

**PREANALYTICAL NONCONFORMITY MANAGEMENT REGARDING
PRIMARY TUBE MIXING IN BRAZIL**
UPRAVLJANJE PREANALITIČKOM NESAOBRAZNOŠĆU U POGLEDU MEŠANJA
PRIMARNIH EPRUVETA U BRAZILU

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Summary

Background: The multifaceted clinical laboratory process is divided in three essential phases: the preanalytical, analytical and postanalytical phase. Problems emerging from the preanalytical phase are responsible for more than 60% of laboratory errors. This report is aimed at highlighting and discussing nonconformity (e.g., nonstandardized procedures) in primary blood tube mixing immediately after blood collection by venipuncture with evacuated tube systems.

Methods: From January 2015 to December 2015, fifty different laboratory quality managers from Brazil were contacted to request their internal audit reports on nonconformity regarding primary blood tube mixing immediately after blood collection by venipuncture performed using evacuated tube systems.

Results and Conclusions: A minority of internal audits (i.e., 4%) concluded that evacuated blood tubes were not accurately mixed after collection, whereas more than half of them reported that evacuated blood tubes were vigorously mixed immediately after collection, thus magnifying the risk of producing spurious hemolysis. Despite the vast majority of centers declaring that evacuated blood tubes were mixed gently and carefully, the overall number of inversions was found to be different from that recommended by the manufacturer. Since the turbulence generated by the standard vacuum pressure inside the primary evacuated tubes seems

Kratak sadržaj

Uvod: Složeni klinički laboratorijski proces podjeljen je na tri osnovne faze: preanalitičku, analitičku i postanalitičku. Problemi koji potiču iz preanalitičke faze odgovorni su za preko 60% laboratorijskih grešaka. Cilj ovog rada bio je da se istaknu i razmotre nesaobraznosti (tj. nestandardne procedure) u mešanju uzoraka krvi u primarnim epruvetama neposredno posle uzimanja krvi venepunkcijom pomoću sistema evakuiranih epruveta.

Metode: Između januara 2015. i decembra 2015. godine, kontaktirano je sa pedeset različitih menadžera za laboratorijski kvalitet u Brazilu i zatraženi su njihovi izveštaji sa internih provera nesaobraznosti u pogledu mešanja primarnih epruveta neposredno po uzimanju uzoraka krvi venepunkcijom obavljenom pomoću sistema evakuiranih epruveta.

Rezultati i zaključci: U manjem broju internih provera (tj. 4%) zaključeno je da mešanje evakuiranih epruveta nije pravilno obavljeno posle sakupljanja uzoraka, dok je više od polovine prijavilo da su evakuirane epruvete snažno mešane neposredno posle uzimanja uzoraka, što povećava rizik od nastanka hemolize. Uprkos tome što je većina centara prijavila da su evakuirane epruvete za uzorke krvi mešane nežno i pažljivo, utvrđeni ukupni broj inverzija razlikovao se od onog koji su preporučili proizvođači. Kako se čini da je turbulencija koja nastaje usled standardnog pritiska vakuuma unutar primarne evakuirane epruvete dovoljna da obezbedi solubilizaciju, mešanje i stabilizaciju između aditi-

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to be sufficient for providing solubilization, mixing and stabilization between additives and blood during venipuncture, avoidance of primary tube mixing probably does not introduce a major bias in tests results and may not be considered a nonconformity during audits for accreditation.

Keywords: accreditation, blood specimen collection, phlebotomy, quality, specimen handling

Introduction

The multifaceted clinical laboratory process is divided into three essential phases (preanalytical, analytical and postanalytical). Despite consolidated evidence that problems emerging from the preanalytical phase are responsible for more than 60% of laboratory errors (1, 2), it is reasonable to suggest that all phases of the testing process should be strictly standardized and continuously monitored.

Process accreditation is the best means to guarantee patient safety in laboratory diagnostics. Accreditation is a process aimed at providing independent appraisal and recognition – by one expert clinical laboratory professional – of the specific competence of testing (i.e., International Organization for Standardization – ISO 15189 standard for medical laboratories) (3). DICQ® is a National System of Accreditation (i.e. accreditation body) of the Brazilian Society of Clinical Analyses (4). This accreditation system is based on the ISO 15189 document (3).

All laboratories accredited by DICQ® (i.e., 279 laboratories so far) are yearly audited by an expert about their full knowledge of ISO 15189 standards. All nonconformities identified by the expert at audit time are reported to the DICQ® coordinator. Briefly, nonconformity is the nonfulfillment of a requirement. Moreover, requirement refers to the need or expectation that is stated, generally implied or obligatory – i.e. standardized procedure from evacuated tubes datasheets regarding primary tubes mix collected by venipuncture (5). Moreover, each accredited laboratory should plan and periodically carry out an internal audit process in order to: i) demonstrate that the pre-examination, examination and post-examination procedures are conducted in a manner that meets the needs and requirements of the stakeholders; ii) ensure conformity to the quality management system; and iii) continually improve the effectiveness of the quality management system. However, when certain nonconformities recur or doubt emerges about laboratory compliance with its own procedures, the laboratory should be proactive to identify, document and eliminate the leading cause(s). Corrective actions to be taken should be determined and clearly documented (3). This report is aimed at highlighting and discussing nonconformity (e.g., nonstandardized procedures) in primary blood tube mixing immediately after blood collection by venipuncture with evacuated tube systems; with the leading principle that nothing is more irksome than work to eliminate a fake nonconformity.

va i krvi tokom venepunkcije, izbegavanje mešanja primarnih epruveta verovatno ne unosi značajna odstupanja u rezultate testova i ne mora se smatrati primerom nesaobraznosti tokom proveru za akreditaciju.

Ključne reči: akreditacija, sakupljanje uzoraka krvi, flebotomija, kvalitet, rukovanje uzorcima

Methods

From January 2015 to December 2015, fifty different laboratory quality managers from Brazil were contacted to request their internal audit reports on nonconformity regarding primary blood tube mixing immediately after blood collection by venipuncture with evacuated tube systems. Were evaluated laboratories without nonconformity regarding pre-analytical phase procedures reported by external audit (i.e., 50 from 279 laboratories, 17.9%). Objective evidence were collected from internal audit reports and then classified by three types of theoretical nonconformities: i) evacuated blood tubes were left in upright position and sent to core laboratory without mixing afterwards; ii) evacuated blood tubes were shaken up vigorously immediately after appropriate filling, independently of the additive type inside the tubes; and iii) evacuated blood tubes were mixed gently and carefully by inverting but the number of times was different from recommended by the manufacturer (Table I). More than one piece of objective evidence could be reported during an internal audit by the same quality manager.

Table I Objective evidence from nonconformity reports regarding primary blood tube mixing immediately after blood collection by venipuncture, as reported by 50 Brazilian clinical laboratories during their internal audit processes.

Objective evidence	Frequency
Evacuated blood tubes were left in upright position and sent to core laboratory without mixing afterwards.*	2/50 (4%)
Evacuated blood tubes were shaken up vigorously immediately after appropriate filling, independently of the additive type inside the tubes.	30/50 (60%)
Evacuated blood tubes were mixed gently and carefully by inverting but the number of times was different from that recommended by the manufacturer.	44/50 (88%)

Note: *neither hemolyzed nor clotted samples (including no fibrin filaments or micro clots) due to this kind of mixing procedure were reported by the evaluated laboratories. This observation is in line with outcomes from Parenmark and Landberg (8), and Lima-Oliveira et al. (10).

Results

The objective evidence from 50 internal audits in Brazilian laboratories are shown in *Table I*. The frequency of different practices was found to be rather dissimilar, highlighting poor harmonization among different facilities. In particular, a minority of laboratories (i.e., 4%) reported that evacuated blood tubes were not accurately mixed after collection, whereas more than half of them indicated that evacuated blood tubes were vigorously mixed immediately after collection, thus magnifying the risk of producing spurious hemolysis. Notably, despite the vast majority of centers declaring that evacuated blood tubes were mixed gently and carefully, the overall number of inversions was different from that recommended by the manufacturer.

Discussion

The internal laboratory audits described in this article showed that the vast majority of phlebotomists (i.e., the health care professionals who usually perform blood collection by venipuncture using evacuated blood tubes) were not performing primary blood tube mixing according to the recommendations of the Clinical Laboratory Standards Institute (CLSI), nor adequately following the instructions of the manufacturers.

The manufacturers of evacuated blood collection tubes recommend that blood tubes for laboratory testing should be gently mixed by inversion immediately after collection (6); *Table II*. Several auditors from Brazil (both internal and external) also followed the recommendations for biological sample collection and processing issued by the Brazilian Society of Clinical Pathology and Laboratory Medicine (SBPC/ML). In particular, this latter guidance has been used as the gold standard for defining whether or not a procedure is in conformity with the standard (7).

Interesting evidence has been published by Parenmark and Landberg about the daily practice of mixing primary evacuated blood tubes (8). In brief, the authors performed and compared three different mixing procedures, concluding that mixing blood samples immediately after collection may not be mandatory for all types of tubes, whereas instant mixing may carry substantial risk of generating spurious hemolysis, thus biasing the test results of those parameters which are more vulnerable to blood cell (especially erythrocyte) injury (8). According to this evidence, a real need for revising and possibly updating the current recommendations for appropriate mixing of primary evacuated blood tubes has been put forward (9). More recently, a study by Lima-Oliveira et al. (10) confirmed the data of Parenmark and Landberg (8) showing that primary evacuated blood tube mixing immediately after blood collection by venipuncture appears to be not strictly or always necessary. More specifically, no fibrin filaments or micro clots were observed in evacuated blood tubes collected with an anticoagulant additive (i.e., EDTA, sodium citrate or lithium heparin) even when the tubes were left unmixed (10). It could hence be concluded that maintaining evacuated blood tubes in an upright position and then shipping the specimens to the central laboratory (i.e., a procedure that was reported by 2 out of the 50 internal audits in our study) should not be considered a nonconformity when the blood collection is performed using evacuated blood tubes. Interestingly, another recent article highlighted the risk of inverting lithium heparin plasma specimens (i.e., after centrifugation), thus confirming that tube placement and inversion may be a critical issue for certain analytes (11, 12).

The vigorous shaking nonconformity-procedure reported by 30 out of 50 internal audits is known to generate a visual alteration (e.g., presence of foam on the top of all types of evacuated tubes before centrifugation, and appearance of a »blood ring« on the tube top after stopper removal from serum tubes) (13). However, since the study of Lima-Oliveira et al. (13) evaluated only a single manufacturer, each labo-

Table II Mixing recommendation from the manufacturers of evacuated tubes used by the laboratories audited.

Tube description	Mix recommendation by manufacturers*	
	Becton Dickinson®	Greiner Bio-one®
without additive	–	from 5 to 10
with sodium citrate	from 3 to 4	from 4 to 5
with clot activator	6	from 5 to 10
with heparin	from 8 to 10	from 5 to 10
with EDTA	from 8 to 10	from 5 to 10
with glycolysis inhibitor	from 8 to 10	from 5 to 10

EDTA, ethylenediaminetetraacetic acid;

*mix the blood gently and thoroughly by inverting the tube for the required number of inversions as specified in the manufacturer's instructions.

ratory should carry out a similar study using other brands of evacuated blood tubes. Moreover, as regards the ISO 15189 standard, independent verification by the laboratory should confirm, using objective evidence (in the form of performance characteristics), that the performance claims for the pre-examination procedure (i.e. primary blood tubes mix) have a real influence on the quality of the total testing process, discounting any additional activity that will not generate a substantial impact on the reliability of laboratory test results (14–16). Evacuated blood tube mixing probably belongs to one of these latter activities.

Another important aspect that has emerged from our study is that 44/50 internal audits reported that evacuated tubes were gently and carefully mixed, but the number of inversions was not in agreement with the current recommendations. Unfortunately, no precise information was available about the difference between the number of recommended tube inversions and those really practiced in the local facility. However, even this aspect may not be necessarily regarded as a nonconformity.

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In conclusion, since the turbulence generated by the standard vacuum pressure present inside the primary evacuated tubes seems to be sufficient for providing solubilization, mixing and stabilization between additives and blood during venipuncture, avoidance of primary tube mixing probably does not introduce major bias in the tests results and may not be considered a nonconformity during audits for accreditation.

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Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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