after a prolonged standing position, thus generating an enhancement of capillary pressure which ultimately leads to ultrafiltration of plasma in the interstitial space. Accordingly, larger and non-diffusible biomarkers remain entrapped within the blood vessels, whereas smaller and filterable elements migrate along with water into the interstitial space [18]. Hence to these evidences, patient posture during venous blood sampling must be uniformed to a reference position, either sitting or supine. Irrespective of the chosen criterion, a recommendation should be given that a 15–20 min minimum period of resting in reference position should be observed before collecting diagnostic blood samples [16, 17]. From an organizational perspective, the resting period (15-20 min) could seriously impact on the workflow at blood collection sites – but this can be obviated by appropriately organizing and/or scheduling the flow of the patients. Contradicting the above evidences, the meaningless bias observed by comparing Troponin T (TnT) values in supine and standing positions also suggests that it may be unnecessary to allow the patient rest for 15-20 min in sitting or supine position, before venipuncture upon admission to the emergency room, thus saving valuable time for diagnosis or rule out of acute myocardial infarction by TnT [19].

Regarding fasting time, an important framework for the harmonization of definitions for fasting requirements for laboratory tests was published by the Working Group on Preanalytical Phase (WG-PRE) of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) [20], this

document highlighted that: i) specimens for all blood tests should be drawn from 7 to 9 am; ii) fasting time for all blood tests should be 12 h; and iii) "no sample is better than a bad sample" should always be the leading principle for laboratory professionals.

Since the degree of bias due to non-fasting state depends on many different mechanisms – rate of food absorption, patient metabolism and type of food – [21] experiments were planned aiming to evaluate whether a light meal (i.e. breakfast) could jeopardize laboratory tests. These experiments were published in Papers I and II.

Methods

The study population consisted of 17 healthy volunteers (8 women and 9 men; mean age ± standard deviation: 29 ± 4 years), who were recruited among the laboratory personnel (Papers I and II). A single expert phlebotomist carried out the collection of diagnostic blood specimens. All volunteers were maintained seated for 15 min prior to phlebotomy in order to eliminate possible interferences of blood distribution due to different postures [16, 17]. After this interval, a vein was located on the forearm by using a subcutaneous tissue transilluminator device (Venoscópio IV plus; Duan do Brasil, Brazil) for preventing venous stasis interference due to tourniquet application (see Chaper 2) [22-24]. All blood samples (Table 1) were collected using a 20 gauge straight needle (Terumo Europe NV, Leuven, Belgium); to eliminate any possible interference due to either the contact phase or tissue factor, about 2 mL of blood were preliminarily collected in a discard tube without additive (Vacuette®; Greiner Bio-One GmbH, Kremsmünster, Austria). The first blood sample was collected between 8:00 and 8:30 a.m., after an overnight fast. Immediately after blood collection, the volunteers consumed a light meal (breakfast), containing standardized amounts of carbohydrates, protein, and lipids. The meal was based on commercial food purchased at a shop to represent a usual breakfast. Each volunteer ate one slice of cheese, one yogurt, two slices of bread, one chocolate snack, and one fruit juice, as previously described by Lippi *et al.* [25]. The exact composition of the meal is shown in Table 4. Subsequent blood samples were collected at 1, 2, and 4 h after the end of the meal.

Table 4. Nutritional composition of the light meal

Nutritional composition	Slice of cheese	Yorgut	Slice of bread	Chocolat snack	Fruit juice	Total
Number (overall weight)	1 (25 g)	1 (125 g)	2 (46.8 g)	1 (20.7 g)	1 (200g)	417.6g
Kcal	64	134	126	105	134	563
KJ	266	562	532	438	572	2370
Protein (g)	4.4	4.1	4.2	1.1	0.8	14.6
Carbohydrate(g)	0.8	19.4	22	12.7	32	86.9
Sugar (g)	0.8	N/A	3	10	N/A	13.8
Total lipids (g)	4.6	4.4	2.4	5.5	0	16.9
Saturated lipids (g)	3.1	N/A	0.8	3.7	0	7.6
Fibre (g)	0	N/A	0.9	0.2	2	3.1
Sodium (g)	0.3	N/A	0.286	0.02	0	0.606
Calcium (g)	0.133	0.131	N/A	N/A	N/A	0.264
Vitamin C (g)	N/A	N/A	N/A	N/A	0.024	0.024

Kcal, kilocalorie; KJ, kilojoules; N/A, not available
This table was previously published by *Annals of Laboratory Medicine* (ISSN 2234-3814) and *Blood Transfusion* (ISSN 1723-2007)

Routine clinical biochemistry (Paper I), and coagulation (Paper II) tests were performed in duplicate, according to the manufacturers' specifications, and using proprietary reagents. Both instruments (cobas 6000, and ACL TOP 700) were calibrated against appropriate proprietary reference standard material, and verified with the use of proprietary quality controls. Results of laboratory tests assayed from blood specimens collected before- and after-meal were compared. Details due to statistical tests applied, instrument used to assay biomarkers, and panel of tests evaluated are shown in Tables 2 and 3.

Results and Discussion

The breakfast with 14.4 g of protein (Table 4) increased significantly the abumin after 4 h (Figure 2-A); whereas, after 2 h of a light meal, serum levels of total bilirubin were significantly decreased as compared to the fasting state (Figure 2-B). Phisiologically, feeding stimulates albumin synthesis and this event might improve the storage of essential amino acids [26-29]. Moreover, a fasting state increases hepatic uptake of non-esterified fatty acids and interferes with the hepatic clearance of bilirubin, thus contributing to unconjugated hyperbilirubinemia of fasting [30]. Furthermore, insulin secretion after a meal induces significant changes in ions [31-35] daily measured at clinical laboratories (Figure 2 -C,-D,-E and -F). Regarding coagulation testing, a significantly higher % activity of AT was observed at 1 h, and 4 h after the ingestion of the

light meal as compared to baseline specimen [113 (104-117) and 111 (107-120) *vs.* 109 (102-118), respectively; P = 0.029 and P = 0.016]. Two hours after the ingestion of the meal, aPTT was found significantly lower than baseline samples [32.0 (29.9-34.8) *vs.* 34.1 (32.2-35.2), respectively; P = 0.041].

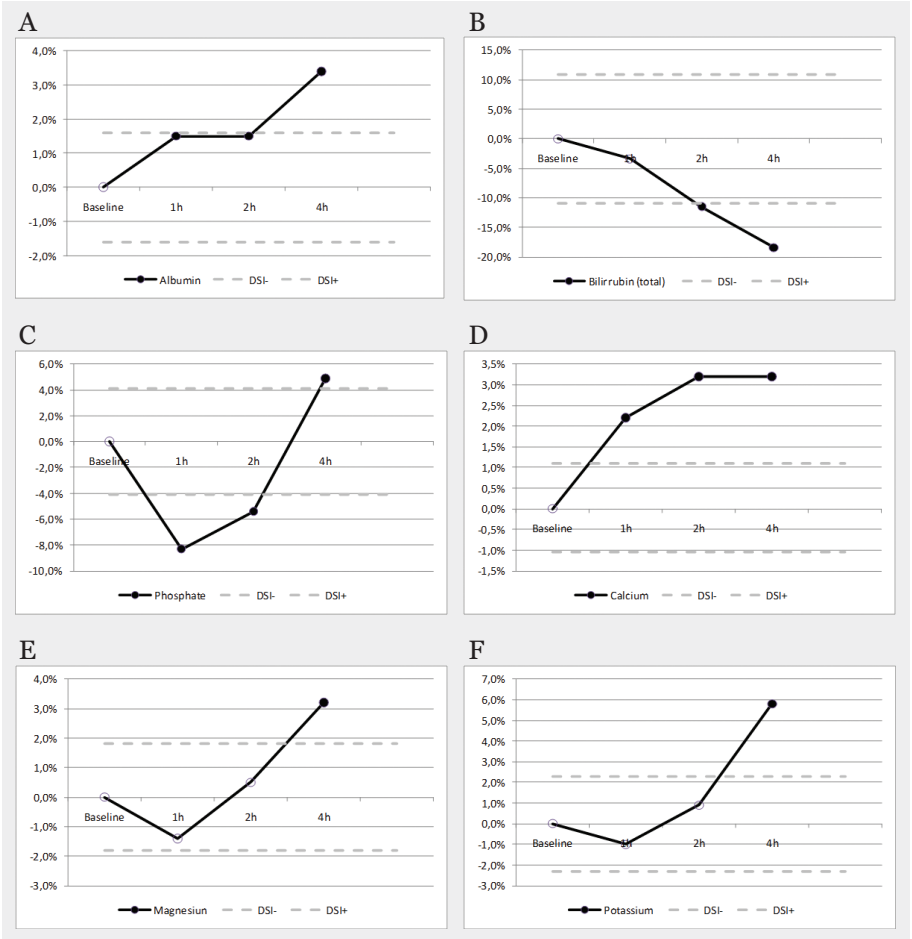

Figura 2. Continued

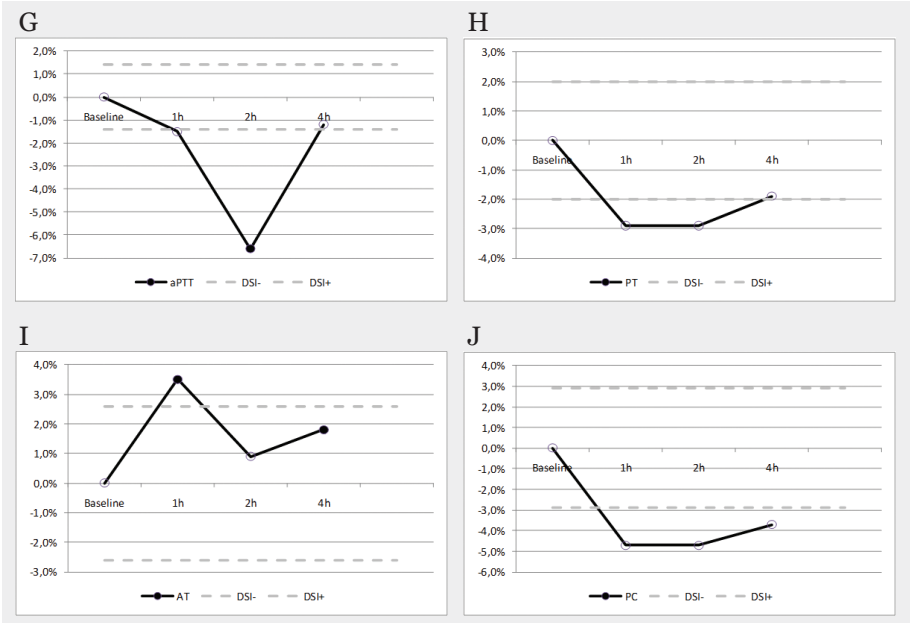

Figure 2. Observed bias for some common laboratory parameters in healthy volunteers after breakfast

Hours after breakfast (x-axis) are plotted against bias values (y-axis). Solid line – bias. Dashed lines --- acceptable criteria based on desirable specification for imprecision (DSI) derived from biologic variation [36]. Solid symbols (•) indicate statistically significance difference ($P<0.05$) when compared with baseline. All interferograms were constructed from results previously published on Papers I and II.

Dasheets from Instrumentation Laboratory reagents notify that ACL TOP Family System (Instrumentation Laboratory, Milan, Italy) are not affected by triglycerides up to: 11.3 mmol/L for both aPTT and PT, 9.9 mmol/L for FIB, 26.0 mmol/L for AT, 10.1 mmol/L for PC, and 17.0 mmol/L for PS. However, the Figure 2 -G, -H, -I and -J had shown that the influence of lipemia was seriously underestimated, meaning that the measured bias at declared triglycerides concentration could be higher than reported by the manufacturer for aPTT, PT, AT and PC. Actually, the concentrations of triglycerides in samples tested were lower than 2.0 mmol/L (Paper II). Moreover, Nikolac *et al.* properly proposed that manufacturers should use evidence based quality specifications for assessing the allowable biases, instead of arbitrary limits [37].

Based on the evidences described above, I suggest laboratory quality managers to standardize the fasting time at 12 h for all blood tests; since fasting time at 4 h as tested was not enough to elimininate the variability caused by lipemia. Moreover, this kind of standardization could prevent possible laboratory variability that was not evaluated by this study (i.e., more caloric food intake as lunch or dinner) and unpredictable analytical bias (Papers I

and II). Indeed, if fasting time is avoided – for complete blood count, albumin, bilirubin, phosphate, calcium, magnesium, and potassium – more risks than benefits can derive for patients [21]. However, in particular situations it is not possible to draw a blood specimen under optimal conditions (i.e., fasting 12 h), since outpatients attending to emergency room often are in a postprandial state – period immediately after the meal – due to singular eating habits, and easy access to food (specially in developed countries) [20, 21]. Besides, patients at afternoon consultation with general practitioner commonly are in after meal time too (e.g., fasting 4 h) – Figure 3 summarizes variability on laboratory tests owing to food intake 4 h before blood sample collection. In this case, a screening question might be asked to patients in order to both reduce bias and assist interpretation of results, particularly for patients admitted to emergency department, i.e., "What time was your last food intake?". This information could be taken into account for interpretation of results, and during patient follow-up at emergency department.

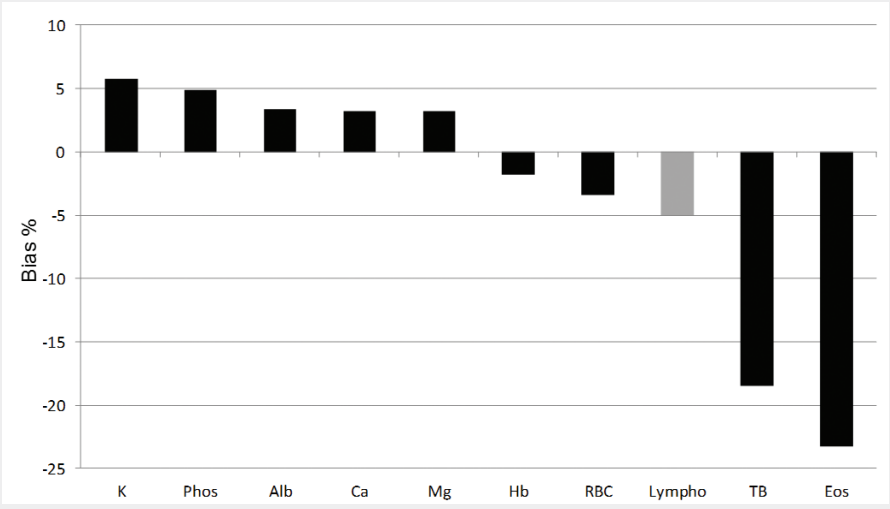

Figure 3. Variability on laboratory tests due to food intake 4 h before blood collection by venipuncture

Variability is expressed as difference (bias, %) from 4 h fasting (during day) compared to the 12 h fasting (overnight). This figure was constructed from results previously published by Paper I, and Lippi *et al.* [27]

• bias higher than desirable specification derived from biologic variation

• bias lower than desirable specification derived from biologic variation

K, potassium; Phos, phosphate; Alb, albumin; Ca, calcium; Mg, magnesium; Hb, haemoglobin; RBC, red blood cells; Lympho, lymphocytes; TB, total bilirubin; Eos, eosinophils.

Chapter 2

Diagnostic blood specimen collection by venipuncture

Overview

Diagnostic blood samples collected by phlebotomy are the most common type of biological specimens drawn and sent to clinical laboratory facilities for being analyzed, thus supporting caring physicians in patient diagnosis, follow-up and/or therapeutic monitoring. Moreover, both diagnosis, management and treatment of patients can be compromised by poor quality of phlebotomy [38]. Furthermore, phlebotomy is the most important activity in the extra-analytical phase [39]. However, procedures involving phlebotomy are poorly studied as regards the major sources of laboratory errors [23].

Since 1977, the CLSI has recognized the need to put significant attention toward the pre-examination components of laboratory testing, including the correct collection of blood specimens by venipuncture – the detailed procedure is shown in Table 5 [14].

Due to phlebotomy procedure both observational and experimental studies were performed (in this PhD thesis) aiming to:

- evaluate the performance of phlebotomists, to identify the major sources of errors during diagnostic blood specimen collection (Paper III);
- verify the compliance with CLSI documents by clinical laboratories from South America, and to assess whether taught phlebotomists are following the exact procedure for blood collection by venipuncture due to CLSI H03-A6 document (Paper IV);
- verify if minor modification of the procedure for collection of diagnostic blood specimens by venipuncture from CLSI H03-A6 document is able to reduce the tourniquet application time (Paper V);
- evaluate the impact of venous stasis on platelet function assessed by multiple electrode aggregometry (Paper VI);
- assess the impact of sodium citrate- and lithium-heparin-blood samples contaminated with different amounts of dipotassium ethylenediaminetetraacetic acid (K_2EDTA blood) on routine coagulation (Paper VII), and clinical chemistry (Paper VIII) laboratory tests;
- evaluate the effect of tubes mixing (gentle vs. vigorous) on diagnostic blood specimens collected in vacuum tube systems by venipuncture (Paper IX); and
- evaluate whether it is really necessary to mix the primary blood tubes immediately after blood collection by evacuated tube systems (Paper X).

Table 5. Procedures for the collection of diagnostic blood specimens by venipuncture from CLSI H03-A6 document

Steps	Procedures	Importance of the procedures
i	prepare accession order	to guarantee patient identity assurance [40-43]
ii	approach and indentify the patient; sanitize hands	
iii	verify the patients' fasting status or diet restrictions, as appropriate, and	fasting status is an important source of variability [25, 44, 45]
	inquire if the patient has a latex sensitivity; select appropriate gloves and tourniquet	to prevent allergic reaction and/or anaphylactic shock attributed to latex allergy [46-48]
iv	assemble necessary supplies and select appropriate tubes according to the requests	to prevent errors in clinical laboratory induced by supplies and additives such as anticoagulants and clot activators [49-52]
v	position the patient	to eliminate possible interferences of blood distribution due to different posture [53]
vi	apply the tourniquet, select the venipuncture site and vein	see Table 7 and Figure 7
vii	put on gloves	preventing phlebotomists' exposure to potentially infectious blood pathogens [54, 55]
viii	cleanse the venipuncture site and allow to dry	cleaning prevents infection by skin microorganisms, waiting for drying prevents hemolysis [56, 57]
ix	perform venipuncture; once blood flow begins, request the patient to open his/her hand	see Table 7 and Figure 7
x	fill tubes using the correct order of draw	to prevent errors by cross contamination between additives [58-61]
xi	release and remove the tourniquet	see Table 7 and Figure 7
xii	place the gauze pad over the puncture site	safe feature for preventing phlebotomists' exposure to potential infections by bloodbome pathogens [54, 55]
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	applying pressure to the site is a efficient prevention of bruise [62]
xv	label the tubes and record the time of collection; some facilities also specify phlebotomist identification on the tubes	to reduce missing identication and guarantee the traceability of the process [2, 40, 41, 43]
xvi	observe special handling requirements (if any required)	to guarantee diagnostic blood specimens stability [63-65]
xvii	send properly labeled blood collection tubes to the appropriate laboratories	

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Methods

Performance of phlebotomists

The checklist (proposed in my Master Degree dissertation, Table 6) [66] was followed to evaluate the performance of phlebotomists during diagnostic blood specimen collection by venipuncture (Paper III), regarding: i) time of tourniquet application; ii) inappropriate requests to patients to clench their fist repeatedly; iii) either excessive or aggressive disinfection of the forearm by the phlebotomist, which can induce venous stasis; iv) the order of drawn of evacuated tubes during specimen collection; and v) the mix of primary blood tubes (with either anticoagulant or clot-activator) in conformity with manufacturer datasheet. Moreover, this checklist allowed to correlate source of laboratory errors with improper phlebotomy procedures witnessed; such as increased potassium concentration in the specimen due to fist clenching by the patient.

The phlebotomist performances were analyzed only during procedures involving blood collection in evacuated tubes containing additives (i.e., clot activator, sodium citrate, ethylenediaminetetraacetic acid [EDTA], heparin, or sodium fluoride). To standardize the approach, and to reduce the 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), not undergoing chemotherapy, or catheterization, and neither 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 venipunctures; the time of tourniquet application was measured with a calibrated chronometer. The time interval between tourniquet application and removal were recorded in seconds. Moreover, the laboratory quality managers were the only laboratory personnel informed about this research; the phlebotomists were unaware that data were being collected.

Table 6. Checklist to assess the performance of phlebotomists during diagnostic blood specimen collection by venipuncture

Procedure	Verification	
1) Tourniquet application time	Patient I	_____seconds
	Patient II	_____seconds
	Patient III	_____seconds
	Patient IV	_____seconds
	Patient V	_____seconds
2) Did the phlebotomist inappropriately request the patient to clench the fist repeatedly?	1 Yes ( )	2 No ( )
3) Did the phlebotomist make the friction movement of the forearm, during the cleaning of the venipuncture site, to induce venous stasis?	1 Yes ( )	2 No ( )
4) Did the phlebotomist use the correct sequence of vacuum tubes during blood collection?	1 Yes ( )	2 No ( )
5) What was the sequence of tubes used by the phlebotomist?*	( ) sodium citrate [#] ( ) sodium fluoride [#] ( ) EDTA [#] ( ) clot activator and gel separator [#] ( ) the phlebotomist does not have a standardized sequence; the tubes are randomly inserted into the vacuum collection system.	
6) Did the phlebotomist mix the diagnostic blood specimens?	1 Yes ( )	2 No ( )

* This item is evaluated only if the answer to procedure 4 was "no".
[#] Enumerate the order of the sequence used.
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Survey

To verify the use of CLSI standards (Paper IV) in South America, a survey was sent by mail to 3674 laboratories, with the five questions following:

i) Do you use standardized operating procedures in all your laboratory activities?

ii) If yes, what steps of your laboratory process are based on CLSI guidelines? #

( ) pre-analytical ( ) analytical ( ) post-analytical

( ) my processes are not based in CLSI guidelines

If you marked "my processes are not based in CLSI guidelines", then specify where your procedures are based. If you had checked the above preanalytical option, do you currently employ the CLSI H03-A6 document [14] to standardize your procedures for blood collection by venipuncture?

iii) If yes, do your phlebotomists perform the blood collection by venipuncture following the exact *venipuncture procedure* from page 5 item 8 of CLSI H03-A6 document [14]?

iv) If not, what did you change in this procedure? And why did you change this procedure?

Phlebotomy training program

After analyzing the survey results, thirty phlebotomists from São Paulo state, Brazil, (previously evaluated on Paper III) were invited to be trained according to CLSI H03-A6 standard aiming to eliminate phlebotomy errors (Paper IV). These professionals had 5 (4.8-5.6) years of experience in diagnostic blood specimen collection by venipuncture. The above professionals were operational at institutions where approximately 200 blood collections by venipuncture are performed per work-day. Each phlebotomist was trained individually to perform exactly the venipuncture procedure from CLSI H03-A6 document – page 5 item 8 [14]. The phlebotomy-training program was realized during 8 h where the importance of each step of the procedure was explained (Table 5). Only one external/expert auditor from DICQ [67] trained all phlebotomists in one month. DICQ is a National System of Accreditation from the Brazilian Society of Clinical Analyses. This accreditation system is based on ISO 15189:2007 [68]. After the training, all phlebotomists were monitored for twenty work-days to guarantee the assimilation of the correct procedures for the collection of diagnostic blood specimen, in conformity with the CLSI H03-A6 document (Table 5). Only after this period of time, the phlebotomists were re-evaluated, using the same checklist (Table 6) and method described above. Moreover, laboratory quality managers consider this period of time

sufficient for incorporating new procedures.

A second training (Paper V) was executed for the same thirty phlebotomists (previously appraised on both Paper III and Paper IV). Each phlebotomist was retrained individually to perform the new venipuncture procedure proposed in Paper IV (Table 7). The phlebotomy-training program was delivered over 8 h, during which the importance of each step of the updated procedure was clearly explained. After training, all phlebotomists were monitored and appraised with the same methodology, and checklist described above.

Table 7. The most important proposed changes to venipuncture procedure

	Steps from CLSI H03-A6	Changes proposed (Paper IV)
vi	Apply the tourniquet and select the venipuncture site and vein	Put on gloves
vii	Put on gloves	Cleanse the venipuncture site
viii	Cleanse the venipuncture site and allow to dry	Request the patient to just close his/her hand (never request the patient to "pump")
ix	Perform venipuncture; once blood flow begins, request the patient to open his/her hand	Apply the tourniquet and select the venipuncture site and vein
x	Fill tubes using the correct order of draw	Perform venipuncture; once blood flow begins, request the patient to open his/her hand Also release and remove the tourniquet
xi	Release and remove the tourniquet	Fill tubes using the correct order of draw

The steps identification (Roman numbers) from the original CLSI H03-A6 standard (presently replaced by the CLSI GP41-A6 document) [14].
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Impact of venous stasis on platelet function

The study population consisted of 20 healthy volunteers (11 women and 9 men; mean age ± SD: 29 ± 4 years), who were recruited among our laboratory personnel (i.e., 74 individuals). The inclusion criteria were based on the anamnestic exclusion of blood coagulation disorders, platelets dysfunction, diabetes, dyslipidemias, thalassemia syndromes and other hemoglobinopathies; as regards women, that they had regular menstrual cycles and were not using hormonal contraceptives (Paper VI). None of the volunteers took any medication. BMI was less than 30.0 kg/m^2 in all volunteers. The collection of diagnostic blood specimen was performed by a single, expert phlebotomist, following the previously published protocols [22-24]. Briefly, all volunteers after 12 h fasting were maintained seated for 15 min to

eliminate interferences due to posture [16, 17]. After this time frame, 6 mL of blood was collected by venipuncture with a 20 G straight needle (Terumo Europe, Leuven, Belgium) directly into two 3 mL Hirudin blood tubes for Multiplate analysis following two different procedures:

Procedure 1 (no stasis) – a radial vein was localized on the right forearm by a subcutaneous tissue transilluminator device (Venoscopio IV plus, Duan do Brasil, Sao Paulo, Brazil) without tourniquet, to prevent any interference from venous stasis [22-24];

Procedure 2 (stasis) – an antecubital vein was localized on the left forearm by tourniquet application during 60 s prior to venipuncture.

To eliminate any potential interference due to either the contact phase or the tissue factor, a first 2 mL discard tube was drawn from each volunteer for both procedures. All samples were processed for the assessment of platelet function by multiple electrode aggregometry (MEA) after collection (<15 min) on the same Multiplate instrument equipped with electronic pipette, and using proprietary software by Multiplate. The instrument had been previously calibrated by the manufacturer (during production). The calibration maintenance was contextually verified with the use of proprietary quality controls (liquid control set, ref 06675999 190, Roche Diagnostics GmbH).

Principle: in the Multiplate Test Cell, activated platelets adhere to, and aggregate on, the sensor wires. This leads to an increased resistance between the sensor wires, which is continuously recorded and expressed via the area under the curve in arbitrary units (i.e., AU*min or U). Approximately 8 AU correspond to 1 Ω[69]. The panel of tests included ADP-test (without prostaglandin E1), ADP HS-test (with prostaglandin E1), ASPI-test, COL-test, RISTO H-test (high concentration, 0.77 mg/mL), RISTO L-test (low concentration, 0.20 mg/mL), and TRAP-test (Table 8).

Table 8. Multiplate® portfolio to assay platelet function by MEA	
Tests	Description
ADP	ADP induced platelet activation sensitive to clopidogrel, prasugrel and other ADP receptor antagonists
ASPI	Cyclooxygenase dependent aggregation (using arachidonic acid) sensitive to Aspirin®, NSAIDs and other inhibitors of platelet cyclooxygenase
COL	Collagen induced aggregation
RISTO	Von Willebrand factor (vWF) and glycoprotein Ib (GpIb) dependent aggregation using ristocetin
TRAP	Platelet stimulation via the thrombin receptor using a protease-activated receptor 1 agonist (TRAP-6), sensitive to IIb/IIIa receptor antagonists
Prostaglandin E1 reagent	For the assessment of ADPtest HS (high sensitivity). For the assessment of positive (i.e. abnormal) controls of the ADPtest

Ethylenediaminetetraacetic acid (EDTA) contamination and order of drawn

Fifteen healthy volunteers, were enrolled among the laboratory personnel to assess the impact of sodium citrate- and lithium heparin-blood samples contaminated with different amounts of dipotassium ethylenediaminetetraacetic acid (K_2EDTA blood) on routine coagulation (Paper VII), and clinical chemistry (Paper VIII) laboratory tests. The blood was drawn at rest following the procedure proposed at Paper IV (Table 7), after overnight fast, by an experienced phlebotomist. The tubes were filled up to their nominal volume (Table 1), and all phases of sample collection were standardized.

Five citrate tubes of each subject were pooled to obtain 10.8 mL of sodium citrate-anticoagulated blood (Paper VII), and two lithium-heparin tubes of each subject were pooled to obtain 12 mL of heparinised blood (Paper VIII), which were then divided in 5 aliquots of 2 mL each. The whole blood of the K_2EDTA tube was then added in scalar amounts to the autologous blood aliquots, to obtain different degrees of K_2EDTA blood contamination (Table 9).

Table 9. Volumes used to obtain different degrees of K_2EDTA blood volume contamination

	Aliquots				
Samples	A	B	C	D	E
Sodium citrate (Paper VII)	2 mL	2 mL	2 mL	2 mL	2 mL
Litium heparin (Paper VIII)	2 mL	2 mL	2 mL	2 mL	2 mL
K_2EDTA*	-	0.1 mL (5%)	0.3 mL (13%)	0.8 mL (29%)	1.5 mL (43%)
Concentration (mg/dL)*	0.00	0.09	0.24	0.51	0.77

*percentage values regard the proportionality of autologous blood (volume/volume). EDTA concentration was estimated from the preceding EDTA-containing tube. It was predicted taking into account a mass concentration of 1.8 mg of K_2EDTA per 1 mL of blood, as declared by BD®.

The aliquots were then mixed by gentle inversion and centrifuged at room temperature. All parameters were measured in one single analytical run according to manufacturer's specifications (Table 2); and using proprietary reagents with the same lot number. The analyzers were also previously calibrated against appropriate proprietary reference standard material and verified by third-party internal quality control.

Mixing of primary blood tubes

Blood samples were collected for routine coagulation, immunochemistry, and hematological testing to evaluate both the effect of tubes mix – gentle vs. vigorous – (from 100 volunteers, Paper IX), and whether it is really necessary to mix the primary blood tubes immediately after blood collection (from 300 volunteers, Paper X) by evacuated tube system.

Paper IX – Immediately after the venipuncture all evacuated tubes (one from each additive type) were processed through two different procedures:

Procedure 1 — Gold Standard [63, 65]: all diagnostic blood specimens collected in tubes with K_2EDTA or sodium-citrate were gently inverted five times, as recommended by the manufacturer [70, 71]. Specimens collected in evacuated tubes with clot activator and gel separator (serum tubes) were gently inverted ten times, since the manufacturer recommends to gently invert each tube several times to maximize the contact between blood and silica (clot activator) after blood collection [72].

Procedure 2 — Vigorous mixing: immediately after collection all blood specimens were shaken up vigorously during 3–5 seconds, independently on the additive type inside the evacuated tubes.

Paper X – All evacuated tubes were processed due to three different procedures:

Procedure 1 – Gold Standard: all specimens were mixed gently and carefully by inverting five times as recommended, then properly centrifuged (Figure 4A);

Procedure 2 – Rest time: all specimens remained 5 min in the upright position, followed by gentle careful mixing by inverting five times, then properly centrifuged (Figure 4-B);

Procedure 3 – No mix: all specimens were left in upright position without mixing afterwards, then properly centrifuged (Figure 4-C).

Details from both evacuated tubes, and instruments used to assay biomarkers for Papers IX and X were shown in Tables 1 and 2.

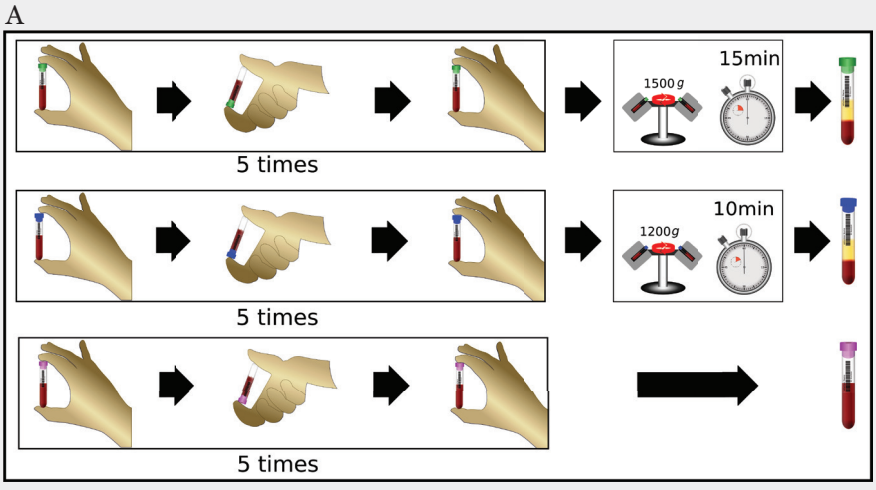

Figure 4. Continued

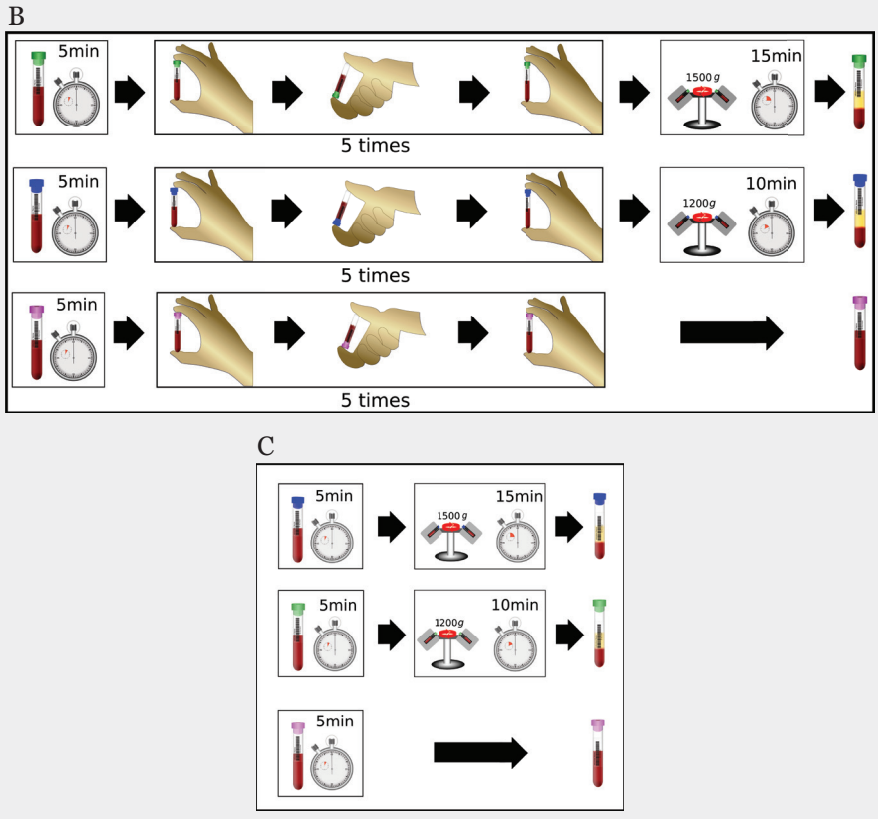

Figure 4. Processing of evacuated tubes

Color of stopper means additives as follow: blue, sodium-citrate; green, lithium-heparin; and purple, EDTA.

Results and Discussion

The answers from 2781 laboratories (Paper IV) were received throughout the study period (i.e., 60 days), that is 76% of the total previously predicted (Figure 5-A). The results of the survey are shown in Figure 5-B. Briefly, the survey shows that CLSI documents are widely used in South America since 2622 (94%) from 2781 laboratories appear compliant with these documents to standardize their procedures.

A

Figure 5. Representativeness of CLSI documents in South America

Figure 5-A: Geographic distribution of evaluated laboratories by survey.

All evaluated countries are shown textured. The absolute number represents the group of laboratories evaluated by countries.

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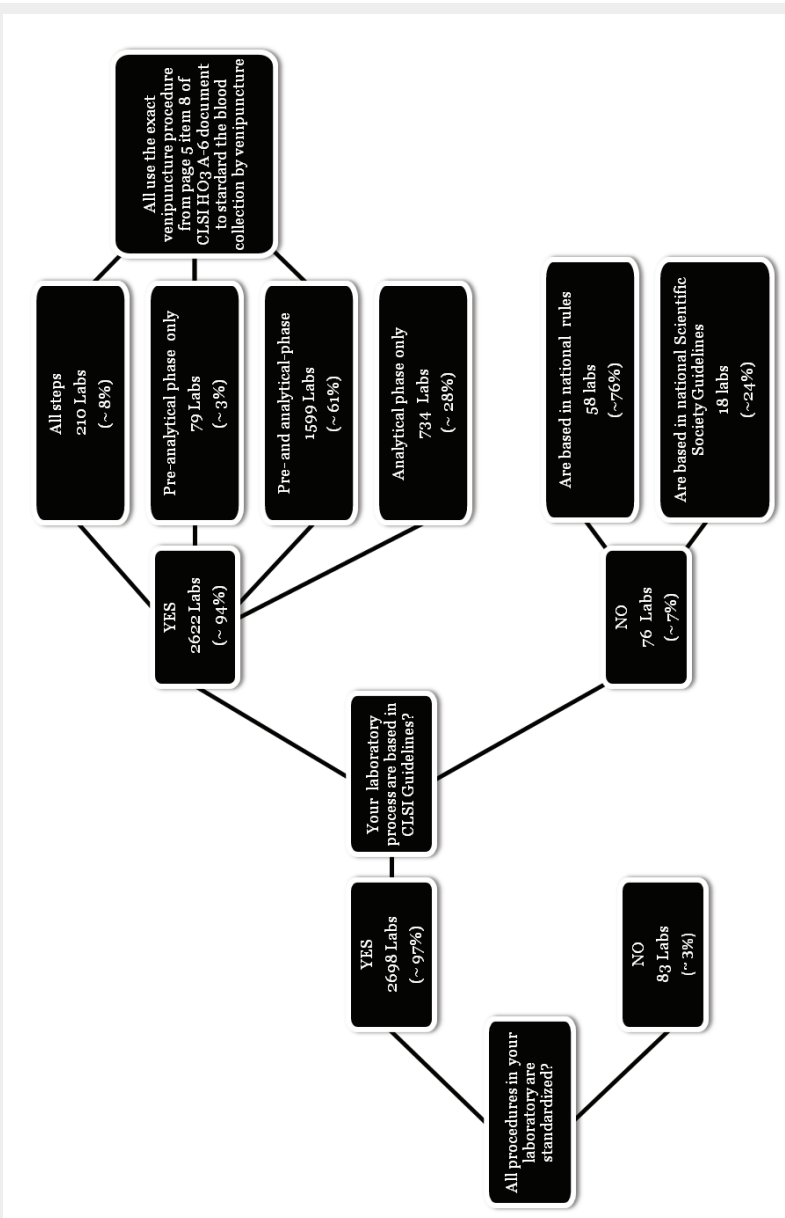

Figure 5-B. Survey results
Labs, laboratories.
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The major nonconformities observed during phlebotomies are summarized in Table 9. The first phlebotomists' evaluation (Paper III) had shown that the overall rate of errors was over 60%. There were no significant differences observed between public and private laboratories except for adequate mix of primary blood tubes, for which private laboratories had fewer errors (P = 0.04).

Phlebotomists after training according to CLSI H03-A6 (Paper IV) completely eliminated several nonconformities, including: i) incorrect friction on the forearm during the cleaning of the venipuncture site (e.g., this procedure induce venous stasis, making easy the vein location); ii) incorrect order of draw; and iii) inadequate mix of primary blood tubes (i.e., with either anticoagulants, or clot activator). Moreover, all phlebotomists inappropriately requested the patient to clench the fist repeatedly (i.e., more than twice) (Table 10).

Regarding tourniquet time (Figure 6), the overall means were 84.4 s (Paper III), 118 s (Paper IV), and 30 s (Paper V). Persistently, private laboratories applied the tourniquet for significantly shorter time than public laboratories: 69.9 s vs. 98.9 s, $P < .001$ (Paper III); 87 s vs. 148 s, $P < 0.001$ (Paper IV); and 28 s vs. 32, $P = 0.002$ (Paper V).

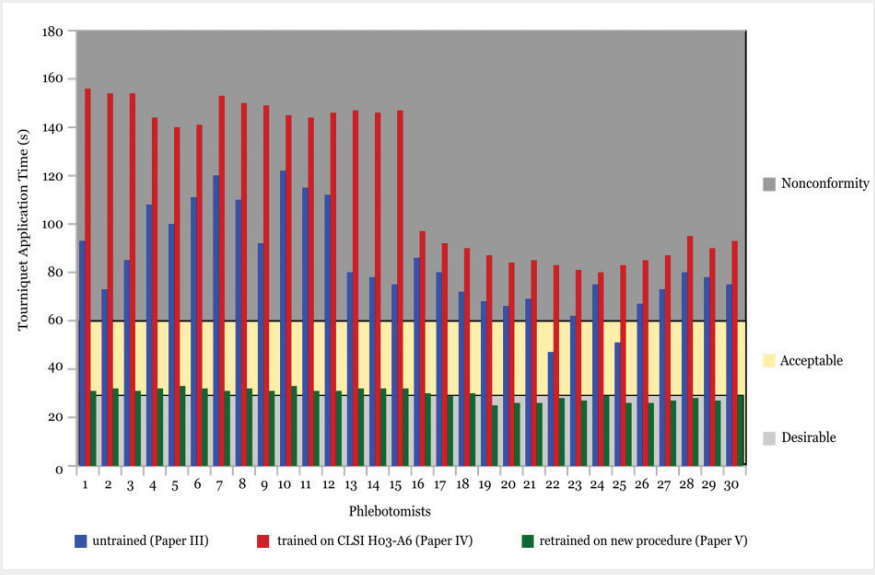

Figure 6. Assessment of tourniquet time application during diagnostic blood specimen collection by venipuncture

Tourniquet application time in seconds from 30 different phlebotomists. The performance of phlebotomists due to tourniquet application time is shown in three different colors: in blue phlebotomists without specific train (Paper III), in red phlebotomists after being trained on CLSI H03-A6 procedure (Paper IV), and in green the phlebotomists' performance after training on new procedure that improved the steps sequence from CLSI H03-A6 document (Paper V). Tourniquet time were classified: higher than 60 s, as nonconformity; between 30 s and 60 s, as acceptable; and less than 30 s, as desirable.

Table 10. Improvement of phlebotomy error rates pre- and post- training on CLSI H03-A6 and proposed new procedure

Error description	Phlebotomists without specific training (Paper III)				Phlebotomists after being trained on CLSI H03-A6 procedure (Paper IV)				Phlebotomists after training on new procedure (Paper V)			
	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 veni-puncture site	27/30	13/15	14/15	1.000	0/30**	0/15	0/15	---	0/30	0/15	0/15	---
Incorrect order of draw	26/30	13/15	12/15	1.000	0/30**	0/15	0/15	---	0/30	0/15	0/15	---
Incorrect mixing of evacuated 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). ---, not calculated.
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These evidences mirror that the venipuncture procedure from CLSI H03-A6 document page 5 item 8 [14] induces venous stasis due to tourniquet application time > than 60 s. Briefly, venous stasis by tourniquet application (> than 60 s) promotes the outflow of water, diffusible ions and low molecular weight substances from the blood vessels, thus increasing the concentration of biomarkers at the venipuncture site (Figure 7) [73]. This source of laboratory variability has been extensively investigated in the past decade [74-76]. Sixty seconds of venous stasis are *per se* able to significantly modify (i.e., $P < 0.05$) both routine clinical biochemistry, haematology, and coagulation tests [22-24]. Moreover, tourniquet application during 60 s generates bias higher than sought by quality specifications derived from biological variation for: i) total protein (2.8% vs. 1.2%, respectively, P= 0.001); ii) albumin (2.4% vs. 1.3%, respectively, P= 0.001); iii) potassium (6.5% vs. 1.8%, respectively, P=0.001), and iv) calcium (1.3% vs. 0.8%, respectively, P=0.001) [24].

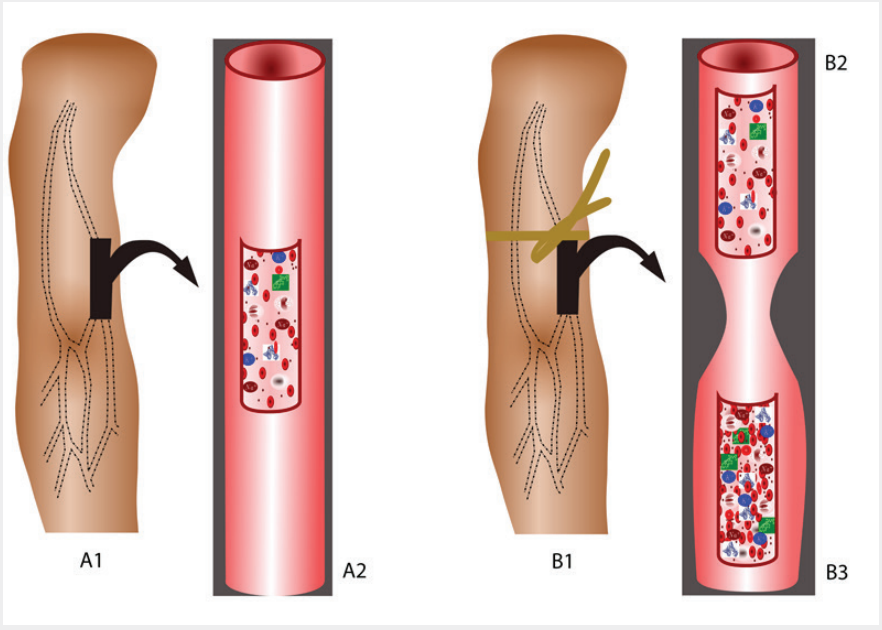

Figure 7. Venous stasis due to tourniquet application

A1. Schematic upper limb without tourniquet application; A2. Schematic blood vessels environment at physiological condition. B1. Schematic upper limb after tourniquet application; B2. Schematic blood vessels over tourniquet application; B3. Schematic blood vessels at venous stasis condition due to tourniquet application by more than one minute.

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A device able to minimize the variability induced by venous stasis was validated (i.e., Venoscopio from Duan®, a transilluminator device) [22-24]. The

transilluminator device is essentially based on cold near infrared light-emitting diodes (LEDs), whose light is absorbed by intra-erythrocyte haemoglobin flowing along the veins. Interestingly, Weiss and Goldman had proposed the transillumination for mapping veins to be cannulated prior to ambulatory phlebotomy, because this practice would allow a more accurate visualization of the vein course [77]. Presently IVD companies that provide evacuated tubes and needles, also provide transilluminator systems. Becton, Dickinson and Company is exclusive partner of AccuVein (Huntington, NY) in 30 countries [78], whereas Greiner Bio-One GmbH (Kremsmünster, Austria) is the official distributor of VeinViewer (Christie Medical Holdings, Inc., Memphis, Tennessee) in Brazil and Italy [79]. Since transilluminator is not broadly used in phlebotomy services in South America (mainly due to the incremental cost), a critical evaluation of international adherence to the phlebotomy procedure described in the CLSI H03-A6 standard was carried out (Paper IV), showing that the minor modifications proposed to the standard were effective to decrease tourniquet application time to less than 35 s and 30 s in public- and private-laboratories, respectively (Paper V), thus substantially contributing to reducing the bias generated by venous stasis. Presently, the proposed new procedure for collection of diagnostic blood specimens by venipuncture (Table 7) should be strongly suggested for use by all quality laboratory managers and/or phlebotomy coordinators in their services, in order to avoid preanalytical errors due to venous stasis. Moreover, Bölenius *et al.* [80] used the hemolysis index (HI) to assess the efficiency of a large-scale 2 h educational intervention. The authors concluded 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 both the national and local venous blood specimen collection guidelines, that is similar to international standards (CLSI H03-A6 document) [80]. This kind of training program should instead be strongly recommended and performed worldwide. Furthermore, previous investigations had shown that educational program, and technological interventions for phlebotomists are relevant and promote decrease of error rates – consequently resulting in quality improvement [81-84]. Maybe Bölenius *et al.* [80] found only minor effects because the procedure from CLSI H03-A6 document increase the tourniquet application time.

As regards the impact of venous stasis on MEA (Paper VI), a difference was observed between the responses of platelets collected under- and without-stasis using both ADP (a weak platelet agonist) [85] and low-dose of ristocetin (Table 11). Briefly, ristocetin is an antibiotic known to induce thrombocytopenia and platelet agglutination. In the presence of ristocetin platelets bind to von Willebrand's factor (VWF) through glycoprotein Ib (GPIb) receptors. *In vitro*, ristocetin forms complexes with VWF molecules, which bind to GPIb and trigger platelet activation and aggregation. Reduced or lack of agglutination in the presence of ristocetin – by RISTO-test for Multiplate

– is attributed to absence or reduction of VWF, or to lack or deficit of platelet GPIb receptors, such as in Bernard–Soulier syndrome [86]. RISTO-test is a sensitive assay for diagnosing both Bernard–Soulier syndrome and severe von Willebrand's disease. Moreover, Table 10 shows that platelet agregation due to RISTO L-test is significantly reduced by approximately 15% after 1 min of venous stasis. A possible explanation of such a subtle, although significant, reduction might be attributable to local flow disruption caused by venous stasis (Figure 7) on one hand, and the consequently enhanced proteolysis by ADAMTS13 on the largest and more reactive VWF molecules on the other [87].

Table 11. Impact of venous stasis on Multiplat® e tests

Test	Procedure		Mean % difference	Bland–Altman		P value
	Stasis	No stasis		Mean bias	95 % CI	
ADP	62.5 [48.2–75.0]	67.0 [53.8–78.2]	-7.2	3.75	-5.15–12.6	**0.040**
ADP HS	61.0 [34.2–72.0]	61.5 [40.5–73.8]	-0.8	0.38	-14.5–15.3	0.888
ASPI	83.5 [74.0–93.0]	86.5 [73.8–89.5]	-3.6	-0.38	-16.0–15.3	0.889
COL	72.0 [60.0–88.8]	74.0 [60.5–82.2]	-2.8	0.75	-14.8–16.4	0.944
RISTO L	9.50 [3.88–18.4]	12.2 [6.12–19.8]	-28.4	1.75	-1.07–4.57	**0.015**
RISTO H	130 [122–154]	128 [116–162]	1.5	2.31	-20.9–25.5	0.853
TRAP	92.5 [75.0–95.0]	91.5 [80.5–106.5]	1.1	6.12	-7.51–19.8	0.031

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ADP-test and ADP HS-test on Multiplate has been developed for evaluating platelet activation via platelet ADP receptors. The ADP stored in the dense granules of platelets is released during activation, a phenomenon accompanied by shape change. The released ADP further activates adhered platelets in addition to activating local free circulating platelets by binding to purinergic receptors – P2Y1 and P2Y12 on platelet membranes. When platelets are activated, the individual components of the glycoprotein IIb/IIIa receptors on platelet membrane physically alter their conformation, thus producing the high-affinity fibrinogen-binding site glycoprotein IIb/IIIa. Fibrinogen, both freely circulating and released from alpha granules, subsequently binds to the glycoprotein IIb/IIIa receptors forming platelet-to-platelet bridges resulting in platelet aggregation. Thus, the effect of 1 min venous stasis on ADP-test (without-prostaglandin E1) is consistent with a significant reduction

of platelet sensitivity to ADP agonist. Significantly, platelets collected without stasis were statistically more responsive to ADP and low-dose of ristocetin than platelets collected after stasis (ADP-test and RISTOL-test, Table 11). Moreover, no differences between stasis vs. no stasis in platelet responses to ADP were apparent if prostaglandin E1 was added to activated platelets (ADP HS-test). Furthermore, the two groups of platelets (stasis, and no stasis) were less responsive to ADP in the presence of prostaglandin E1 than in its absence (Table 11), as was expected. A likely reason for the dampening effects of prostaglandin E1 on platelet responses to ADP is the well known ability of prostaglandin E1 to dampen platelet responses to all platelet agonists [88]. Consequently – the results suggest that – tourniquet use by 60 s during blood specimens collection could induce inappropriate changes in antiplatelet/anticoagulant therapy, as a consequence of biased aggregation data. Moreover, the recommendation from CLSI GP41-A6 document (formerly CLSI H03-A6) [14] regarding tourniquet time application – anyhow not to be extended over 60 s – is not enough to assure the quality of aggregation data by Multiplate, and thus patient safety. As such, the tourniquet should be avoided when assessing platelet function by MEA.

EDTA salt (e.g. K_2- or K_3-EDTA) is an anticoagulant widely used, especially for hematological testing or to stabilize the whole blood for the plasma assessment of both unstable and fragile molecules (i.e., cytokines, peptides and cardiac biomarkers) [89]. The current blood collection guidelines incorporate the so-called 'order of drawn' originally proposed by Calan and Cooper [61] to reduce the risk of cross-contamination of additives (i.e., EDTA) from a previously filled tube (Figure 8). The suggested sequence entails 1st blood culture bottle or tube without additive; 2nd sodium citrate tube; 3rd serum tube with or without clot activator and with or without gel separator; 4th heparin tube with or without gel separator and/or dedicated-syringe with heparin; 5th EDTA tube with or without gel separator; and 6th glycolytic inhibitor tube [14].

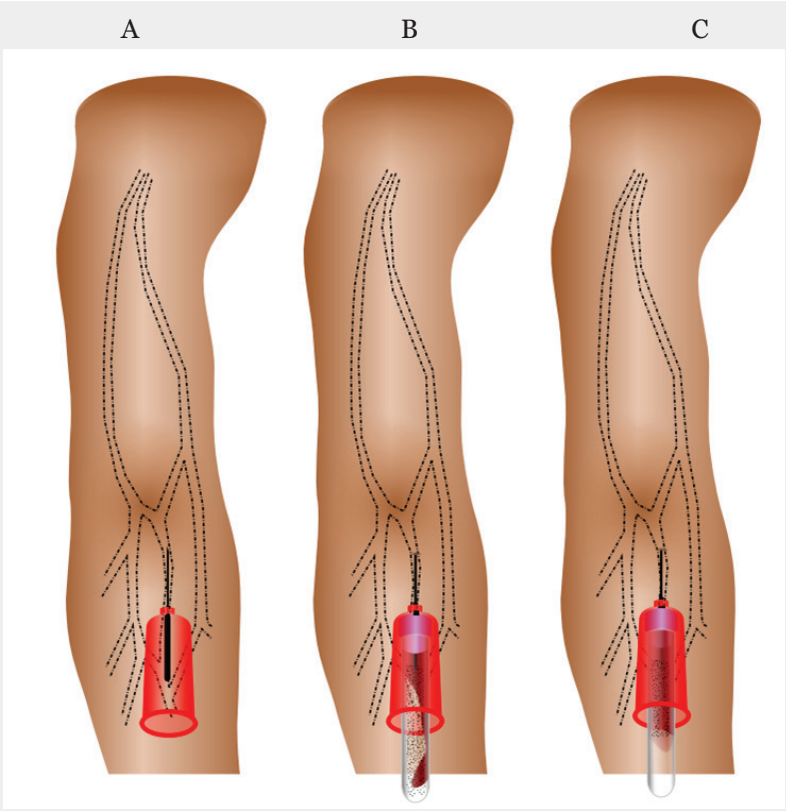

Figure 8. Theoretical principle of EDTA carryover during blood collection using evacuated tube system

A. Schematic multi-sample blood collection needle connected to holder placed on venipuncture site at upper limb. Briefly the multi-sample blood collection needle show two different serial needles: one “external” to insert into the vein and other on the opposite, covered by rubber to fill the evacuated tubes. The rubber sheath allows multiple tubes of blood to be collected and covers the needle in between tube changes, so blood lacks contact with holder.

B. Schematic blood collection by venipuncture at begin – external needle is into median cubical vein and EDTA evacuated tube was inserted into the holder. The EDTA salt (spray-dried) on the internal-walls of the evacuated tube is both dissolving (solubilization process), mixing and stabilizing by the blood collected, due to blood turbulence generated by the standardized negative pressure inside evacuated tubes.

C. Schematic blood collection by venipuncture at the end – EDTA evacuated tube was full filled till the nominal volume, consequently the internal needle directly contacted the blood collected with EDTA salt due to evacuated tube orientation (e.g., with the stopper placed on a lower plane than the bottom, between 15° and 30°), thus exposing the following tube in the sequence of draw to a theoretical risk of additive carryover.

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Experimentally K_2EDTA blood volume contamination had shown that the free carboxylic acid form of EDTA – after K_2EDTA dissociation – strongly chelates divalent metal ion as Ca^{++} and Mg^{++} on lithium heparin blood tubes (impacting on clinical chemistry determination, Paper VIII). Moreover, K_2EDTA blood volume contamination on sodium citrate blood tubes may hence jeopardize coagulation testing (Paper VII). Statistically and clinically significant prolongation was observed for both aPTT and PT between 29% and 43% of K_2EDTA contamination, whereas the decrease of fibrinogen values became statistically and clinically significant at 43% of K_2EDTA contamination. Moreover, significant variations starting from 5% of K_2EDTA contamination were observed for: calcium, chloride, iron, LD, magnesium (all decreased) and potassium (increased). The variation of phosphate and sodium (both increased) were significant after 13% and 29% of K_2EDTA contamination, respectively (Figure 9). Furthermore, the presence of K_2EDTA or K_3EDTA in either serum or plasma specimens may mask true cases of hypokalaemia or hypercalcaemia, whereas true cases of hypokalaemia may be misjudged as significant hyperkalaemias [90]. Cornes *et al.* performed a multicentre observational study and showed that spurious hyperkalaemia due to EDTA contamination is relatively frequent. This evidence led the authors to suggest an education program to: i) correct blood collection technique; and ii) prevent EDTA sample contamination [91]. Afterwards, the same team of authors evaluated a series of blood collections – blood were drawn by evacuated tube system, sequentially into a serum tube, followed by a EDTA tube, and followed by another serum tube – where potassium EDTA contamination was not evidenced [92]. Similar data were published in a subsequent study, demonstrating that serum tubes collected after either a K_2EDTA or a sodium citrate blood tube do not show a clinically meaningful interference on potassium or calcium assessment [93]. At variance with this conclusion, a case report, where a dedicated-syringe for blood gas analyses was contaminated by EDTA evacuated tube and lead to inaccurate results of both potassium and calcium was published [94]. Possibly the EDTA carryover during blood collection could be correlated with either phlebotomy practice or evacuated tubes producers', since each trademark provided different results on routine hematological testing (i.e., complete blood count, see Chapter 3 as regards Papers XVI and XVII). Since evidences regarding EDTA contamination were rather consistently different, the need to strictly follow the order of drawn to guarantee patient safety is questioned. As a reliable approach to prevent that unreliable data are generated, the measurement of EDTA in hyperkalaemic, hypocalcaemic and hypomagnesaemic samples may be advisable in order to exclude EDTA salt contamination. Indeed, the assessment of EDTA in serum is inexpensive and can be easily implemented in the vast majority of automated analyzers [95].

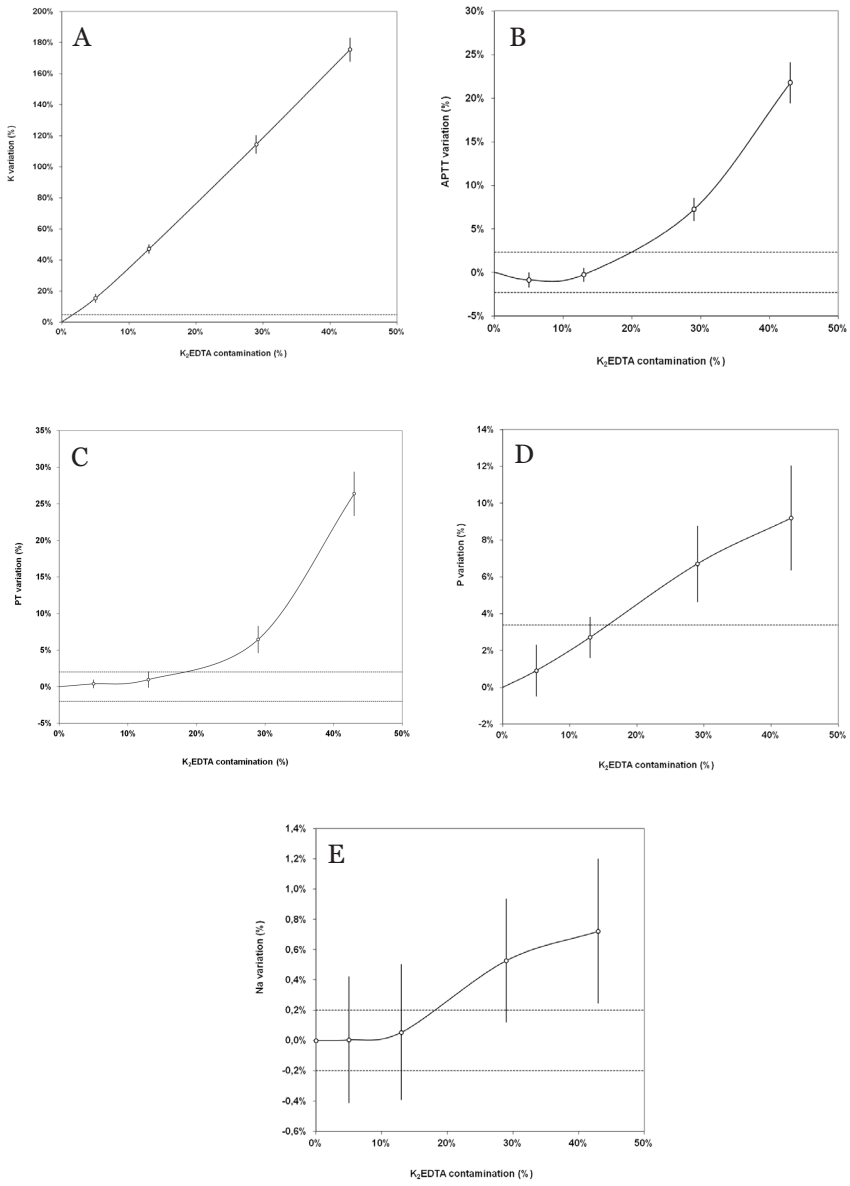

Figure 9. Continued

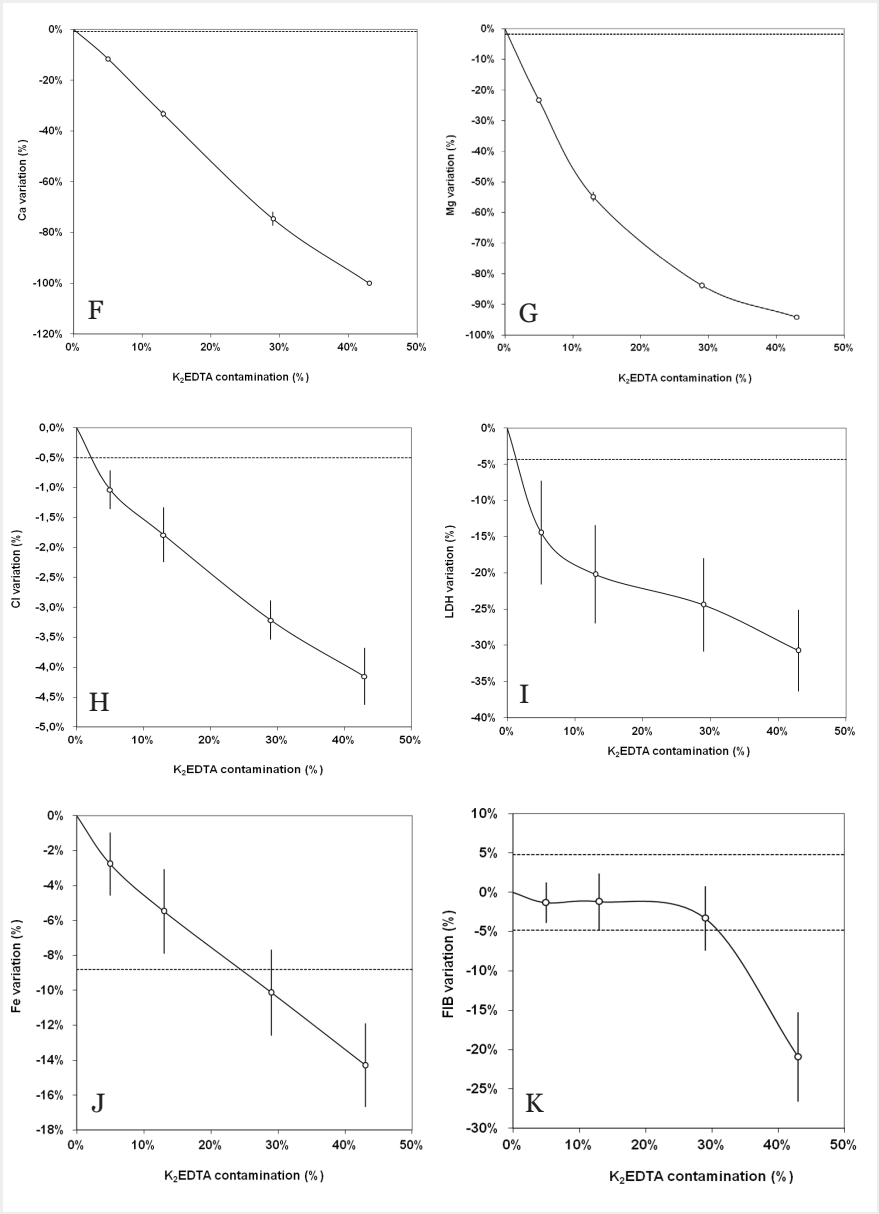

Figure 9. Effect of different extents of samples with K2EDTA blood volume contamination on potassium, activated partial thromboplastin time (aPTT), prothrombin time (PT), phosphate, sodium, calcium, magnesium, chloride, lactate dehydrogenase (LDH), iron, and fibrinogen.

The percentage bias is shown as mean and 95% confidence interval (95% CI).
The dotted lines delimit the desirable quality specifications for bias.
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The gentle inversion to mix primary blood tubes is currently advocated by the CLSI [14]. In theory: i) when blood tubes are mixed by gentle inversion, the risk of formation of micro clots, clots or fibrin filaments is limited; and ii) a vigorous mixing (or shaking) promotes either erythrocyte injury or spurious hemolysis [14]. Experimentally these theories were demystified. Since, no significant differences ($P > 0.05$) were detected between the mix procedures 1 and 2 – gold standard *vs*. vigorous mix – for routine coagulation, immunochemistry, or hematology testing (Paper IX). Therefore, either vigorous- or gentle-mix tubes immediately after blood collection have similar effect on laboratory results. Surprisingly, all samples vigorously mixed before centrifugation had shown only a visual alteration (presence of foam on the top, Figure 10 -A2.2, -B2.2, C2.2); and all serum tubes from vigorous mix procedure had shown a "blood ring" on the tube top after stopper removal (Figure 10-B3.2).

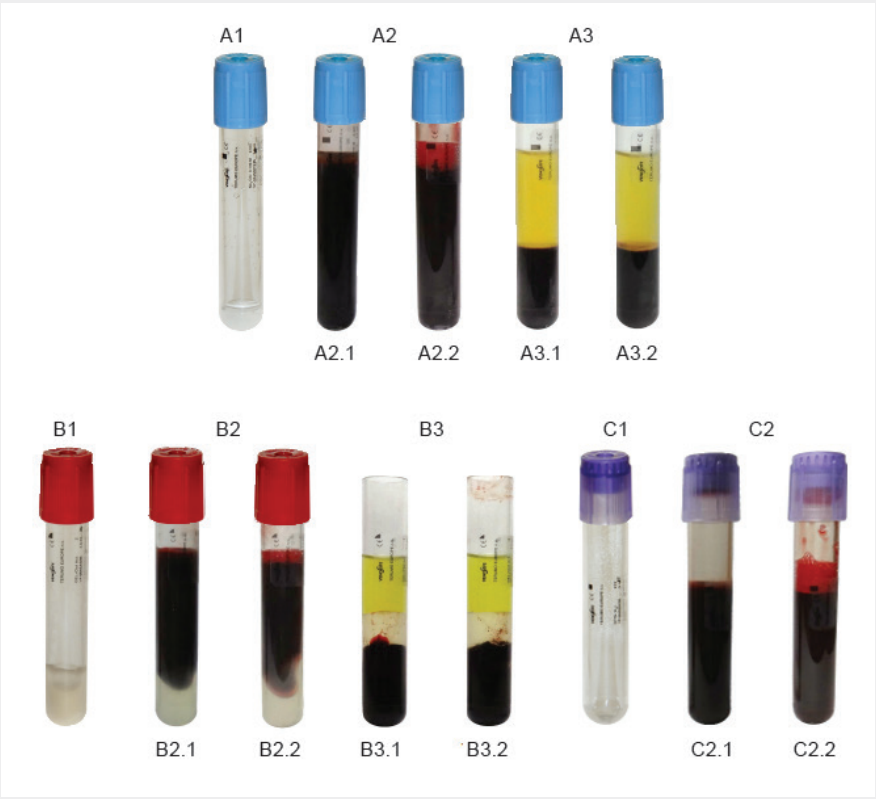

Figure 10. Three types of evacuated tubes with additive in the two different mix procedures

A. Sodium citrate evacuated tube. B. Serum evacuated tube with clot activator and gel separator. C. K_2EDTA evacuated tube. For each evacuated tube type (A–C), 1. Empty tube. 2. Tubes before centrifugation, respectively with gentle inversion/gold standard (2.1) and vigorously (2.2) mix (both manually). 3. Tubes after centrifugation respectively with inversion/gold standard (3.1) and vigorously (3.2) mix , both manually too.
This figure was previously published by *Clinical Biochemistry* (ISSN 0009-9120)

As regards the mixing of primary blood tubes immediately after blood collection by evacuated tube systems; it appears to be unnecessary (Paper X). Since, neither fibrin filaments, microclots nor clots were observed in samples not mixed, avoidance of mixing primary evacuated tubes does not carry a substantial risk of clotting. Moreover, significant statistical differences were found for: i) RBC and hematocrit when Procedure 1 (gold standard) was compared with Procedure 2 (rest time); ii) ALT and ESR when Procedure 1 (gold standard) was compared with Procedure 3 (no mix); and iii) RBC, HCT and HI when Procedure 2 (rest time) was compared with Procedure 3 (no mix). Surprisingly, clinically significant differences were found only for Na when Procedure 1 (gold standard) was compared with Procedure 2 (rest time), and Procedure 2 (rest time) was compared with Procedure 3 (no mix), Table 12. Moreover, these results confirmed the outcomes published by Parenmark and Landberg [96], Table 13. Tube mixing by gentle inversion or through horizontal mixing tray immediately after blood collection may increase the value of the hemolysis index compared with no mixing. Moreover, the blood turbulence inside evacuated tubes (due to negative pressure) is itself sufficient to provide solubilization, mixing and stabilization of additives in blood.

Table 12. Effect of three different procedures of primary blood evacuated tubes mixing in laboratory testing

Test (Units)	Desirable Bias (%)[29]	CVa (%)	Procedure 1 Gold Standard	Procedure 2 Rest Time	Procedure 3 No Mix	Procedure 1 Gold Standard vs Procedure 2 Rest Time		Procedure 1 Gold Standard vs Procedure 3 No mix		Procedure 2 Rest Time vs Procedure 3 No mix	
						Mean % difference	P values	Mean % difference	P values	Mean % difference	P values
Clinical chemistry											
ALT** (µkat/L)	12.0	1.3	0.31 ± 0.09	0.30 ± 0.09	0.30 ± 0.10	3.2	0.1036	-3.3	**0.0150**	0.0	0.1868
Na* (mmolL)	0.3	1.0	141 [139-142]	140 [139-141]	141 [139-142]	**0.7**	0.1121	0.0	0.3363	**-0.7**	0.1161
Hematology											
RBC* (102/L)	1.7	1.5	4.67 [4.36-4.87]	4.64 [4.34-4.74]	4.68 [4.35-4.80]	0.6	**0.0037**	0.2	0.5374	-0.9	**0.0068**
HCT** (%)	1.7	1.5	43.9 ± 1.02	43.5 ± 4.50	43.8 ± 1.01	0.9	**0.0012**	-0.2	0.4540	-0.7	**0.0051**
ESR* (mm/h)	NA	8.9	24.5 [17.2-38.0]	24.0 [17.2-34.8]	24.0 [18.0-35.0]	2.0	0.0676	-2.1	**0.0033**	0.0	0.7272
Coagulation											
HI* (NA)	NA	NA	3.0 [2.0-4.0]	2.0 [2.0-3.0]	4.0 [2.0-4.0]	33.3	0.3360	25	0.1839	-100	**0.0106**

*Non-normal distribution; the values are presented as median [interquartile range]; P value represents the significance by Wilcoxon ranked-pairs test. ** Normal distribution; the values are presented as mean ± standard deviation; P value represents the significance by paired Student's *t*-test.
The bold P values are statistically significant (P < 0.017), [29] according to Bonferroni correction for multiple comparisons. Mean % difference in bold was higher than the desirable bias. CVa (%): Analytical coefficient (within-run precision), by internal quality control on the respective instrument.
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Table 13. Mean % difference calculated from Parenmark and Landberg data [96]

Parameter	instant mixing	5 min rest before mixing	no mix	Desirable bias (%) [36]	Mean % difference: Instant mixing vs 5 min rest before mixing	Mean % difference: Instant mixing vs no mix
Chemistry						
CRP	1.40	1.40	1.40	21.8	0.0	0.0
AST	0.41	0.40	0.40	5.4	2.4	2.4
LDH	3.61	3.51	3.43	4.3	2.8	**5.0***
CK	1.46	1.45	1.45	11.5	0.7	0.7
TBil	7.10	7.15	7.20	11.4	- 0.7	- 0.1
Fe	12.0	12.1	11.9	8.8	- 0.8	0.8
Na	142	140	140	0.3	**1.4***	**1.4***
K	4.08	4.01	4.03	1.8	1.7	1.2
Hematology						
RBC	4.57	4.53	4.53	1.7	0.9	0.9
HGB	138	139	138	1.8	- 0.7	0.0
HCT	0.43	0.42	0.42	1.7	**2.3****	**2.3****
MCV	93.5	93.4	93.5	1.2	0.11	0.1
WBC	7.73	7.55	7.39	5.6	2.3	4.4
PLT	326	340	334	5.9	4.3	- 2.4
Coagulation						
PT	1.00	1.01	1.02	2.0	- 1.0	**-2.0****
aPTT	27.0	26.9	26.8	2.3	0.3	0.7

*P < 0.05; **P value not communicated by authors [96]. Bold mean % differences represent clinically significant variations, when compared with desirable bias

Chapter 3

Verification of *in vitro* diagnostic devices

Overview

The processes of qualification, validation, verification, and nonconformity management are essential in accredited laboratories [2] (Table 14). However, seldom the verification process is regarded as an issue in the preanalytical management. Indeed, the laboratory staff may start to use new *in vitro* diagnostic devices – e.g., a brand/kind of evacuated tubes, new medical devices, or transport systems – without (or prior to) any verification. The ISO 15189:2012 document contains clear indication that "all necessary improvements and potential sources of nonconformities, either technical or concerning the quality management system, shall be identified and all laboratory process shall be validated" [2].

Table 14. Terms and definitions from ISO 9000:2005 standard [97]

Terms	Definition
Requirement	The need or expectation, which is explicitly declared or implied, to be achieved
Non-conformity	Non fulfillment of a requirement
Qualification	The process to demonstrate the ability to fulfill specified requirements.
Verification	The confirmation, that specified requirements have been fulfilled.
Validation	The confirmation, that the specified requirements for a specific intended use or application have been fulfilled.

Presently, diagnostic products can be divided into either of two major categories: i) *in vitro* diagnostic (IVD) devices, such as laboratory instruments, reagents, assays, and blood collection tubes; ii) medical devices, such as specimen collection devices (needles and sets), whilst each one has its own technical characteristics devised for specific use [98]. Although most blood tubes are traded and available worldwide, their use should require previous verification by laboratory managers.

An IVD device called Buzzy® (MMJ Labs LLC, Atlanta GA USA) has been proposed for phlebotomy, which combines a cooling ice pack and a vibrating motor in order to relieve the venipuncture pain [99-101]. Since, Buzzy® is worldwide distributed, also delivered directly to patients [102], laboratories are using Buzzy® without previously verification.

In order to save resources (human, equipments, and financial), many health services have centralized a large number of the main analytical activities in the so-called core-laboratories: where blood specimens are delivered from peripheral collections sites. This implicitly means that a large

number of samples are convoyed from one facility to another. Moreover the core-laboratory may be settled inside a hospital where the various clinical departments – i.e., intensive care unit, emergency department, cardiology, and others – can be considered as peripheral collection sites. Alternatively, the main laboratory can be located externally to hospitals, thus providing activity to collection sites that are strategically located for collecting samples from large geographical areas (e.g., private laboratories servicing regional healthcare).

Inside a hospital, the sample transport is frequently done by pneumatic tube transport systems, able to deliver specimens from departments to core-laboratory, thus reducing the overall turnaround time [103-105]. However, this kind of specimen transportation is frequently performed without previous verification, as regards the potential source of variability. Furthermore, plasma samples are frequently collected in gel tubes, centrifuged, and transported in safety bags, since the gel should virtually create a barrier between plasma, and blood cellular components. Recent evidence has been provided that centrifuged heparin gel tubes should be maintained in closure-up position to avoid errors in clinical diagnosis, especially in longitudinal comparison of patient's data using aspartate aminotransferase, lactate dehydrogenase or potassium determination, as biomarkers [106]. Serum tubes with gel separator should be preferably used in standard protocols of clinical research, when there is a high risk that the maintenance in closure-up position during the transport after centrifugation cannot be ensured [106].

At the begin of the 21st century, robotic automation is poised to revolutionize laboratory practices [107]. The final goal of automation and consolidation in clinical laboratories is to ensure reliable information to assist physicians in patient management and improving clinical outcomes [108-113]. Presently, most technological devices are increasingly devised to perform repetitive tasks so far pertaining to human operators, with advantages in terms of accuracy, speed, convenience, and cost. Moreover, there is a long history of quality requirements in clinical laboratory, yet the concern has mainly regarded the analytical phase of this process [114]. Owing to substantial advances in technology, laboratory automation, and analytical quality, there is mounting evidence that further quality improvements should be targeted to extra-analytical activities of the total testing process (i.e., automation for pre-analytical process) [39, 115-118].

Experimental studies were performed regarding laboratory verification (in this PhD thesis) aiming:

- to evaluate the impact of Buzzy® use during diagnostic blood specimen collection by venipuncture for both routine immunochemistry (Paper XI) and hematological testing (Paper XII);
- to verify if the same kind of dedicated-syringes for blood gas, and evacuated tubes – i.e., with- sodium citrate, clot activator, lithium heparin, and EDTA – from different brands could be a source of variability for laboratory testing (Papers: XIII, XIV, XV, XVI, XVII, and XVIII);

- to evaluate the impact of a pneumatic tube transport system for routine hematological testing (Paper XIX); and
- to evaluate whether automation for the pre-analytical phase improves data quality (Paper XX).

Methods

Verification of Buzzy® impact on laboratory tests

To evaluate the impact of Buzzy® use during diagnostic blood specimen collection by venipuncture on immunochemistry (Paper XI) and hematology (Paper XII), blood samples were drawn from 100 volunteers by a single, expert phlebotomist. A vein was located in the left forearm without applying a tourniquet. This was done by a subcutaneous tissue transilluminator device (Venoscópio IV plus, Duan do Brasil, Sao Paulo, Brazil), thus avoiding the venous stasis. Blood samples were collected with a 20G straight needle directly into evacuated tubes: one 5 mL tube with clot activator and acrylic gel separator, and one 4 mL tube with 5.9 mg of K_2EDTA (Table 14). Nearly 2 mL of blood were preliminarily collected into a discard tube without additive to exclude potential interference from contact phase activation of blood, or tissue factor. In sequence, external cold and vibration was established by Buzzy® on the right forearm – 5 cm above the venipuncture site – for 1 minute before venipuncture and continued until the end of blood collection by venipuncture with the same procedure performed in the left forearm, as described above (Figure 11). Results of immunochemistry (Paper XI), and hematological (Paper XII) tests assayed from blood specimens collected without- and with-Buzzy® use were compared. Details from instrument used to assay biomarkers are shown in Table 2.

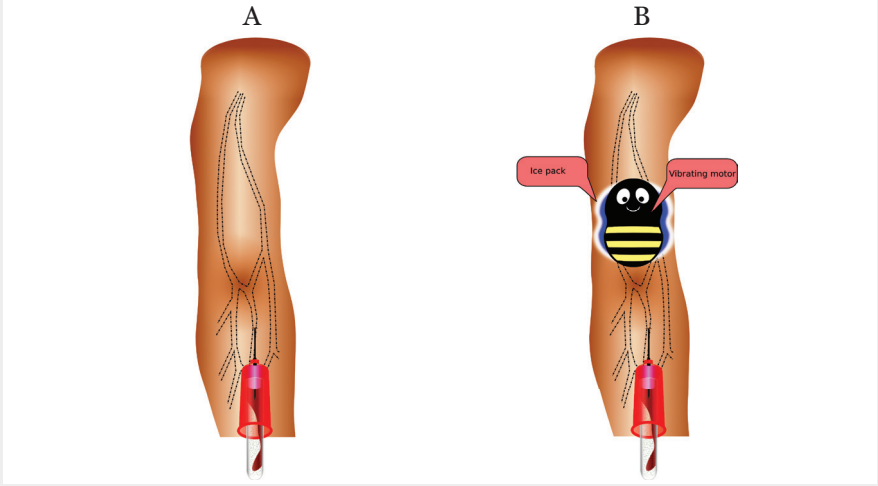

Figure 11. Schematic representation of Buzzy® use evaluation during diagnostic blood specimen collection by venipuncture

A. left forearm, blood specimens were drawn without either tourniquet or Buzzy® . B. right forearm, tourniquet application was avoided, and blood specimens were drawn after Buzzy® use during one minute 5 cm above the venipuncture site.

Verification of *in vitro* diagnostic devices

The collection of all diagnostic blood specimens to verify evacuated tubes (Papers: XIII, XIV, XV, XVI, and XVII) were performed by a single, expert phlebotomist. All volunteers, after 12 h fasting, were maintained seated for 15 min prior to phlebotomy. After this time interval, a vein was located on forearm by a subcutaneous tissue transilluminator device (Venoscópio IV plus, Duan®, Sao Paulo, Brazil), then blood samples were collected by venipuncture with a 20 G straight needle directly into the appropriate IVD device (evacuated tubes) described in Table 15. To eliminate any potential interference due to either the contact phase or the tissue factor, ~2 mL of blood were preliminarily collected in a discard tube without additive. In order to verify blood gas syringes (Paper XVIII), all volunteers after 8 h fasting, were maintained seated near the blood gas analyzer during 15 min. A single expert nurse performed all blood specimen collection following the CLSI H11-A4 [119] procedure. Briefly, after puncture of radial artery using 21 G butterfly, 9.6 mL of blood were drawn directly into four different heparinized-syringes (Table 15).

Table 15. Different brands of *in vitro* diagnostic devices studied

Papers	Brand 1	Brand 2	Brand 3	Brand 4	Brand 5
XIII	4.0 mL; 18 IU of lithium heparin; Vacuette® (Greiner Bio-one GmbH, Kremsmunster, Austria)	5.0 mL; not declared; Labor Import® (Guangzhou Improve Medical Instruments Co. Ltda, Zhejiang, China)	4.9 mL; ~16 IU of lithium heparin; S-Monovette® (Sarstedt, Numbrecht, Germany)	4.0 mL; 14-17 USP of lithium heparin; PST® (Becton, Dickinson and Company Franklin Lakes, NJ, USA)	3.0 mL; 17 IU of lithium heparin; PST II® (Becton, Dickinson and Company Franklin Lakes, NJ, USA)
XIV	4.0 mL; clot activator; Vacuette® (Greiner Bio-one GmbH, Kremsmunster, Austria)	6.0 mL; clot activator; Labor Import® Guangzhou Improve Medical Instruments Co. Ltda, Zhejiang, China)	4.9 mL; clot activator; S-Monovette® Sarstedt, Numbrecht, Germany)	4.0 mL; clot activator; SST® Becton, Dickinson and Company Franklin Lakes, NJ, USA)	5.0 mL; clot activator; SST II Advance® Becton, Dickinson and Company Franklin Lakes, NJ, USA).
XV	3.6 mL; 0.4 mL buffered sodium citrate (9NC) 0.109 mol/l: 3.2W/V%; Venosafe® (Terumo Europe NV, Leuven, Belgium)	2.0 mL; (9NC) sodium citrate 3.2%; Vacuette® (Geiner Bio-one GmbH, Kremsmunster,Austria)	2.7 mL; (9NC) sodium citrate 0.109 mol/L; BD Vacutainer® Becton, Dickinson and Company, FranklinLakes, NJ, USA)	3.6 mL; sodium citrate 3.2%; Labor Import® (Shandong Weigao Group Medical Polymer, Peoples Republic of China)	1.4 mL; sodium citrate 3.2%, 0.105 mol/mL; S-Monovette® (Sarstedt, Numbrecht, Germany)
XVI	3.0 mL; 5.9 mg of K2EDTA; Venosafe® (Terumo Europe, Leuven, Belgium)	4.0 mL; concentration of K2EDTA not declared; Vacuette® (Greiner Bio-One, Kremsmünster, Austria)	3.0 mL; 5.4 mg of K2EDTA; Vacutainer® (BD Vacutainer, Becton Dickinson Diagnostics, Plymouth, UK)	–	–
XVII	4.5 mL; concentration of K3EDTA not declared; Labor Import® (Shandong Weigao Group Medical Polymer, Weihai, China)	1.2 mL; 1.6 mg of K3EDTA; S-Monovette® (Sarstedt, Numbrecht, Germany)	–	–	–
XVIII	50 IU of heparin calcium balanced/mL of blood; S-Monovette® (Sarstedt, Nümbrecht, Germany)	in house (common 5 mL syringe) washed with sodium heparin 5000 IU (Becton, Dickinson and Company, Franklin Lakes, NJ, USA)	80 IU of spray-dried calcium-balanced lithium heparin; BD Preset® (Becton, Dickinson and Company, Franklin Lakes, NJ, USA)	80 IU of lyophilized electrolyte-balanced lithium heparin; Pico 50® (Radiometer Medical ApS, Denmark)	–

Note: Characteristics are shown as: volume (evacuated tube only); additive; product name; producer.

The sequence of syringes was randomized, however, syringe from Brand 2 were always the last to be collected, to eliminate the possible contamination from liquid sodium heparin. Before connecting each syringe, ~1.5 mL of blood volume were discarded in order to replenish the butterfly extension tube and to wash out possible interferences due to residual heparin. All the samples were processed following CLSI H18-A4 [65]. Moreover, blood collection materials (i.e., IVD devices, and medical devices) were accurately standardized in these studies (Papers: XIII, XIV, XV, XVI, XVII, and XVIII) – syringes, butterfly, needle, evacuated tubes, and sodium heparin were from a single lot – and samples were assayed according to the manufacturer's specifications, using proprietary reagents. Results of Immunochemistry (Papers XIII and XIV), hematological (Papers XVI and XVII), coagulation (Paper XV) tests assayed, and blood gas analyses (Paper XVIII) from blood specimens collected with different Brands of *in vitro* diagnostic devices were compared. Details from instrument used to assay biomarkers are shown in Table 2.

Verification of pneumatic tube transport system for routine hematological testing

A group of 50 apparently healthy volunteers of both genders were enrolled to blood withdrawal after 8 h fasting. A single, expert phlebotomist performed the collection of all diagnostic blood specimens. All volunteers were maintained seated during 15 min prior to phlebotomy. After this interval, a vein was located on forearm by a subcutaneous tissue transilluminator device without tourniquet, and venipuncture with 20 G straight needles were performed: 2 mL of blood were preliminarily collected in an evacuated tube without additive; then two identical 3.0 mL evacuated tubes containing 5.9 mg of K_2EDTA were drawn (Table 1). Samples from each volunteer were transported according to two parallel procedures (Paper XIX).

Transport-procedure I: Diagnostic blood specimens were kept in vertical, closure-up position, and hand carried by laboratory personnel in an appropriate biohazard container at room temperature (20 ± 1 °C) from the phlebotomy site to the core laboratory. The mean transport time was 7 min.

Transport-procedure II: Samples were transported by a pneumatic tube transport system (Op1000, Oppent S.p.A, Milan, Italy), which connects the phlebotomy site (outside the hospital) with the core laboratory on the hospital at 1st floor (point-to-point). There are neither heat nor cold sources along this route. The system generates a maximum speed of 3 m/s. The mean transport time from phlebotomy site to core laboratory was 85 s.

Paired samples (from transport procedures I and II) were analyzed at the same time for routine hematological testing (<15 min after blood collection), and erythrocyte sedimentation rate (Table 2).

Verification of laboratory automation for pre-analytical phase

A group of 100 adult ambulatory outpatients, 54 female and 46 male, with an average age of 49 years (range, 46–69 years) were enrolled as volunteers in this study. A single, expert phlebotomist performed the collection of all blood samples. All volunteers, after 12 h fasting, were maintained seated for 15 min prior to phlebotomy. After this time interval, a vein was located on forearm by a subcutaneous tissue transilluminator device, without tourniquet placement, and 7 mL of whole blood was collected by venipuncture with a 20 G straight needle, directly into two 3.5 mL evacuated tubes with 52.5 USP lithium heparin and gel separator. To eliminate any potential interference due to either the contact phase or tissue factor, ~2 mL of blood were preliminarily collected in a discard tube without additive. The lithium heparin tubes, one from each volunteer, were separately processed according to traditional-, and automated-processing (Paper XX).

Traditional Processing [65]

One hundred tubes (one from each volunteer) were left in the upright position for 10 min at room temperature (20 °C) to allow complete *in vitro* anticoagulation before centrifugation at 1200g for 10 min at room temperature, on the same centrifuge Rotanta 460R (Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). All primary tubes processed by traditional method were uncapped manually and immediately placed into the cobas 6000 <c501> module (Roche Diagnostics GmbH, Mannheim, Germany) to assay the clinical chemistry tests (Table 2).

Automated Processing

The other 100 tubes, one from each volunteer, were left in the upright position for 10 min at room temperature (20 °C) to allow complete *in vitro* anticoagulation. The tubes were then placed in racks suited for the following process into one MODULAR PRE-ANALYTICALS EVO (MPA) system (Roche Diagnostics GmbH, Mannheim, Germany). The MPA system includes modules that centrifuge, aliquot patient samples, automatically perform removal and insertion of container stoppers, and add barcode label (Figure 12) [120]. The automatic centrifuge unit (ACU) is a module inside MPA used to centrifuge samples based on parameters (such as rotation rate and duration) defined in the total system manager.

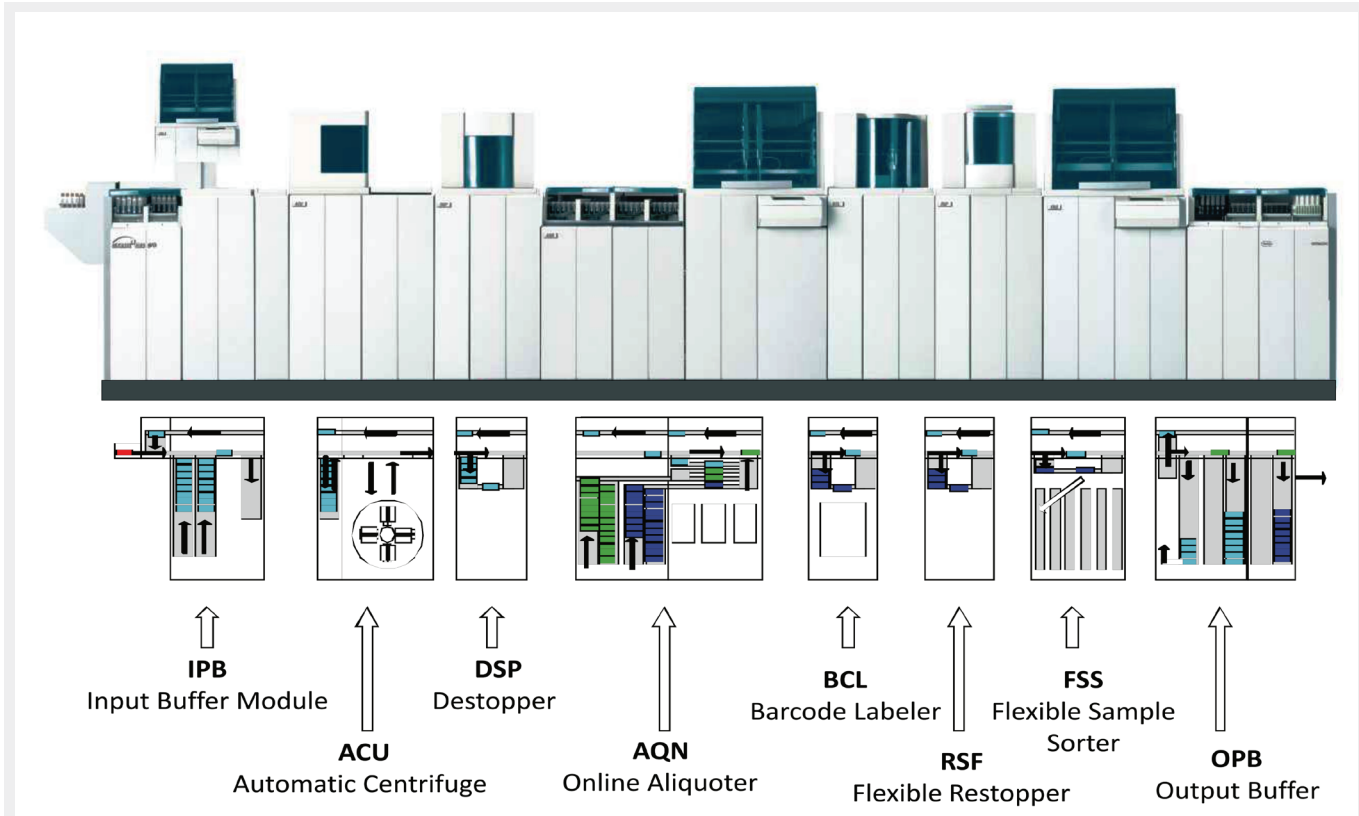

Figure 12 Schematic diagram of the MODULAR® PRE-ANALYTICALS EVO - MPA system (Roche Diagnostics GmbH)

The centrifuge is self-balancing, and a single ACU can accommodate up to 40 samples (8 racks) at a time. A MPA system can be configured with either one or two ACU modules. For this evaluation, only an ACU was used for centrifugation at 1200g for 10 min at room temperature. Then only one aliquot from each primary evacuated tube was performed by MPA system. Samples from the MPA system were fed directly into the same cobas 6000 <c501> module to assay the clinical chemistry tests (Table 2), without further staff intervention.

When all clinical chemistry testing was finished: i) the samples from the traditional processing were stored at +4 °C, as common practice in clinical laboratory, and as prescribed in accredited laboratories; ii) the primary tubes from the automated processing (i.e., MPA) were stored inside the output buffer (OPB) in the same MPA system. Briefly, OPB stores racks contain the primary samples intended for offline processing at room controlled temperature [120]. All samples were stored during 6 h, and the same panel of tests (Table 2) was then performed on the same cobas 6000 <c501> module. Results from both different processing procedures and different storing procedures were compared.

Results and Discussion

The new device to relieve venipuncture pain – that combines a cooling ice pack and a vibration motor – called Buzzy®, can be used during diagnostic blood specimens' collection by venipuncture for the majority of the routine immunochemistry tests (Papers XI). This device shall be avoided when blood samples are collected for: protein, albumin, transferrin, or complete blood count determination, Table 16 (Papers XI and XII). Recently, the findings on the bias induced by this device were challenged [121]. However, a strong evidence had shown that the combination of cold and vibration close to the venipuncture site generate by Buzzy® device should be considered a potential source of pre-analytical variability – significant decreases (i.e., $P < 0.05$) were observed for: white blood cells (WBC), neutrophils (NEU), and lymphocytes, whereas significant increases (i.e., $P < 0.001$) were observed only for red blood cells (RBC), haemoglobin (Hb) and haematocrit (Hct) (Paper XII). In contrast, I had shown that the tourniquet application during either 60, 90, 120, or 180 s caused significant increases on: platelets, WBC, NEU, monocytes, and eosinophils (i.e., $P < 0.05$, when compared with the parallel blood collection with transilluminator, Table 17) [22]. The physiology disturbance on vein environment caused by either of variability sources – i.e., vein stasis by tourniquet application (Figure 7), or that due to the combination of cold and vibration close to the venipuncture site – during blood collection should be avoided to guarantee both patient safety and the best laboratory outcomes.

Table 16. Impact of Buzzy® on routine laboratory testing (Papers XI and XII)

Tests	Units	Desirable bias (%)	CVa (%)	Blood specimen collection		Mean % difference	P-value
				Left forearm with Buzzy®	Right forearm without Buzzy®		
RBC**	(10^{12}/L)	1.7	1.5	4.90 ± 0.55	4.80 ± 0.55	**2.0**	**0.0006**
Hb**	(g/L)	1.8	1.0	141.4 ± 13.2	137.9 ± 12.7	**2.5**	**0.0002**
Hct**	(%)	1.7	1.5	41.5 ± 4.0	40.6 ± 4.0	**2.2**	**0.0005**
COL**	(mmol/L)	4.0	1.8	4.73 ± 0.7	4.58 ± 0.7	3.2	**0.0002**
HDL*	(mmol/L)	5.2	4.3	1.35 (1.11–1.64)	1.30 (1.07–1.63)	3.7	**0.0079**
TG**	(mmol/L)	10.7	2.0	1.55 ± 0.6	1.51 ± 0.6	2.6	**0.0101**
TP**	(g/L)	1.2	1.2	80.1 ± 4.4	78.9 ± 3.6	**1.5**	**0.0244**
ALB**	(g/L)	1.3	1.2	48.9 ± 3.7	47.9 ± 3.4	**2.0**	**0.0135**
UREA**	(mmol/L)	5.5	2.6	9.35 ± 1.8	9.64 ± 1.7	-3.1	**0.0004**
ALP**	(µkat/L)	6.4	2.7	1.19 ± 0.3	1.14 ± 0.3	4.2	**0.0232**
AMYL**	(µkat/L)	7.4	0.8	1.29 ± 0.3	1.24 ± 0.3	3.9	**0.0069**
AST*	(µkat/L)	5.4	1.2	0.49 (0.37–0.60)	0.44 (0.37–0.61)	**10.2**	0.8217
GGT*	(µkat/L)	10.8	1.6	0.54 (0.37–0.81)	0.53 (0.37–0.80)	1.8	**0.0232**
CK**	(µkat/L)	11.5	3.3	2.25 ± 1.5	2.14 ± 1.4	4.9	**0.0035**
TBil*	(µmol/L)	11.4	2.2	10.3 (5.1–13.7)	8.6 (5.1–13.7)	**16.5**	0.1736
Transferrin**	(g/L)	1.3	1.2	2.31 ± 0.3	2.27 ± 0.3	**1.7**	**0.0266**
Cortisol**	(nmol/L)	12.5	3.7	223.9 ± 99.0	198.4 ± 104.2	11.4	**0.0317**
TSH**	(mU/L)	7.8	3.5	1.33 ± 0.6	1.28 ± 0.6	3.8	**0.0074**

* Non-normal distribution; the values are presented as median (interquartile range); **Normal distribution; the values are presented as mean ± standard deviation; The P-values in bold are statistically significant (P <0.01 and P <0.05, for hematology and clinical chemistry, respectively) and bold mean % differences represent clinically significant variations, when compared with desirable bias[36].

Table 17. Impact of physiology disturbance on vein caused by two different source of variability (vein stasis by tourniquet application, and cold with vibration by Buzzy®) on routine haematological testing

Parameters	Desirable bias (%)	Source of variability				Cold and vibration by Buzzy® Paper XII
		Tourniquet application time [24]				
		60 s	90 s	120 s	180 s	
PLT	5.9	3.4 (P<0.05)	3.6 (P<0.01)	4.9 (P<0.01)	4.1 (P<0.01)	- 0.7 (NS)
WBC	5.6	2.5 (P<0.01)	4.8 (P<0.01)	4.9 (P<0.01)	3.1 (P<0.01)	- 3.5 (P<0.01)
NEU	9.1	3.3 (P<0.01)	4.2 (P<0.01)	6.6 (P<0.01)	3.9 (P<0.01)	- 2.9 (P<0.05)
MONO	13.2	4.3 (P<0.01)	3.9 (P<0.05)	3.6 (NS)	3.7 (NS)	- 3.6 (NS)
EOS	19.8	4.2 (P<0.05)	24.1 (P<0.01)	7.5 (NS)	3.8 (P<0.05)	0.0 (NS)
LYMP	7.4	0.8 (NS)	2.6 (P<0.01)	5.6 (P<0.01)	2.8 (P<0.05)	- 3.9 (P<0.05)
RBC	1.7	1.5 (P<0.01)	2.8 (P<0.01)	2.8 (P<0.01)	4.7 (P<0.01)	2.0 (P<0.001)
Hb	1.8	1.3 (P<0.01)	2.6 (P<0.01)	2.7 (P<0.01)	4.1 (P<0.01)	2.5 (P<0.001)
Hct	1.7	1.5 (P<0.01)	2.9 (P<0.01)	2.9 (P<0.01)	4.3 (P<0.01)	2.2 (P<0.001)

Legend: Results are expressed as percentage of variation (%) from the source of variability compared to the no-stasis by transilluminator. NS: Not Statistically Significant (P>0.05).

The verification of *in vitro* diagnostic devices – evacuated tubes, and syringes for blood gas analysis – had shown that different brands of evacuated tubes provided different laboratory results, Table 18 (Papers: XIII, XIV, XV, XVI, XVII, and XVIII). The presumable cause for these divergences could be attributed to the interaction between blood and evacuated tubes components, e.g., surfactant(s), stopper(s), stopper-lubricant(s), separator gel(s) and active(s) [122]. Moreover, blood gas analysis could be influenced by dedicated syringes produced from different manufacturers due to diverse preparations such as: i) presence of dry heparin inside the syringes; ii) mechanisms for elimination of the air bubbles from samples before mixing; iii) different materials of syringes and stoppers. Furthermore, the sample collection for blood gas analysis using common plastic syringes washed with sodium heparin before arterial puncture should also be avoided.

Table 18. Variability of results in laboratory testing due to different brands of evacuated tube

		Parameters of clinical chemistry (Desirable Bias%)													
		Cre (3.8)	Amyl (7.4)	P (3.2)	Mg (1.8)	Alt (12.0)	Glu (1.8)	Urea (5.5)	ALKP (6.4)	AST (5.4)	LDH (4.3)	TBIL (11.4)	Ca (0.8)	Fe (8.8)	K (1.8)
Brand 1 vs. Brand 2	Paper XIV	_	6.6 (P<0.01)	_	4.9 (P<0.01)	_	_	_	_	_	_	_	_	_	_
	Paper XIII	_	8.0 (P<0.01)	_	6.0 (P<0.01)	- 18.8 (P<0.01)	-2.5 (P<0.01)	_	_	4.8 (P<0.01)	5.7 (P<0.01)	8.1 (P<0.01)	1.3 (P<0.01)	- 1.1 (P<0.01)	3.7 (P<0.01)
Brand 1 vs. Brand 3	Paper XIV	6.6 (P=0.04)	7.8 (P<0.01)	_	4.9 (P<0.01)	_	_	_	_	_	_	_	_	_	_
	Paper XIII	_	_	_	6.0 (P<0.01)	-12.5 (P<0.01)	- 3.6 (P<0.01)	1.8 (P<0.01)	_	7.1 (P<0.01)	10.9 (P<0.01)	_	_	_	1.2 (P<0.01)
Brand 2 vs. Brand 4	Paper XIV	- 2.7 (P<0.01)	8.2 (P<0.01)	-1.6 (P<0.01)	_	_	_	_	_	_	_	_	_	_	_
	Paper XIII	-4.2 (P<0.01)	- 7.0 (P<0.01)	_	_	_	_	_	- 0.9 (P<0.01)	_	_	_	_	- 1.7 (P<0.01)	_
Brand 2 vs. Brand 5	Paper XIV	_	11.0 (P<0.01)	- 1.6 (P<0.01)	_	_	_	_	_	_	_	_	_	_	_
	Paper XIII	_	- 7.0 (P<0.01)	- 1.7 (P<0.01)	_	_	3.1 (P<0.01)	_	_	_	- 11.2 (P<0.01)	_	- 1.3 (P<0.01)	- 0.6 (P<0.01)	- 7.7 (P<0.01)
Brand 3 vs. Brand 4	Paper XIV	- 9.9 (P<0.01)	- 6.2 (P<0.01)	_	_	- 13.6 (P=0.04)	_	_	_	_	_	_	_	_	_
	Paper XIII	- 4.2 (P=0.26)	_	_	_	_	_	- 1.9 (P<0.01)	_	- 5.1 (P<0.01)	- 6.8 (P<0.01)	_	1.3 (P=0.12)	_	_
Brand 3 vs. Brand 5	Paper XIV	- 8.4 (P=0.01)	- 2.9 (P<0.01)	_	_	_	_	_	_	_	_	_	_	_	_
	Paper XIII	_	_	_	_	_	4.1 (P<0.01)	- 1.9 (P<0.01)	_	- 7.7 (P<0.01)	- 17.7 (P<0.01)	_	_	_	- 5.0 (P<0.01)
Brand 4 vs. Brand 5	Paper XIV	_	3.1 (P<0.01)	_	_	_	_	_	_	_	_	_	_	_	_
	Paper XIII	6.6 (P<0.01)	_	_	_	_	3.1 (P<0.01)	_	_	_	-10.2 (P<0.01)	_	-1.3 (P<0.01)	_	-6.3 (P<0.01)
Brand 2 vs. Brand 3	Paper XIV	6.6 (P=0.02)	13.5 (P<0.01)	- 0.8 (P<0.01)	_	_	_	_	_	_	_	_	_	_	_
	Paper XIII	_	-6.1 (P<0.01)	_	_	_	_	_	_	2.5 (P<0.01)	5.6 (P<0.01)	_	- 1.3 (P<0.01)	_	-2.6 (P<0.01)
Brand 1 vs. Brand 4	Paper XIV	-2.7 (P<0.01)	2.1 (P<0.01)	_	4.9 (P<0.01)	_	_	_	_	_	_	_	_	_	_
	Paper XIII	- 4.2 (P=0.09)	_	_	6.0 (P<0.01)	- 15.6 (P<0.01)	-2.5 (P=0.02)	_	- 1.7 (P<0.01)	_	4.9 (P<0.01)	10.3 (P<0.01)	1.3 (P<0.01)	_	2.5 (P<0.01)
Brand 1 vs. Brand 5	Paper XIV	_	5.1 (P<0.01)	_	4.9 (P<0.01)	_	_	_	_	_	_	_	_	_	_
	Paper XIII	_	_	_	4.8 (P<0.01)	- 15.6 (P<0.01)	_	_	_	_	- 4.9 (P=0.11)	_	_	_	- 3.7 (P=0.03)

Table 18. continued

		Coagulation Tests (Desirable bias %)		Hematological parameters (Desirable bias %)							
		PT (2.0)	aPTT (2.3)	RBC (1.7)	HCT (1.7)	Hb (1.8)	MCV (1.2)	WBC (5.6)	PLT (5.9)	MPV (2.3)	PDW (1.4)
Brand 1 vs. Brand 2	Paper XV	8.6 (P<0.01)	2.3 (P<0.01)	–	–	–	–	–	–	–	–
	Paper XVI	–	–	–	-2.3 (P=0.04)	–	- 0.5 (P<0.01)	2.9 (P=0.03)	–	–	- 3.8 (P<0.01)
Brand 1 vs. Brand 3	Paper XV	6.9 (P<0.01)	1.3 (P<0.01)	–	–	–	–	–	–	–	–
	Paper XVI	–	–	–	–	–	1.1 (P<0.01)	2.9 (P=0.03)	–	–	- 2.4 (P<0.01)
Brand 1 vs. Brand 4	Paper XV	–	- 3.0 (P<0.01)	–	–	–	–	–	–	–	–
Brand 1 vs. Brand 5	Paper XV	5.2 (P<0.01)	–	–	–	–	–	–	–	–	–
Brand 2 vs. Brand 3	Paper XV	- 1.9 (P<0.01)	- 1.0 (P=0.02)	–	–	–	–	–	–	–	–
	Paper XVI	–	–	–	1.8 (P<0.01)	–	1.6 (P<0.01)	–	–	–	–
Brand 2 vs. Brand 4	Paper XV	- 5.7 (P<0.01)	- 5.5 (P<0.01)	–	–	–	–	–	–	–	–
Brand 2 vs. Brand 5	Paper XV	- 3.8 (P<0.01)	–	–	–	–	–	–	–	–	–
Brand 3 vs. Brand 4	Paper XV	- 3.7 (P<0.01)	- 4.4 (P<0.01)	–	–	–	–	–	–	–	–
Brand 3 vs. Brand 5	Paper XV	1.8 (P<0.01)	–	–	–	–	–	–	–	–	–
Brand 4 vs. Brand 5	Paper XV	1.8 (P<0.01)	4.2 (P<0.01)	–	–	–	–	–	–	–	–
	Paper XVII	–	–	0.8 (P<0.01)	1.4 (P<0.01)	–	0.4 (P<0.01)	–	1.4 (P<0.01)	- 3.6 (P<0.01)	4.8 (P<0.01)

Table 18. continued

		Parameters of blood gas analyses (Desirable bias %)															
		pH (1.0)	pO2 (NA)	sO2 (NA)	pCO2 (1.8)	cHCO3 (NA)	CtCO2 (NA)	ctO2 (NA)	BE (NA)	tHb (1.8)	Na (0.3)	K (1.8)	Ca (0.6)	Glu (2.2)	Lac (8.0)	O2Hb (NA)	p 50 (NA)
Brand 1 vs. Brand 2	Paper XVIII	–	−5.2 (P<0.01)	−0.4 (P <0.01)	2.2 (P <0.01)	2.9 (P <0.01)	2.8 (P <0.01)	–	−62.5 (P<0.01)	1.5 (P<0.01)	−1.5 (P<0.01)	5.1 (P<0.01)	22.75 (P<0.01)	3.0 (P<0.01)	2.0 (P=0.01)	−0.8 (P<0.01)	0.8 (P=0.04)
Brand 1 vs. Brand 3	Paper XVIII	0.15 (P<0.01)	−09 (P=0.40)	–	–	1.7 (P=0.01)	1.6 (P=0.01)	–	−62.5 (P<0.01)	–	−1.0 (P<0.01)	–	–	3.0 (P<0.01)	−10.0 (P<0.01)	–	−0.8 (P<0.01)
Brand 1 vs. Brand 4	Paper XVIII	0.07 (P<0.01)	3.3 (P <0.01)	0.8 (P =0.02)	−1.4 (P =0.01)	–	–	–	−20.8 (P<0.01)	–	−1.2 (P<0.01)	−1.3 (<0.01)	−3.78 (P<0.01)	3.0 (P<0.01)	−12.0 (P<0.01)	0.5 (P<0.01)	–
Brand 2 vs. Brand 3	Paper XVIII	0.14 (P<0.01)	4.1 (P <0.01)	0.7 (P =0.04)	−3.2 (P <0.01)	−1.3 (P=0.04)	−1.2 (P=0.04)	−2.4 (P<0.01)	–	−1.5 (P<0.01)	0.5 (P<0.01)	−5.1 (<0.01)	−29.78 (P<0.01)	–	−12.2 (P<0.01)	–	−1.5 (P<0.01)
Brand 2 vs. Brand 4	Paper XVIII	0.05 (P=0.02)	8.1 (P <0.01)	1.3 (P <0.01)	−3.7 (P <0.01)	−2.6 (P <0.01)	−2.5 (P<0.01)	–	25.6 (P<0.01)	−1.5 (P<0.01)	0.3 (P<0.01)	−6.7 (<0.01)	−34.33 (P<0.01)	–	−14.3 (P<0.01)	1.3 (P<0.01)	−0.8 (P<0.01)
Brand 3 vs. Brand 4	Paper XVIII	−0.08 (P=0.04)	4.2 (P <0.01)	0.5 (P <0.01)	–	−1.3 (P <0.01)	−1.2 (P<0.01)	1.9 (P<0.01)	25.6 (P<0.01)	–	−0.2 (P=0.02)	−1.6 (<0.01)	−3.51 (P<0.01)	–	–	0.6 (P<0.01)	0.8 (P=0.04)

With regard to the verification of pneumatic tube transport system (PTS) (Paper XIX) neither statistical nor clinical significant differences were observed between samples from Transport-procedure I (hand carried) and Transport-procedure II (PTS) for routine hematological testing, erythrocyte sedimentation rate, and reticulocytes (Table 19).

Table 19. Variability in routine hematological testing from two different transport procedures

Tests	Units	Comprehensive results		*P*-value
		PTS	Hand carrying	
RBC*	(10^{12}/L)	4.90 ± 0.51	4.91 ± 0.51	0.1691
HGB*	(g/L)	14.1 ± 1.86	14.1 ± 1.88	0.2521
HCT*	(%)	44.8 ± 5.42	44.9 ± 5.46	0.1747
MCV*	(fL)	91.5 ± 7.13	91.5 ± 7.16	0.2752
RDW†	(%)	12.9 [12.5–14.7]	12.8 [12.6–14.6]	0.2815
WBC†	(10^9 /L)	6.28 [5.61–9.06]	6.27 [5.54–9.09]	0.1212
NEU*	(10^6 /L)	4.42 ± 1.85	4.34 ± 1.78	0.0308
LYMPHO†	(10^6 /L)	1.95 [1.51–2.63]	1.95 [1.48–2.52]	0.8999
MONO*	(10^6 /L)	0.46 ± 0.18	0.47 ± 0.20	0.8949
EOS*	(10^6 /L)	0.21 ± 0.12	0.21 ± 0.12	0.6452
BASO†	(10^6 /L)	0.04 [0.03–0.07]	0.04 [0.03–0.06]	0.5494
LUC*	(10^6 /L)	0.14 ± 0.05	0.14 ± 0.06	0.5071
PLT*	(10^9 /L)	219 ± 45.0	218 ± 46.6	0.8548
MPV*	(fL)	8.94 ± 0.66	8.92 ± 0.64	0.8813
PDW*	(%)	48.7 ± 5.44	49.0 ± 5.65	0.7629
RETIC†	(10^9 /L)	71.4 [63.5–80.7]	68.6 [60.0–76.0]	0.0383
ESR†	(mm/h)	29.5 [24.0–39.5]	30.0 [22.5–38.5]	0.3765

PTS, pneumatic tube transport system.
*Normal distribution; the values were mean± standard deviation; P-value represents the significance by paired Student's t-test. †Non-normal distribution; the values were median (interquartile range); P-value represents the significance by Wilcoxon ranked-pairs test. This table was previously published by *International Journal of Laboratory Hematology* (ISSN 1751-553X)

To my best knowledge this was the first study that evaluated the impact of PTS on erythrocyte sedimentation rate determination by TEST 1 YDL (Alifax, Polverara, Italy). Others five main studies have investigated the effects of PTS on complete blood count, and my results (Table 18) are generally comparable with these studies [105, 123-126]. Moreover, laboratory quality managers before start using the PTS should evaluate important procedures such as the correct filling of primary blood evacuated tubes, the speed of transport, and the impact endured by the container on arrival. Furthermore, previous investigation

showed that the use of PTS for diagnostic blood transport increases the frequency of hemolyzed samples [127-129] and could jeopardize patient safety regarding the hemostasis tests, and platelet functions [130-132]. However, the highest rates of hemolyzed samples are seen in systems with intermediate switching stations that accelerate and decelerate specimens [133], unlike the point-to-point (direct phlebotomy to core-laboratory) pneumatic tube system. The apparent limitation of my study (i.e., the exclusive use of a point-to-point PTS, Paper XIX) might instead further substantiate the outcomes of other studies where increased hemolysis index was observed after using PTS with switching stations that accelerate and decelerate specimens.

Laboratory automation and consolidation both aim to ensure consistent information to support physicians in patient management and improve clinical outcomes [108-113]. Since the preanalytical phase is responsible for several sources of laboratory variability, the laboratory automation for this phase can improve both quality of sample and patient safety; provided that the best practices (as described in Chapter 1 and Chapter 2) are followed during both blood collection and specimens handling. Various types of preanalytical automation are able to sort primary blood tubes and perform centrifugation when required (e.g., to obtain plasma or serum specimens). In these systems the primary blood tube types are recognized by the cap color. The color-coding for blood collection tube closing is used as indicator to identify additives. Nevertheless, the present cap colors from different producers of blood collection tubes lack standardization, and this fact is a potential cause of laboratory errors [134]. The MPA system effectively reduces the labor associated with specimen processing and decreases the number of laboratory errors that occur with specimen sorting, labeling, and aliquoting. This is a predictable advantage, if one considers that the instrument (Figure 12) does not require routine human manipulation. The more interesting finding is that the MPA system preserves the integrity of sample handling throughout the activities of specimen processing, Table 19 (Paper XX). Some suggestions can be advanced to explain these findings: i) the primary tubes are recapped and stored for further analyses (when needed) more rapidly in the MPA system than in the traditional processing; ii) when the process takes place starting after the MPA system, all tests are directly performed without a break on aliquoted-barcoded cups, whereas the primary tube remains open inside the cobas 6000 <c501> module (as in all brands of clinical chemistry analyzers) during the measuring process, using traditional processing; iii) blood tubes are manually recapped and stored at the end of the process, when the rack tray in the analyzer is full. The evidence of biases increases in COL, TG, TP, ALB, CRE, UA, ALP, AST, LDH, CA, FE, NA, and K, when traditional processing was compared with automated processing after storage, can be explained by the fact that either high- or low-molecular-weight analytes are affected by water evaporation in open tubes (Table 20). This is confirmed by previous evidence that tubes which remain open for a long time display significant variation in the concentration

of macromolecules, enzymes, and ions [135, 136]. This hypothesis also supports the significant biases increases observed between traditional processing before storage and traditional processing after storage for COL, TG, TP, ALB, CRE, UA, AST, LDH, P, CA, MG, FE, NA, and K (Table 20). Although it might be provocatively concluded that the results of this verification (Table 20) may be considered clinically irrelevant, this would not consider the current quality specifications for bias, derived from biological variation [137]. Obviously, the quality specifications derived from biological variation are considered of pivotal importance, and their usefulness is acknowledged in daily practice by quality managers of medical laboratories [36, 137-139]. As such, the caring physicians, who are unaware of the real patient situation, might abstain from appropriate treatments as a consequence of biased results from traditional processing of diagnostic blood specimens. It is also noteworthy that the relative (percentage) increases of the various analytes was rather broad and heterogeneous (i.e., ranging from -1.9% to +15.9%) and thereby *a priori* unpredictable. For a rational explanation of the consequences of uncontrolled evaporation, it should be considered that in serum/plasma where the pH is about 7.4, the interfacial water molecules between air (which is over the interface) and liquid bulk (which is under the interface) are polarized with stronger hydrogen bonding in the surface than in the bulk. This creates water solvation and ionization properties that change at the interface, with a depth of about 250 nm, where ions and hydrophilic solutes such as proteins are generally becoming less soluble. Moreover, plasma proteins that are mainly in the form of anions at pH 7.4 are progressively repelled by the evaporating surface, thus concentrating in the liquid bulk. During the process, the proteins more proximal to the interface can partially dehydrate and shrink, with conformational changes possibly involving chemical reactivity. Complex molecules, such as lipoproteins, are expected to behave according to the lipid/apoprotein ratio [140, 141]. The results (Table 20) had shown that the MPA system can really improve the quality in the pre-analytical phase of laboratory testing.

Table 20. Processing of diagnostic blood specimen's variability as mean % differences in a routine clinical chemistry laboratory test

Tests	Desirable Bias (%)	CVa (%)	MPA before Storage vs Traditional before Storage				MPA after Storage vs Traditional after Storage			
			Mean % Difference	P value	Mean Bias (Bland Altman)	95 CI (Bland Altman)	Mean % Difference	P value	Mean Bias (Bland Altman)	95 CI (Bland Altman)
Col	4.0	1.8	0.6	0.20	-0.01	(−0.15 to 0.13)	**5.6**	**<0.01**	-0.09	(−0.26 to 0.08)
HDL	5.2	4.3	0.0	1.00	0.00	(−0.05 to 0.05)	1.3	0.83	0.00	(−0.07 to 0.07)
TG[b]	10.7	2.0	-0.8	0.94	0.00	(−0.06 to 0.06)	**11.3**	**<0.01**	-0.03	(−0.08 to 0.02)
TP[a]	1.2	1.2	0.1	0.90	-0.31	(−34.19 to 33.57)	**13.6**	**<0.01**	-13.90	(−14.50 to 13.71)
ALB[a]	1.3	1.2	0.2	0.21	-1.38	(−16.12 to 13.37)	**11.4**	**<0.01**	-6.42	(−21.71 to 8.88)
BUN[b]	5.5	2.6	1.5	0.70	0.00	(−0.22 to 0.23)	1.8	**0.04**	-0.09	(−0.25 to 0.06)
CRE[b]	3.8	2.5	-1.2	0.18	0.53	(−4.20 to 5.25)	**7.8**	**<0.01**	-2.35	(−7.32 to 2.62)
UA[b]	4.9	1.0	0.0	0.80	0.87	(−8.75 to 10.48)	**5.8**	**<0.01**	-6.69	(−16.20 to 2.82)
ALP[b]	6.4	2.7	3.2	**<0.01**	-0.01	(−0.08 to 0.05)	**8.2**	**<0.01**	-0.03	(−0.09 to 0.02)
AMY- P[b]	8.0	1.0	0.0	0.15	0.01	(−0.02 to 0.03)	2.4	**0.04**	-0.01	(−0.04 to 0.02)
AST[B]	5.4	1.2	0.0	0.87	-0.01	(−0.05 to 0.02)	**11.4**	**<0.01**	-0.01	(−0.06 to 0.03)
ALT[b]	12.0	1.3	0.0	0.85	0.00	(−0.02 to 0.02)	8.1	**<0.01**	-0.03	(−0.09 to 0.04)
GGT[b]	10.8	1.6	0.0	0.88	0.00	(−0.04 to 0.04)	2.5	**0.04**	0.00	(−0.05 to 0.04)
LDH[b]	4.3	1.5	**11.3**	**<0.01**	-0.70	(−1.57 to 0.17)	**6.5**	**<0.01**	-0.44	(−1.32 to 0.44)
CK[b]	11.5	3.3	1.5	0.56	0.00	(−0.06 to 0.06)	3.6	**<0.01**	-0.02	(−0.12 to 0.07)
P[a]	3.2	3.0	1.0	**<0.01**	-0.01	(−0.04 to 0.02)	0.0	0.99	0.00	(−0.10 to 0.10)
CA[a]	0.8	0.7	0.0	0.97	0.00	(−0.08 to 0.08)	**2.4**	**<0.01**	-0.02	(−0.10 to 0.06)
MG[b]	1.8	1.2	-1.3	**0.02**	-0.01	(−0.05 to 0.03)	1.2	0.36	0.00	(−0.04 to 0.03)
FE[a]	8.8	2.6	-1.2	**<0.01**	0.50	(−0.96 to 1.95)	**10.9**	**<0.01**	-0.29	(−1.17 to 0.59)
NA[a]	0.3	1.0	-0.1	0.32	0.21	(−2.59 to 3.01)	**3.4**	**<0.01**	-2.98	(−5.80 to −0.16)
K[a]	1.8	1.5	0.0	0.55	0.01	(−0.21 to 0.23)	**2.6**	**<0.01**	0.00	(−0.34 to 0.34)
HI[a]	NA	0.8	**23.2**	**<0.01**	-1.04	(−6.19 to 4.10)	**28.8**	**<0.01**	-1.5	(−6.44 to 3.44)

Table 20. Continued

Tests	Desirable Bias (%)	CVa (%)	Traditional before Storage vs Traditional after Storage				MPA before Storage vs MPA after Storage			
			Mean % Difference	P value	Mean Bias (Bland Altman)	95 CI (Bland Altman)	Mean % Difference	P value	Mean Bias (Bland Altman)	95 CI (Bland Altman)
Col	4.0	1.8	**6.0**	**<0.01**	-0,13	(−0.25 to −0.02)	1.0	0.18	-0.06	(−0.22 to 0.10)
HDL	5.2	4.3	3.9	**<0.01**	-0.04	(−0.10 to 0.02)	2.6	0.25	-0.04	(−0.10 to 0.02)
TG[b]	10.7	2.0	11.3	<0.01	-0.02	(−0.10 to 0.05)	-0.8	0.36	0.00	(−0.05 to 0.06)
TP[a]	1.2	1.2	**13.8**	**<0.01**	-16.42	(−41.73 to 8.90)	0.4	0.24	-2.83	(−34.91 to 29.25)
ALB[a]	1.3	1.2	**12.2**	**<0.01**	-9.62	(−22.95 to 3.70)	1.2	0.20	-4.58	(−19.74 to 10.58)
BUN[b]	5.5	2.6	3.1	**<0.01**	-0.18	(−0.39 to 0.03)	2.7	**<0.01**	-0.09	(−0.34 – 0.17)
CRE[b]	3.8	2.5	**8.9**	**<0.01**	-2.01	(−6.96 to 2.94)	0.0	0.56	0.86	(−5.75 to 7.48)
UA[b]	4.9	1.0	**5.8**	**<0.01**	-8.06	(−18.94 to 2.83)	0.0	0.77	-0.50	(−8.77 to 7.78)
ALP[b]	6.4	2.7	5.9	0.37	-0.01	(−0.06 to 0.04)	0.8	0.50	0.01	(−0.04 to 0.07)
AMY- P[b]	8.0	1.0	2.4	0.99	-0.02	(−0.04 to 0.01)	0.0	0.97	0.00	(−0.02 to 0.02)
AST[b]	5.4	1.2	**15.9**	**<0.01**	-0.03	(−0.07 to 0.01)	5.1	**<0.01**	-0.03	(−0.07 to 0.02)
ALT[b]	12.0	1.3	8.1	**<0.01**	-0.03	(−0.09 to 0.04)	0.0	0.81	0.00	(−0.03 to 0.02)
GGT[b]	10.8	1.6	2.5	**0.04**	-0.02	(−0.08 to 0.04)	0.0	1.00	-0.02	(−0.06 to 0.03)
LDH[b]	4.3	1.5	**5.2**	**<0.01**	-0.22	(−0.86 to 0.42)	**10.1**	**<0.01**	-0.48	(−1.13 to 0.18)
CK[b]	11.5	3.3	1.5	0.21	0.02	(−0.12 to 0.16)	-0.8	0.78	0.04	(−0.13 to 0.21)
P[a]	3.2	3.0	**4.0**	**<0.01**	-0.04	(−0.14 to 0.06)	**5.0**	**<0.01**	-0.05	(−0.11 to 0.01)
CA[a]	0.8	0.7	**4.9**	**<0.01**	-0.08	(−0.15 to −0.01)	**1.7**	**<0.01**	-0.06	(−0.15 to 0.03)
MG[b]	1.8	1.2	**2.5**	**<0.01**	-0.02	(−0.05 to 0.02)	0.0	0.74	-0.02	(−0.05 to 0.01)
FE[a]	8.8	2.6	**12.0**	**<0.01**	-0.90	(−1.97 to 0.18)	0.0	0.42	-0.11	(−1.99 to 1.76)
NA[a]	0.3	1.0	**3.8**	**<0.01**	-3.58	(−5.97 to −1.20)	0.3	0.08	-0.40	(−3.04 to 2.25)
K[a]	1.8	1.5	**2.8**	**<0.01**	-0.12	(−0.31 to 0.06)	0.2	0.25	-0.12	(−0.39 to 0.16)
HI[a]	NA	0.8	**8.0**	**<0.01**	-0.39	(−2.92 to 2.14)	0.9	0.78	0.06	(−2.84 to 2.97)

The bold P values are statistically significant (P < 0.05), and bold mean % differences represent clinically significant variations when compared with desirable bias [36]. NA, not available; a, Normal distribution; P value represents the significance by paired Student t test; b, Nonnormal distribution; P value represents the significance by Wilcoxon ranked-pairs test; CVa, analytical coefficient of variability; MPA, MODULAR PREANALYTICALS EVO-MPA system (Roche Diagnostics GmbH). This table was previously published by Journal of Laboratory Automation (ISSN 2211-0682)

Concluding remarks

Key concepts

In order to prevent laboratory errors and to optimize the preanalytical phase management it must be considered that laboratory tests influence as many as ~70% of medical decisions 13, thus any errors throughout the total testing process can jeopardize patient safety. In this context, the accurate management of the preanalytical phase is the way forward to guarantee the best outcomes regarding the prevention of the Laboratory errors. Moreover, the patient preparation for clinical laboratory analyses, the procedure for diagnostic blood specimen collection by venipuncture, and the verification of *in vitro* diagnostic devices are essential to properly report a laboratory test.

On the above basis, this Thesis was aimed to highlight some preanalytical priorities to prevent laboratory errors and to optimize the preanalytical phase management, as listed:

- **Patient preparation:** fasting time should be mandatory for the mainstream laboratory tests (Papers: I and II);
- **Phebotomists' performance:** laboratory quality managers should evaluate the performance of their phlebotomists to generate quality indicators using a check list (Paper III);
- **Prevention of venous stasis:** tourniquet should be applied after cleansing the venipuncture site; once blood flow begins tourniquet should be released and removed to guarantee best laboratory outcomes (Papers: IV and V); wherears tourniquet shall be avoided to evaluate platelet function by MEA (Paper VI). Moreover, either cold and vibration near venipuncture site to eliminate pain, should be avoided (Papers: XI and XII);
- **EDTA contamination and order of tube collection:** the assessment of EDTA in serum is inexpensive and could be implemented in automated analyzers. Moreover, the order of draw should be still followed (Papers: VII and VIII);
- **Mixing primary blood tubes:** blood turbulence inside evacuated tubes due to negative pressure is itself sufficient to provide solubilization, mixing and stabilization of additives in blood; additional mixing procedure could be unnecessary (Papers: IX and X);
- **Assembly of the necessary devices for *in vitro* diagnostics:** verification should be performed before local routine implementation of blood collection tubes, or new devices (Papers: XI-XVIII);
- **Transport of the blood specimens:** the kind of transport (pneumatic- vs. hand-transport) should be validated by local laboratory for each assay (Paper XIX);
- **Laboratory automation for preanalytical phase:** the laboratory automation of this phase can improve both quality of sample and patient safety, if the best practices (as described above) are correctly followed, during both blood collection and specimens handling (Paper XX).

General Conclusion

This thesis could represent a (pre)pre-analytical management-tool to prevent laboratory errors, with the concept that laboratory results from inappropriate blood specimens are inconsistent and do not allow proper treatment nor monitoring of the patient. The most important question when dealing with the laboratory sources of error is not "how big is one single source of variability?" but, "how big could be the error if all sources of variability impact together over a single laboratory outcome?". General practitioners, nurses, technicians, and laboratory professionals all need to operate together to reduce laboratory variability in each single step described above, with the aim to guarantee the reliability of the results and thus the safety of patients.

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References

1. International Organization for Standardization. Quality management systems - Requirements ISO 9001 document. Geneva, Switzerland: International Organization for Standardization; 2008.

2. International Organization for Standardization. Medical laboratories - Requirements for quality and competence ISO document 15189. Geneva, Switzerland: International Organization for Standardization; 2012.

3. Huisman W. European medical laboratory accreditation. Present situation and steps to harmonisation. Clin Chem Lab Med 2012;50:1147-52.

4. Lima-Oliveira G, Lippi G, Salvagno GL, Brocco G, Guidi GC. *In vitro* diagnostic company recalls and medical laboratory practices: an Italian case. Biochem Med 2015;25:273-8.

5. Clinical Laboratory Standards Institute. Quality management system: development and management of laboratory documents. CLSI QMS02-A6 document. 6th ed. Wayne, PA: Clinical Laboratory Standards Institute; 2013.

6. Lundberg G. Acting on significant laboratory results. J Am Med Assoc 1981;245:1762-63.

7. Valenstein PN, Sirota RL. Identification errors in pathology and laboratory medicine. Clin Lab Med 2004;24:979-96.

8. Schiff GD, Hasan O, Kim S, Abrams R, Cosby K, Lambert BL, Elstein AS, Hasler S, Kabongo ML, Krosnjar N, Odwazny R, Wisniewski MF, McNutt RA. Diagnostic error in medicine: analysis of 583 physician-reported errors. Arch Intern Med 2009;169:1881-7.

9. Nilsson K, Grankvist K, Juthberg C, Brulin C, Söderberg J. Deviations from venous blood specimen collection guideline adherence among senior nursing students. Nurse Educ Today 2014;34:237-42.

10. Lippi G, Guidi GC, Mattiuzzi C, Plebani M. Preanalytical variability: the dark side of the moon in laboratory testing. Clin Chem Lab Med 2006;44:358-65.

11. Hallworth MJ. The ' 70% claim ': what is the evidence base? Ann Clin Biochem 2011;48:487-8.

12. Hallworth M, Hyde K, Cumming A, Peake I. The future for clinical scientists in laboratory medicine. Clin Lab Haematol 2002;24:197-204.

13. Graber ML, Franklin N, Gordon R. Diagnostic error in internal medicine. Arch Intern Med 2005;165:1493-9.

14. Clinical Laboratory Standards Institute. Procedures for the collection of diagnostic blood specimens by venipuncture. CLSI H3-A6 document. 6th ed. Wayne, PA: Clinical Laboratory Standards Institute; 2007.

15. World Health Organization. WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy. World Health Organization press 2010;Geneva.

16. Lippi G, Salvagno GL, Lima-Oliveira G, Brocco G, Danese E, Guidi GC. Postural change during venous blood collection is a major source of bias in clinical chemistry testing. Clin Chim Acta 2015;440:164-8.

17. Lippi G, Salvagno GL, Lima-Oliveira G, Danese E, Favaloro EJ, Guidi GC. Influence of posture on routine hemostasis testing. Blood Coagul Fibrinolysis 2015;26:716-9.

18. Dixon M, Paterson CR. Posture and the composition of plasma. Clin Chem 1978;24:824-6.

19. Lippi G, Salvagno GL, Lima-Oliveira G, Montagnana M, Danese E, Guidi GC. Circulating cardiac troponin T is not influenced by postural changes during venous blood collection. Int J Cardiol 2014;177:1076-7.

20. Simundic AM, Cornes MP, Grankvist K, Lippi G, Nybo M. Standardization of collection requirements for fasting samples: For the Working Group on Preanalytical Phase (WG-PA) of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM). Clin Chim Acta. 22014;432:33-7.

21. Guidi GC, Simundic AM, Salvagno GL, Aquino JL, Lima-Oliveira G. To avoid fasting time, more risk than benefits. Clin Chem Lab Med. 2015;53:e261-4.

22. Lima-Oliveira G, Lippi G, Salvagno GL, Montagnana M, Scartezini M, Guidi GC, Picheth G. Transillumination: a new tool to eliminate the impact of venous stasis during the procedure for the collection of diagnostic blood specimens for routine haematological testing. Int J Lab Hematol 2011;33:457-62.

23. Lima-Oliveira G, Salvagno GL, Lippi G, Montagnana M, Scartezini M, Picheth G, Guidi GC. Elimination of the venous stasis error for routine coagulation testing by transillumination. Clin Chim Acta 2011;412:1482-4.

24. Lima-Oliveira G, Lippi G, Salvagno GL, Montagnana M, Mangueira C, Sumita N, Picheth G, Guidi GC, Scartezini M. New ways to deal with known preanalytical issues: use of transilluminator instead of tourniquet for easing vein access and eliminating stasis on clinical biochemistry. Biochem Med 2011;21:152-9.

25. Lippi G, Lima-Oliveira G, Salvagno GL, Montagnana M, Gelati M, Picheth G, Duarte AJ, Franchini M, Guidi GC. Influence of a light meal on routine haematological tests. Blood Transfus 2010;8:94-9.

26. De Feo P, Horber FF, Haymond MW. Meal stimulation of albumin synthesis: a significant contributor to whole body protein synthesis in humans. Am J Physiol 1992;263:E794-9.

27. Caso G, Feiner J, Mileva I, Bryan LJ, Kelly P, Autio K, Gelato MC, McNurlan MA. Response of albumin synthesis to oral nutrients in young and elderly subjects. Am J Clin Nutr 2007;85:446-51.

28. Hunter KA, Ballmer PE, Anderson SE, Broom J, Garlick PJ, McNurlan MA. Acute stimulation of albumin synthesis rate with oral meal feeding in healthy subjects measured with [ring-2H5]phenylalanine. Clin Sci (Lond) 1995;88:235-42.

29. Jefferson LS. Lilly Lecture 1979: role of insulin in the regulation of protein synthesis. Diabetes 1980;29:487-96.

30. Cowan RE, Thompson RP. Fatty acids and the control of bilirubin levels in blood. Med Hypotheses 1983;11:343-51.

31. Al-Rubeaan K, Siddiqui K, Abu Risheh K, Hamsirani R, Alzekri A, Alaseem A, Saleh SM, Al-Yami Z, Al-Ghamdi A, Alayed K. Correlation between serum electrolytes and fasting glucose and Hb1Ac in Saudi diabetic patients. Biol Trace Elem Res 2011;144:463-8.

32. Rohrscheib M, Tzamaloukas AH, Ing TS, Siamopoulos KC, Elisaf MS, Murata HG. Serum potassium concentration in hyperglycemia of chronic dialysis. Adv Perit Dial 2005;21:102-5.

33. Gozansky DM, Herman RH. Water and sodium retention in the fasted and refed human. Am J Clin Nutr 1971;24:869-71.

34. Bloom WL. Inhibition of salt excretion by carbohydrate. Arch Intern Med 1962;109:26-32.

35. Fernandez-Real JM, Lopez-Bermejo A, Ricart W. Cross-talk between iron metabolism and diabetes. Diabetes 2002;51:2348-54.

36. Westgard J. Desirable Biological Variation Database Specifications. 2014 [updated january 2014; cited 2014 28th March]; Available from: http://www.westgard.com/biodatabase1.htm.

37. Nikolac N, Simundic AM, Miksa M, Lima-Oliveira G, Salvagno GL, Caruso B, Guidi GC. Heterogeneity of manufacturers' declarations for lipemia interference - an urgent call for standardization. Clin Chim Acta 2013;426:33-40.

38. Nikolac N, Supak-Smolcic V, Simundic AM, Celap I. Croatian Society of Medical Biochemistry and Laboratory Medicine: national recommendations for venous blood sampling. Biochem Med 2013;23:242-54.

39. Simundic AM, Bilic-Zulle L, Nikolac N, Supak-Smolcic V, Honovic L, Avram S, Beregovaja E, Dobreanu M, Guimaraes JT, Kovacs GL, Singh NM, Sierra-Amor RI, Sypniewska G, Zima T. The quality of the extra-analytical phase of laboratory practice in some developing European countries and Mexico - a multicentric study. Clin Chem Lab Med 2011;49:215-28.

40. Carraro P, Plebani M. Errors in a stat laboratory: types and frequencies 10 years later. Clin Chem 2007;53:1338-42.

41. Plebani M, Carraro P. Mistakes in a stat laboratory: types and frequency. Clin Chem 1997;43:1348-51.

42. Lippi G, Simundic AM. Total quality in laboratory diagnostics. It's time to think outside the box. Biochem Med 2010;20:5-8.

43. Plebani M. Laboratory errors: how to improve pre- and post-analytical phases? Biochem Med 2007;17:5-9.

44. ADA. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2010;33:S62-S9.

45. Cohn JS, McNamara JR, Cohn SD, Ordovas JM, Schaefer EJ. Postprandial plasma lipoprotein changes in human subjects of different ages. J Lipid Res 1988;29:469-79.

46. Peixinho CM, Tavares-Ratado P, Gabriel MF, Romeira AM, Lozoya-Ibanez C, Taborda-Barata L, Tomaz CT. Different in vivo reactivity profile in health care workers and patients with spina bifida to internal and external latex glove surface-derived allergen extracts. Br J Dermatol 2012;166:518-24.

47. Meade BJ, Weissman DN, Beezhold DH. Latex allergy: past and present. Int Immunopharmacol 2002;2:225-38.

48. Lee W, Lee JH, Park do J, Kim HH. A case of anaphylactic shock attributed to latex allergy during gastric cancer surgery. J Korean Surg Soc 2011;81 Suppl 1:S30-3.

49. Gosselin RC, Janatpour K, Larkin EC, Lee YP, Owings JT. Comparison of samples obtained from 3.2% sodium citrate glass and two 3.2% sodium citrate plastic blood collection tubes used in coagulation testing. Am J Clin Pathol 2004;122:843-8.

50. Kratz A, Stanganelli N, Van Cott EM. A comparison of glass and plastic blood collection tubes for routine and specialized coagulation assays: a comprehensive study. Arch Pathol Lab Med 2006;130:39-44.

51. Lippi G, Salvagno GL, Montagnana M, Guidi GC. Influence of two different buffered sodium citrate concentrations on coagulation testing. Blood Coagul Fibrinolysis 2005;16:381-3.

52. Lippi G, Salvagno GL, Montagnana M, Poli G, Guidi GC. Influence of the needle bore size on platelet count and routine coagulation testing. Blood Coagul Fibrinolysis 2006;17:557-61.

53. Guder WG, Narayanan S, Wisser H, Zawta B. Diagnostic samples: from the patient to the laboratory: the impact of preanalytical variables on the quality of laboratory results. 4 ed: Wiley-Blackwell; 2009.

54. Ionescu G, Negut M, Combiescu AA. [Biosafety and biosecurity in the medical laboratory. Update and trends]. Bacteriol Virusol Parazitol Epidemiol 2007;52:91-9.

55. Ishak R, Linhares AC, Ishak MO. [Biosafety in the laboratory]. Rev Inst Med Trop Sao Paulo 1989;31:126-31.

56. Simundic AM, Topic E, Nikolac N, Lippi G. Hemolysis detection and management of hemolyzed specimens. Biochem Med 2010;20:154-9.

57. Koseoglu M, Hur A, Atay A, Cuhadar S. Effects of hemolysis interferences on routine biochemistry parameters. Biochem Med 2011;21:79-85.

58. Rushing J. Drawing blood with vacuum tubes. Nursing 2004;34:26.

59. Majid A, Heaney DC, Padmanabhan N, Spooner R. The order of draw of blood specimens into additive containing tubes not affect potassium and calcium measurements. J Clin Pathol 1996;49:1019-20.

60. Lippi G, Salvagno GL, Brocco G, Guidi GC. Preanalytical variability in laboratory testing: influence of the blood drawing technique. Clin Chem Lab Med 2005;43:319-25.

61. Calam RR, Cooper MH. Recommended “order of draw” for collecting blood specimens into additive-containing tubes. Clin Chem 1982;28:1399.

62. Godwin PG, Cuthbert AC, Choyce A. Reducing bruising after venepuncture. Qual Health Care 1992;1:245-6.

63. Clinical Laboratory Standards Institute. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays. CLSI H21-A5 document. 5th ed. Wayne, PA: Clinical Laboratory Standards Institute; 2008.

64. Salvagno GL, Lippi G, Montagnana M, Franchini M, Poli G, Guidi GC. Influence of temperature and time before centrifugation of specimens for routine coagulation testing. Int J Lab Hematol 2009;31:462-7.

65. Clinical Laboratory Standards Institute. Procedures for the handling and processing of blood specimens for common laboratory tests. CLSI H18-A4 document. 4th ed. Wayne, PA: Clinical Laboratory Standards Institute; 2010.

66. Lima-Oliveira GdS, Picheth G, Sumita NM, Scartezini M. Quality control in the collection of diagnostic blood specimens: illuminating a dark phase of preanalytical errors. J Bras Patol Med Lab 2009;45:441-7.

67. National System of Accreditation from Brazilian Society of Clinical Analyses. Manual for accreditation of the quality management system for clinical laboratories. 6th ed. http://www.dicq.org.br/pdfs/manual_dicq.pdf: Brazilian Society of Clinical Analyses; 2013.

68. International Organization for Standardization. Medical laboratories — Particular requirements for quality and competence ISO document 15189. 2nd ed. Geneva, Switzerland: International Organization for Standardization; 2007.

69. Sibbing D, Byrne RA, Kastrati A. Role of platelet function testing in clinical practice: current concepts and future perspectives. Curr Drug Targets 2011;12:1836-47.

70. Terumo. Venosafe® Plastic Tubes: Hematology - VF-053SDK. Terumo Europe N.V.; 2012; Available from: http://www.terumo-europe.com/laboratory/products/vf_053sdk.php

71. Terumo. Venosafe® Plastic Tubes: Coagulation - VF-054SBCS07. Terumo Europe N.V.; 2012; Available from: http://www.terumo-europe.com/laboratory/products/vf_054sbcs07.php

72. Terumo. Venosafe® Plastic Tubes: Serum Gel - VF-054SAS. Terumo Europe N.V.; 2012; Available from: http://www.terumo-europe.com/laboratory/products/vf_054sas.php.

73. Lima-Oliveira G, Lippi G, Salvagno GL, Picheth G, Guidi GC. Laboratory diagnostics and quality of blood collection. J Med Biochem 2015;34:288–294

74. Lippi G, Salvagno GL, Montagnana M, Franchini M, Guidi GC. Venous stasis and routine hematologic testing. Clin Lab Haematol 2006;28:332-7.

75. Lippi G, Salvagno GL, Montagnana M, Guidi GC. Short-term venous stasis influences routine coagulation testing. Blood Coagul Fibrinolysis 2005 ;16:453-8.

76. Lippi G, Salvagno GL, Montagnana M, Brocco G, Guidi GC. Influence of short-term venous stasis on clinical chemistry testing. Clin Chem Lab Med 2005;43:869-75.

77. Weiss RA, Goldman MP. Transillumination mapping prior to ambulatory phlebectomy. Dermatol Surg 1998;24:447-50.

78. AccuVein. Exclusive Distribution by Becton, Dickinson and Co. 2015 [cited 2015 June 16th]; Available from: http://www.accuvein.com/how-to-buy/international/exclusive-distribution-by-becton-dickinson-and-co/.

79. Chrstie Medical Holding. Christie distributor inquiry form. 2015 [cited 2015 June 16th]; Available from: https://www.christiemed.com/how-to-buy/veinviewer-distributors.

80. Bölenius K, Söderberg J, Hultdin J, Lindkvist M, Brulin C, Grankvist K. Minor improvement of venous blood specimen collection practices in primary health care after a large-scale educational intervention. Clin Chem Lab Med 2012;DOI 10.1515/cclm-2013;51:303-10.

81. Lillo R, Salinas M, Lopez-Garrigos M, Naranjo-Santana Y, Gutierrez M, Marin MD, Miralles M, Uris J. Reducing preanalytical laboratory sample errors through educational and technological interventions. Clin Lab 2012;58:911-7.

82. Lowe G, Stike R, Pollack M, Bosley J, O'Brien P, Hake A, Landis G, Billings N, Gordon P, Manzella S, Stover T. Nursing blood specimen collection techniques and hemolysis rates in an emergency department: analysis of venipuncture versus intravenous catheter collection techniques. J Emerg Nurs 2008;34:26-32.

83. Bailey IR, Thurlow VR. Is suboptimal phlebotomy technique impacting on potassium results for primary care? Ann Clin Biochem 2008;45:266-9.

84. Straszewski SM, Sanchez L, McGillicuddy D, Boyd K, Dufresne J, Joyce N, Wolfe R, Lee AW, Fisher J, Mottley JL. Use of separate venipunctures for IV access and laboratory studies decreases hemolysis rates. Intern Emerg Med 2011;6:357-9.

85. Siess W. Molecular mechanisms of platelet activation. Physiol Rev 1989;69:58-178.

86. Valarche V, Desconclois C, Boutekedjiret T, Dreyfus M, Proulle V. Multiplate whole blood impedance aggregometry: a new tool for von Willebrand disease. J Thromb Haemost 2011;9:1645-7.

87. Zheng XL. Structure-function and regulation of ADAMTS-13 protease. J Thromb Haemost 2013;11:11-23.

88. Gachet C. ADP Receptors of Platelets and their Inhibition. Thromb Haemost 2001;86:222-32.

89. Banfi G, Salvagno GL, Lippi G. The role of ethylenediamine tetraacetic acid (EDTA) as *in vitro* anticoagulant for diagnostic purposes. Clin Chem Lab Med 2007;45:565-76.

90. Davidson DF. Effects of contamination of blood specimens with liquid potassium-EDTA anticoagulant. Ann Clin Biochem 2002;39:273-80.

91. Cornes MP, Davidson F, Darwin L, Gay C, Redpath M, Waldron JL, Ford C, Gama R. Multi-centre observational study of spurious hyperkalaemia due to EDTA contamination. Clin Lab 2010;56:597-9.

92. Cornes MP, Sulaiman RA, Whitehead SJ, Othonos N, Ford C, Gama R. Incorrect order of draw of blood samples does not causes potassium EDTA sample contamination. Brit J Biomed Sci 2012;69:1-2.

93. Salvagno GL, Lima-Oliveira G, Brocco G, Danese E, Guidi GC, Lippi G. The order of draw: myth or science? Clin Chem Lab Med 2013;51:2281-5.

94. Lima-Oliveira G, Lippi G, Salvagno GL, Montagnana M, Picheth G, Guidi GC. Incorrect order of draw could be mitigate the patient safety: a phlebotomy management case report. Biochem Med 2013;23:218-23.

95. Sharratt CL, Gilbert CJ, Cornes MC, Ford C, Gama R. EDTA sample contamination is common and often undetected, putting patients at unnecessary risk of harm. Int J Clin Pract 2009;63:1259-62.

96. Parenmark A, Landberg E. To mix or not to mix venous blood samples collected in vacuum tubes? Clin Chem Lab Med 2011;49:2061-3.

97. International Organization for Standardization. Quality management systems — Fundamentals and vocabulary. ISO document 9000. 3rd ed. Geneva, Switzerland: International Organization for Standardization; 2005.

98. Stankovic AK, Silvestri J, Mails M, Najork C. Total quality in laboratory diagnostics: the role of commercial companies. Biochem Med 2010;20:207-14.

99. Inal S, Kelleci M. Relief of pain during blood specimen collection in pediatric patients. MNC Am J Matern Child Nurs 2012;37:339-45.

100. Baxter A, Cohen L, McElvery H, Lawson M, von Baeyer C. An integration of vibration and cold relieves venipuncture pain in a pediatric emergency department. Pediatr Emerg Care 2011;27:1151-6.

101. Baxter A, Leong T, Mathew B. External thermomechanical stimulation versus vapocoolant for adult venipuncture pain: pilot data on a novel device. Clin J Pain 2009;25:705-10.

102. MMJ Labs. Store: Buy a Buzzy. MMJ Labs; 2014 [Accessed August 7th 2015]; Available from: http://www.storefront-buzzy4shots.com/HOME-BUZZY/products/12/.

103. Guss DA, Chan TC, Killeen JP. The Impact of a pneumatic tube and computerized physician order management on laboratory turnaround time. Ann Emerg Med 2008;51:181-5.

104. Fernandes CM, Worster A, Eva K, Hill S, McCallum C. Pneumatic tube delivery system for blood samples reduces turnaround times without affecting sample quality. J Emerg Nurs 2006;32:139-43.

105. Keshgegian A, Bull G. Evaluation of a soft-handling computerized pneumatic tube specimen delivery system. Am J Clin Pathol 1992;97:535-40.

106. Lippi G, Salvagno GL, Danese E, Lima-Oliveira G, Brocco G, Guidi GC. Invertion of lithium heparin gel tubes after centrifugation is a significant source of bias in clinical chemistry testing. Clin Chim Acta 2014;436:183-7.

107. Boyd J. Tech.Sight. Robotic laboratory automation. Science 2002 18;295:517-8.

108. Zaninotto M, Plebani M. The "hospital central laboratory": automation, integration and clinical usefulness. Clin Chem Lab Med 2010;48:911-7.

109. Hawker CD. Laboratory automation: total and subtotal. Clin Lab Med 2007;27:749-70, vi.

110. Hawker CD, Roberts WL, DaSilva A, Stam GD, Owen WE, Curtis D, Choi BS, Ring TA. Development and validation of an automated thawing and mixing workcell. Clin Chem 2007;53:2209-11.

111. Hawker CD, Roberts WL, Garr SB, Hamilton LT, Penrose JR, Ashwood ER, Weiss RL. Automated transport and sorting system in a large reference laboratory: part 2. Implementation of the system and performance measures over three years. Clin Chem 2002;48:1761-7.

112. Hawker CD, Garr SB, Hamilton LT, Penrose JR, Ashwood ER, Weiss RL. Automated transport and sorting system in a large reference laboratory: part 1. Evaluation of needs and alternatives and development of a plan. Clin Chem 2002;48:1751-60.

113. Hawker CD, Schlank MR. Development of standards for laboratory automation. Clin Chem 2000;46:746-50.

114. Lippi G, Simundic A-M. Total quality in laboratory diagnostics. It's time to think out side the box. Biochem Med 2010;20:5-8.

115. Bilic-Zulle L, Simundic AM, Supak-Smolcic V, Nikolac N, Honovic L. Self reported routines and procedures for the extra-analytical phase of laboratory practice in Croatia -cross-sectional survey study. Biochem Med 2010;20:64-74.

116. Lippi G, Salvagno GL, Montagnana M, Franchini M, Guidi GC. Phlebotomy issues and quality improvement in results of laboratory testing. Clin Lab 2006;52:217-30.

117. Lippi G, Lima-Oliveira G, Nazer SC, Moreira ML, Souza RF, Salvagno GL, Montagnana M, Scartezini M, Picheth G, Guidi GC. Suitability of a transport box for blood sample shipment over a long period. Clin Biochem 2011;44:1028-9.

118. Lippi G, Salvagno GL, Montagnana M, Lima-Oliveira G, Guidi GC, Favaloro EJ. Quality Standards for Sample Collection in Coagulation Testing. Semin Thromb Hemost 2012;38:565-75.

119. Clinical Laboratory Standards Institute. Procedures for the collection of arterial blood specimens. NCCLS H11-A4. 4 ed2004.

120. RocheDiagnostics. Modular Pre-Analytics EVO: Operator's Manual - Version 8.3. Mannheim, Germany: Roche Diagnostics; 2011.

121. Baxter A, Lawson M. Concerns with the methodology, analysis and discussion of the Buzzy® and transillumination comparison article. Blood Transfus 2014;12:s3-s5.

122. Bowen RAR, Remaley AT. Interferences from blood collection tube components on clinical chemistry assays. Biochem Med 2014;24:31-44.

123. Sari I, Arslan A, Ozlu C, Hacioglu S, Dogu MH, Isler K, Keskin A. The effect of pneumatic tube system on complete blood count parameters and thombocyte donation in healthy donors. Transfusion and Apheresis Science 2012;47:81-3.

124. Wallin O, Soderberg J, Grankvist K, Jonsson PA, Hultdin J. Preanalytical effects of pneumatic tube transport on routine haematology, coagulation parameters, platelet function and global coagulation. Clin Chem Lab Med 2008;46:1443-9.

125. Kratz A, Salem R, Van Cott EM. Effects of a pneumatic tube system on routine and novel hematology and coagulation parameters in healthy vollunteers. Arch Pathol Lab Med 2007;131:293-6.

126. Weaver DK, Miller D, Leventhal EA, Tropeano V. Evaluation of a computer directed pneumatic-tube system for pneumatic transport of blood specimens. Am J Clin Pathol 1978;70:400-5.

127. Steige H, Jones J. Evaluation of pneumatic tube systems for delivery of blood specimens. Clin Chem 1971;17:1160-4.

128. Sodi R, Darn SM, Stott A. Pneumatic tube system induced haemolysis: assessing samples type susceptibility to haemolysis. Ann Clin Biochem 2004;41:237-40.

129. Ellis G. An episode of increased hemolysis due to a defective pneumatic air tube delivery system. Clin Biochem 2009;42:1265-9.

130. Bolliger D, Seeberger MD, Tanaka KA, Dell-Kuster S, Gregor M, Zenklusen U, Grapow M, Tsakiris D, Filipovic M. Pre-analytical effects of pneumatic tube transport on impedance platelet aggregometry. Platelets 2009;20:458-65.

131. Hubner U, Bockel-Frohnhofer N, Hummel B, Geisel J. The effect of a pneumatic tube transport system on platelet aggregation using optical aggregometry and PFA-100. Clin Lab 2010;56:59-64.

132. Martin J, Schuster T, Moessmer G, Kochs EF, Wagner KJ. Alterations in rotation thromboelastometry (ROTEM) parameters: point-of-care testing vs analysis after pneumatic tube system transport. British Journal of Anaesthesia 2012;109:540-5.

133. Pragay DA, Edwards L, Toppin M, Palmer RR, Chilcote ME. Evaluation of an improved pneumatic-tube system suitable for transportation of blood specimens. Clin Chem 1974;20:57-60.

134. Simundic AM, Cornes MP, Grankvist K, Lippi G, Nybo M, Ceriotti F, Theodorsson E, Panteghini M. Colour coding for blood collection tube closures - a call for harmonisation. Clin Chem Lab Med 2015;53:371-6.

135. Young DS. Effects of preanalytical variables on clinical laboratory tests 3ed. Washington: AACC Press; 2007.

136. Lambert D, Berrahmoune H, Herbeth B, Siest G, Visvikis-Siest S. Preanalytical variations of proteic biomarkers [in French]. Med Sci (Paris) 2007;23 Spec No 1:9-12.

137. Ricos C, Cava F, Garcia-Lario JV, Hernandez A, Iglesias N, Jimenez CV, Minchinela J, Perich C, Simon M, Domenech MV, Alvarez V. The reference change value: a proposal to interpret laboratory reports in serial testing based on biological variation. Scand J Clin Lab Invest 2004;64:175-84.

138. Cembrowski GS, Tran DV, Higgins TN. The use of serial patient blood gas, electrolyte and glucose results to derive biologic variation: a new tool to assess the acceptability of intensive care unit testing. Clin Chem Lab Med 2010;48:1447-54.

139. Plebani M, Lippi G. Biological variation and reference change values: an essential piece of the puzzle of laboratory testing. Clin Chem Lab Med 2012;50:189-90.

140. Valdez D, LeHuereu J-Y, Gindre M, Urbach W, Waks M. Hydration and Protein Folding in Water and in Reverse Micelles: Compressibility and Volume Changes. Biophys J 2001;80:2751-60.

141. Chaplin M. Interface water and water-gas interfaces. 2012 [Accessed March 22th 2013]; Available from: http://www.lsbu.ac.uk/water/interface.html.

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