

## REVIEW

# Phlebotomy Issues and Quality Improvement in Results of Laboratory Testing

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## SUMMARY

Laboratory testing is an integral part of the decision-making process, and results of laboratory testing often strongly influence medical diagnoses and therapies. There is a long history of quality requirements in laboratory medicine, which have mainly concerned the analytic phase of this process. Owing to the substantial advances in technology, laboratory automation and analytic quality, there is increasing evidence that further quality improvements should be targeted to extra-analytic phases of laboratory testing. Objective difficulties to monitor most of the preanalytic variables which lie outside the direct control or supervision of the laboratory personnel, such as phlebotomy, call for effective educational and preventive policies. Owing to high personnel turnover rates, lack of understanding about good laboratory practices, and inadequate training, there are several opportunities for making errors during phlebotomy, which mainly concern patient misidentification and collection of unsuitable specimens for testing due to unsuited venous accesses, venous stasis, inappropriate collection devices and containers. Improved standardization of phlebotomy techniques, along with operative guidelines dissemination, continuous education, certification, and training of health care professionals involved in blood drawing responsibilities would enhance the chance of obtaining specimens of consistent quality, with favorable revenues for the health care system and the patient's outcome. (Clin. Lab. 2006;52:XXX-XXX)

## KEY WORDS

Laboratory testing, medical error, phlebotomy, preanalytic variability, quality.

## INTRODUCTION

It is undeniable that errors in medicine do exist and whatever solution for limiting their prevalence is a necessary challenge to prevent unjustified expenditure and to enable effective clinical reasoning and decision making (1). While errors due to misuse of drugs or mishandled surgeries resonate prominently, various more silent errors can also affect the diagnostic process as well as any other part of the clinical reasoning. Laboratory testing is an integral part of the decision-making process,

and results of laboratory testing strongly influence medical diagnoses and therapies (2-3). A reasonable definition for laboratory errors, recently acknowledged by the International Organization for Standardization (ISO), is "any defect from ordering tests to reporting results and appropriately interpreting and reacting on these" (4-5). Although there are heterogeneous data on the error probability rate within the whole laboratory workout, they always reflect meaningful numbers, owing to the huge amount of tests performed everyday in clinical laboratories. In practice, the prevalence of laboratory errors has been estimated to range from 1 of every 8300 laboratory results (or 2000 patients) to 1 of every 33-50 laboratory results (5,6).

Over recent years radical changes in laboratory organization have granted medical laboratories major precision and accuracy in test results, reducing the burden of errors within the whole analytic process (5-7). Such a substantial progress was accomplished by developments in technology, informatics and computer science, intro-

duction of laboratory automation and fully-automated analytic platforms, multiple simultaneous pre-analytic workstations, improved testing procedures and compliance with systems of quality management, such as certification and accreditation procedures worldwide. The present issue is that patient care involving non-laboratory personnel and/or steps accounts for the great majority of identifiable errors in the testing process; consequently, extra-analytic quality should be prospectively regarded as the main target for further quality improvements (5-7). Among pre-analytic variables, inappropriate procedures for collection of venous blood specimens account for 60% of the errors, highlighting the need for a more rigid and effective supervision of this pivotal and irreplaceable part of the diagnostic process (7).

### MAJOR SOURCES OF ERROR DURING PHLEBOTOMY

Longitudinal forearm phlebotomy by lancets, an oldest medical practice, was traditionally practiced for blood-letting. Thus, phlebotomy found its origins before the time of Hippocrates, in the fifth century B.C., when the essentials of all medical treatment relied on the four body "humors": blood, phlegm, yellow bile, and black bile (8). Bloodletting was done in sick patients to restore the proper balance among the "humors". By the middle ages, surgeons and barbers were specializing in this bloody practice since the doctors were discouraged by the fact that feudal lords could have them executed in cases of malpractice. These practices achieved unexpected heights in the 18th and early 19th centuries, when a variety of methods were employed (8). The most common one was phlebotomy or venesection, in which blood was drawn from one or more of the larger external veins, such as those in the forearm or neck. In arteriotomy, an artery was punctured, although generally only in the temples. In scarification the "superficial" vessels were attacked, often using a syringe, a spring-loaded lancet (fleam), or a glass cup that contained heated air, producing a vacuum within (8). By the end of the 19th century, phlebotomy evolved through the use of the fleam and was declared quackery. It was only by the beginning of the 20th century that the use of the safer and effective needle and syringe system became commonplace for drawing blood (8). Until the early 1980s, blood collection for analytical purposes continued to be carried out by ordinary straight needles and syringes. The blood was then transferred into sample tubes after needle removal. The introduction of disposable needles and evacuated tube collection systems represented a substantial progress (9). These devices consist of a double-pointed needle, a plastic holder or adapter, and a series of vacuum tubes with stoppers. Blood collection by this procedure produces the best quality samples for laboratory testing, ensuring greater safeness for phlebotomists, since the patient's blood flows directly into appropriate test tubes (9). Additionally, a needle retrac-

table sheath allows sequential drawing of several tubes, thus preventing leakage of blood as tubes are changed. Although the introduction of disposable straight needles and evacuated tube systems has allowed collection of specimens of suitable quality, with additional general advantages from both a safety and a practical point of view, the overall procedures linked to blood sample collection, and the phlebotomy success rate itself, are as yet influenced by several aspects. The main reason for such a high prevalence of problems is that it is currently difficult to monitor most of the pre-analytic variables, including phlebotomy, which are not always under direct control or supervision of the laboratory staff. The phlebotomy activity is rather heterogeneous worldwide; in the German speaking area of Europe only physicians are presently allowed to draw venous and arterial blood and are therefore trained and supervised by the elder colleagues in the ward. In Britain, phlebotomists are educated like technicians and are partially supervised by laboratory professionals (10). Owing to the expanding trend towards consolidation of laboratory testing, which will inevitably entail outsourcing plans for specimen collection and transportation, improved vigilance of decentralized phlebotomy procedures is expected to gain further relevance in the future (11).

Phlebotomy seems one of the most neglected procedure in healthcare, though it suffers from a high degree of pre-analytic variability and still involves serious health risks, mainly represented by accidental needlestick injuries to the operator and casual lesions inflicted to the patient (nerve or tendon injury, hemorrhage, vertigo/syncope, lymphedema) as a result of improperly performed venipuncture (12). Although the prevalence of injuries to both patient and operator is still a matter of concern for most health systems, discussion of this topic is beyond the aim of this review, which is basically focused on the variability in laboratory testing introduced by poorly standardized procedures for drawing blood. Due to high personnel turnover rates, lack of understanding about good laboratory practices, and inadequate training, phlebotomists can incur a wide series of errors that compromise specimen integrity, sometimes in ways that laboratory personnel cannot detect during the analytic process (13). Phlebotomy success, a critical prerequisite for suitable specimens, is dependent upon the degree of laboratory supervision on this procedure. Laboratory personnel submitted significantly fewer rejected specimens than other in-hospital personnel groups, when compared with the respective frequency with which they collected specimens (14). Thus, outpatient phlebotomies by laboratory operators are usually more successful, as reflected by the considerable difference in the error rate between in- and outpatients (0.60% versus 0.039%) (6). Most unsuitable samples result from hemolysis (18.1%), insufficient quantity (16.0%), clotting (13.4%), lost or not received in the laboratory (11.5%), inadequately labeled (5.8%), at variance with previous or expected results (4.8%) (15).

### Patient identification

Shortages of skilled staff and overloaded systems create new occasions for basic but easily preventable errors that might compromise patient safety and introduce unjustified health expenditure. The Institute of Medicine (IOM) 1999 report, "To Err Is Human: Building a Safer Health System", concluded that most medical errors resulted from systematic problems and not from poor performance by individual providers (16). Specimen collection policies are supposed to comply with accurate verification of the patient's identity, which is also a safety goal for the Joint Commission on Accreditation of Healthcare Organizations (JCAHO) (17). Although the College of American Pathologists guidelines currently require two patient identifiers before collecting a specimen (18), misidentification of patients for laboratory testing is still acknowledged as a major cause of medical errors (19). Identification errors can occur during any part of the test cycle; however, most involve the preanalytic phase and, especially, those phlebotomy services where a great deal of specimens is daily drawn and the turnover of phlebotomists is particularly high (20). Thus, proper patient identification remains a central issue for phlebotomy facilities and is an essential requirement for ensuring the accuracy of laboratory results, and for preventing misdiagnosis or unsuited diagnostic and therapeutic procedures, which occasionally lead to unfavorable and life-threatening consequences, for instance in patients receiving incompatible blood products (21). In several Countries, the physician responsible for the patient will draw blood and therefore knows and correctly identifies the patient himself before proceeding with venipuncture. Although this is what each phlebotomist, physician or not, is expected to do before performing venipuncture, the enormous number of patient misidentifications testifies that the reality is obviously different worldwide. In these contexts, the adoption of bar-coded wristbands has a great potential for decreasing patient identification errors (22). Using barcodes for positive patient identification appears so far to be a fast and easy way to ensure accuracy in collecting patient samples. Wristband errors might still occur with some frequency, due to absent wristbands (70% of the cases), wrong wristbands or more than one wristband; partially missing, incomplete or illegible information on the wristband (19). Suggestions to reduce wristband error rates include immediate feedback on errors, admitting clerks and not nurses to place wristbands on patients, and a patient checklist that contains wristband confirmation.

In a developing policy toward zero tolerance for patient misidentification, some health care providers worldwide already require the placement of a patient identification band or other visible means of identification on individuals at the time of admission to a hospital. Barcode wristbands are currently available in a wide range of choices and, hopefully, their wide usage will be further encouraged, as it might allow easy capture of data for

specimen collection for both laboratory and point of care testing, administration of medication, verification of transfusion, patient tracking and patient charging. Along with wristbands, new hand-held computer resources for both patient and sample identification might also be profitable, as this system will enable recognition of potentially mismatched samples, prompting further investigations by specimen integrity departments (11).

### Location of the most suited venous access

Location and detection of an appropriate venous access is an essential condition to ensure successful attempts and quality specimens. The rate of success in venous blood drawing is influenced by two strictly related variables: (i) availability of suitable and easy venous accesses, which is mainly dependent upon the anatomic characteristics of the patient and (ii) the operator's skill and training. The antecubital area is the most suited for phlebotomy, and the vein of choice for venous blood drawing should be the median cubital vein. In the absence of a prominent median cubital vein, the cephalic vein should be considered. The basilic vein should be selected only when no other veins in the antecubital area are more easily accessible (23). Besides the antecubital area, there are a few secondary venous accesses, such as some superficial forearm and hand veins (back of the hand, thumb and palm side of the wrist) (24). Veins of the legs, ankles and feet should preferably not be accessed as blood coming from the inferior limb veins can undergo changes in coagulability mainly due to passage down atherosclerotic plaques in the arteries (25). A particular case is external jugular venipuncture in the neck, which can be performed in surgical or emergency settings (26,27). Puncture of a secondary site involves a variety of additional concerns, including the use of small-bore needles (usually equal to or less than 22- or 23-gauge), the difficulty for the operator to place the supporting hand, and the serious possibility of generating phlebotomy injuries due to the presence of underlying anatomic structures, such as tendons, nerves and arteries. Fistulas, shunts, arterial lines, locks, arteries, femoral and varicose veins, veins of the arm or hand from the site of a mastectomy and limbs with indwelling artificial access devices are not recommended sites, unless traditional and secondary sites have been ruled out and a permission of the primary care physician can be obtained (28,29). An improper choice of the site, such as drawing venous blood from a site distal to the antecubital region of the arm rather than drawing from an antecubital site, has also been shown to result in more hemolysis, as well as cleansing the venipuncture site with alcohol and not allowing it to dry properly (30).

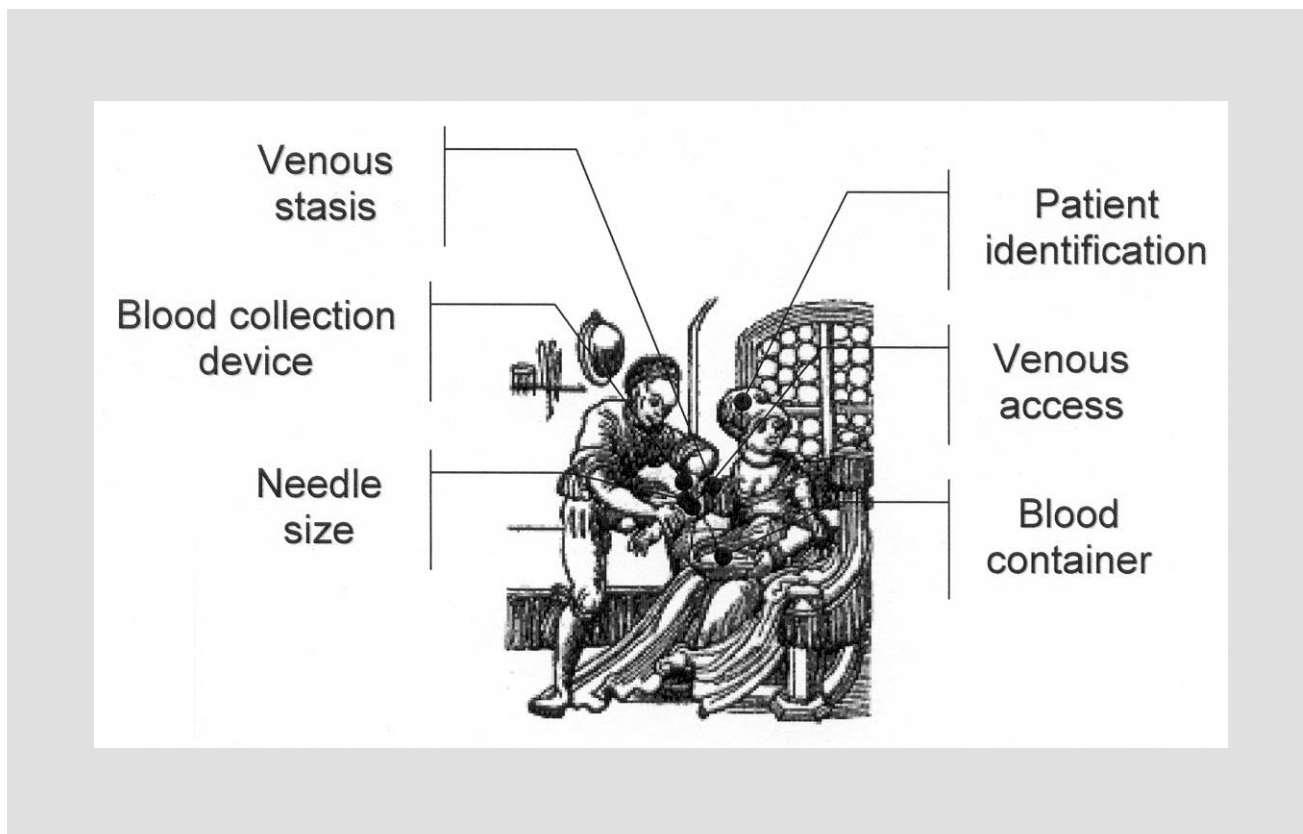


Figure 1: Main sources of phlebotomy errors.

### Tourniquet placement and venous stasis

A tourniquet can be defined as a constricting or compressing device used to temporarily occlude blood circulation of an extremity (usually of the upper limb), for a given period of time. The pressure which is applied circumferentially upon the skin is transferred to the walls of the underlying vessels, causing a transitory vessel occlusion. Two types of tourniquet are currently available: non-inflatable or non-pneumatic tourniquets, constructed of rubber or elasticized cloth, and pneumatic tourniquets, which have cuffs that are inflated by compressed air or gases (31).

Tourniquet placing is commonplace before routine venipuncture to assist phlebotomists in locating a suitable vein, though it can also be useful in the surgical setting to prevent excessive hemorrhage and to provide a relatively bloodless operative field. The tourniquet, applied approximately one hand width (7.5 cm) above the anticipated puncture site in the arm, should be tight enough to obstruct venous but not arterial flow (approximately 20-30 mm Hg under the systolic blood pressure), without causing discomfort to the patient. It should be removed as soon as blood flow is established in the collection system, and under no circumstance should it be left in place for more than one minute. When additional

time is required to complete the blood drawing process, the tourniquet must be released, so that blood flow can resume and normal skin color returns to the extremities (32,33). According to current practice, the tourniquet should thus only be applied when necessary and, if possible, removed as soon as the needle is safely in the vein (32). However, the tourniquet is infrequently released before blood drawing is completed. Although phlebotomy is expected to be the fastest possible, several causes might contribute to lengthen the time since tourniquet tying, including location of an appropriate venous access, selection of the most suitable blood collection system, needle insertion into the vein, and collection of many tubes.

Regardless of substantial evidence that a prolonged venous stasis influences the concentration and/or the activity of several analytes in the blood, the tourniquet time is rarely regarded as a potential source of preanalytic variability. The pattern of changes observed after a prolonged venous stasis, mimicking a prolonged tourniquet time, depends mostly upon the length of the stasis, the size and the protein-binding characteristic of the analytes. The increased intravascular pressure is the major mechanism responsible for increments of high-molecular-weight blood constituents and protein-bound substances in blood due to hemoconcentration (hemoglo-

bin, enzymes, albumin, calcium, total cholesterol, creatinine, iron), whereas a consistent decrease is usually observed for smaller analytes, especially electrolytes (chloride and potassium) (34,35). As most of these changes do not show an exceptional inter-individual variability, they could be anticipated, and potentially corrected, on the basis of precise records establishing the time elapsed between sample collection and tourniquet placing before venipuncture. Indeed, the most proactive actions to prevent artifacts from prolonged stasis are blood withdrawn without undue venous stasis (non-application of the tourniquet in patients with easy and prominent veins and early release after insertion of the needle) and accurate standardization of the external pressure, for example by adopting easy-to-apply, re/de-inflatable electronic devices, which are widely available in the surgical setting (36). Finally, when many tubes are needed, a rigorous and standardized sequence should be advisable, giving priority to the tubes for analytes which are more influenced by prolonged stasis (34).

### Blood collection devices

The introduction of evacuated and aspirating tube collection systems in Europe in the 1960s almost revolutionized the blood collection technique, yielding substantial advances over ordinary syringes, as these devices produce consistent quality specimens for laboratory testing and contextually ensure greater operator safety, especially when coupled with needle safety devices (9). Butterfly devices, consisting of a small needle attached to flexible plastic wings and connected with extension flexible tubing, have been regarded as an alternative to the classic straight needle to collect blood in a selected category of patients. In fact, an adapter can be easily added to the butterfly device, so that it will fit into a needle holder and a vacuum system. These disposals, originally conceived for administration of infusion therapy, would thus be applied to draw blood in restricted categories of patients, such as those with permanent subcutaneous venous cannulation who undergo permanent hemodialysis treatment or receive long-term infusion therapy (critically ill or cancer patients), invasive medical treatment, general anesthesia or sedation before surgery, noxious clinical procedures, and diagnostic radiological investigations. In such circumstances, conservation of catheter patency is integral to the patient's care and reduces the patient's discomfort resulting from repeated venipuncture, contextually diminishing blood loss. Additionally, blood collection by butterfly systems might be advisable in newborns, children, small animals and patients with small, difficult and atypical venous accesses (37). Finally, the use of a butterfly device, less intimidating because of the reduced dimension of the needle, might be advisable when ap-

proaching nervous or anxious patients, who suffer from so-called "needle phobia" (38).

As compared to traditional straight needles, reasonable disadvantages of the use of butterfly devices for collecting venous blood are basically represented by the greater costs, and the chance to obtain less suitable samples (incomplete filling of the vacuum tube, hemolysis, activated or clotted samples) and the increased health risk for the operators, because the possibility of needle stick injuries is substantially higher. Therefore, the use of butterfly needles and intravenous lines for specimen collection have been traditionally discouraged as a routine practice, unless more conventional routes have failed (39). Although little scientific evidence exists so far on the influence on the results of laboratory testing using alternative techniques to draw blood, aside from a few exceptions (serum sodium, platelets and leukocyte counts), no clinically meaningful variations could be observed in routine hematologic, coagulation and clinical chemistry testing in samples collected by butterfly devices as compared to the use of traditional straight needles (34,35). Therefore, the use of such devices (butterfly needles or winged infusion devices) has little clinical repercussion on the reliability of routine laboratory testing and might be proposed as a suitable alternative to the ordinary needle system when indicated and within certain limitations.

Whatever the device used to collect blood (butterfly and other vascular access devices), the only recommendations are directed to ensure appropriate filling of the tubes and to remove potential contaminants, such as infusion fluids (saline or drugs). Three alternative sampling methods are currently available for the latter purpose: (i) the discard technique which involves discarding of an appropriate volume of the first aspirate of blood, (ii) the reinfusion method, based on returning the discarded specimen after obtaining blood for laboratory analysis and, (iii) the push-pull or mixing technique, which requires mixing three to four times the blood back and forth in a syringe to eliminate contamination (37). Even though definitive guidelines on this topic are lacking so far, either the reinfusion or the mixing methods should not be widely recommended. In fact, although these techniques prevent excessive blood loss they do expose the blood sample to a greater risk of hemolysis and clotting by excessive shaking. Moreover, it may not be always possible to obtain an appropriate volume of blood for reinfusion or for completing the mixing sequence. Thus, when butterfly devices, catheters and extension sets are used to draw venous blood for laboratory testing, the most reliable strategy appears to discard a minimum amount of blood after initial flushing, which ranges from two times (for disposals used exclusively for drawing blood) to six times (for intravenous ports concurrently used for infusion therapies) the dead-space roughly corresponding to 1-3 mL of blood, respectively (40-42).

Although few phlebotomists still prefer to use the traditional syringe for venipuncture, this old-fashioned

practice is so far widely discouraged for both health and technical reasons. Firstly, the probability of needlestick and additional sharp injuries is more enhanced when using traditional syringes for drawing blood (43-44). Accordingly, the use of any needle when transferring blood directly from a syringe to a specimen container is currently discouraged by several health organizations, including the Federal Occupational Safety & Health Administration (45). Then, transferring the blood into a tube by pushing down the syringe plunger creates a positive pressure in the tube, which may induce the stopper to come off and might promote hemolysis or clotting in the specimen, especially when large-bore needles are used (46-47). Finally, venous blood collected by needle and syringe causes more environmental contamination than evacuated container systems do (48).

### Needle size

Venipuncture by using needles is an inevitable requirement, as no venous samples can be as yet collected without direct vein puncturing. Needles are available for evacuated systems and for use with a syringe, single draw or butterfly system. Needles developed to be used with evacuated collection systems have a sharp point at both ends, and are usually covered by a sheath, with one end being shorter than the other (32). The long end of the needle penetrates the vein, while the shorter end is used to pierce the stopper of the vacuum tube. There are some common calibration characteristics that usually identify size and purpose for phlebotomy needles. Needles are basically calibrated by gauge, which refers to the diameter of the needle in millimeters. The larger the gauge number, the smaller the diameter of the bore. Venipunctures are usually performed with needles ranging from 19 to 25 gauge (G); 19-21 G needles are used primarily for large antecubital veins, 23 G needles for smaller antecubitals, medium size forearm, hand and foot veins, and 25 G or smaller needles are used only for the smallest veins or for small children and newborns (32). Although there are no definitive indications nor recommendations on the influence of the needle size used to collect venous blood on the results of laboratory testing, it is widely accepted that blood must be withdrawn carefully to avoid excessive pressure or shear stress, which is associated with damage or rupture of blood cells, especially erythrocytes. Hemolysis is usually defined as the release of hemoglobin from erythrocytes into the surrounding plasma due to loss of integrity of the red cell membrane (46). Although hemolysis may occur both *in vivo* or *in vitro*, the latter is a rather common and mostly unfavorable occurrence, as it spuriously influences accuracy and reliability of laboratory testing due to (I) leakage of hemoglobin and other intracellular components into the surrounding fluid, which induces false elevations of some analytes or dilution effects, (II) chemical interference of free hemoglobin in the analytic reaction, and (III) method and analyte con-

centration-dependent spectrophotometric interference (49-50). The primary determinants of hemolysis are conditions resulting in reduced erythrocyte membrane strength, mechanical damage or deformation, entity and duration of exposure to shear stress. Therefore, *in vitro* hemolysis might be predicted by a simple equation that fits the main characteristic (intensity and duration) of the wall shear stress, and a reliable hemolysis threshold can be established for almost each condition tested (46). The major problem encountered when using small-bore needle in association with evacuated tubes is the large vacuum force applied to the blood, which may cause shear stress on the erythrocytes, enhancing the risk of *in vitro* hemolysis (46,51-52), especially when needleless connectors are used (53). Additionally, due to the slower flow within smaller needles, such as those equal to or more than 25 G, the blood is more likely to clot, causing needle occlusion or spurious variations in the results of some analyses, such as slightly increased coagulation activity or decreased platelet count (54-56). Conversely, the use of an excessively large bore needle, usually greater than 19 G, results in a more forceful and turbulent blood flow through the entire collecting system, which might finally predispose the sample to hemolysis (46,57). Therefore, decision on the most appropriate needle size to be used depends on a balance between the characteristics of the venipuncture site and the risk of obtaining clotted and hemolyzed specimens. Additional variables to be considered are the minimum amount of blood required for testing, the age and the psychological attitude of the patient. In this perspective, a 21 G or slightly larger needle is recommended for easy accessible antecubital veins. In fact, 19-21 G needles allow appropriate flow into the collecting system, thus minimizing the probability of increasing preanalytic variability. The 23 G needle might be reserved for newborns and small children, small and fragile veins, provided that a small amount of blood is required (32). Therefore it seems ideal for children because its size provides an extra measure of reassurance. Moreover, because of its thinness, the needle is less likely to collapse the small, delicate hand veins of adults or to collapse or traumatize the fragile veins of geriatric patients. On the other hand, owing to the concerning hemolysis rate, the use of 22/23 G or smaller needles in adults is not recommended (58,59).

### Tube collection (sequence and immediate management)

Assay interferences from blood collection tubes represent challenges to clinical laboratories because they are not detected by the usual quality control or proficiency testing programs. Thus, a standardized sequence for orderly specimen collection is advisable to ensure reliable results of laboratory testing and meet with increasing quality requirements. Disordered blood specimen collection can constitute a major source of error, especially

when different types of tubes are used. There is still an open debate on the most appropriate sequence of drawing blood into evacuated tubes. Turned on in 1977, the order of draw was at first aimed to prevent the effects on test results that tube additives could introduce in a sequence, with the needle carrying on some contamination to the following tubes. In the past decades, however, the order has undergone radical changes, including separate indications when tubes are filled by syringe (60). In 1998, the standards organization recommended a single order of draw for both tube-holder collection and syringe draws. Since then, the widespread use of plastic serum tubes containing clot activators required the development of a modified order of draw. Accordingly, two distinct indications have been developed for glass and plastic tubes (61). In response to this development, which had contributed to add confusion, in January 2004 the National Committee for Clinical Laboratory Standards (NCCLS) announced further changes affecting the procedures of the collection of diagnostic blood specimens by venipuncture, including a new and simplified order of draw that could apply to both glass and plastic tubes (62). Nevertheless, as even the concentration of the additive in the tube is no longer standard, tubes made on different materials have to be tested separately when new reagents are to be compared. According to this revised document, the following sequence is suggested: blood culture tubes should be collected as firsts, followed by non-additive tubes, coagulation (buffered sodium citrate anticoagulant) tubes and, finally, additive tubes in the following order: tubes containing a gel separator and clot activator, sodium heparin anticoagulant tubes with or without a gel separator, ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes, acid citrate dextrose containing tubes and oxalate/fluoride tubes. If routine coagulation testing is the only test ordered, a single non-additive tube should be firstly drawn and discarded to remove potential contamination by tissue fluids or thromboplastins released during venipuncture. An additional guideline on the collection, transport, and processing of specimens for coagulation tests was later released in 2003, updating prior editions that recommended to discard a tube when drawing blood for prothrombin time and activated partial thromboplastin time testing, on the basis that some studies showed that tissue thromboplastin did not affect results even when the sodium citrate tube was the first or the only tube drawn (63). This is consistent with the recent evidence of a substantial agreement of routine coagulation testing among specimens collected during separate consecutive venipunctures or multiple subsequent blood samples collected on the same phlebotomy (64). The new guideline, however, emphasizes that proof of necessity for drawing a discard tube for other coagulation tests is "circumstantial at best," but data suggesting that this practice is unnecessary have not yet been published. The second issue when dealing with procedures immediately associated with venous blood collection is the use of inappropriate containers. The use of an incorrect

tube for a given test still accounts for as much as 13% to 16% of the unsuitable specimens (5,15). To ease blood collection within the appropriate tube, manufacturers have finally featured a wide and heterogeneous series of different color coded tops, but this problem still continues to plague clinical laboratories (65). Additionally, according to the NCCLS guidelines, blood drawn into an evacuated tube system is not acceptable for analysis of arterial blood gases, because there is no way to anaerobically introduce the blood from the tube into the blood gas analyzer (66).

Besides the order of drawn and collection of blood within the proper tubes, some additional pre-analytic problems might arise from inappropriate procedures for collection and handling of the specimen within or immediately after phlebotomy. A major issue is the appropriate filling and mixing of the tubes, as insufficient specimen quantity to perform the test and clotted specimens are, respectively, the second and third most frequent reasons for rejection, right behind hemolysis (14, 15). This is crucial when a proper anticoagulant to blood ratio should be established, such as in specimens for coagulation testing, or a homogeneous mixture of blood and clot activator is needed to allow complete clotting of the specimen. Anticoagulant-containing blood collection tubes must be filled to the proper level (usually to complete vacuum volume) and then gently inverted several times (six to eight times) to allow effective mixing of blood and anticoagulant (spurious variations of laboratory testing can arise if the mixing of blood and additive is not done carefully) but without provoking hemolysis or clotting (63). The use of blood collection tubes with calibrated fill-lines on the outside should be encouraged to prevent under-filling or unnecessary laboratory blood loss (67). Accurate mixing of clot-activator tubes is also necessary to prevent development of fibrin strands in the specimen, which requires re-centrifugation and delays the time of analysis (68). The use of applicator sticks to dislodge the fibrin clot should be avoided, as it may cause further damage to blood cells and hemolysis.

It has been reported that under-filled tubes with blood may significantly affect some results of laboratory testing, especially the activated partial thromboplastin time (APTT) and the prothrombin time (PT), resulting in artifactual prolongation of these tests. However, there are no univocal indications on minimum tube filling that should be ensured to achieve reliable results, as results are largely dependent on the final concentration of the buffered sodium citrate in the specimen and the sensitivity of the reagents employed (69). Reneke et al. demonstrated that accurate PT values can be obtained from normal or pathologic specimens if the tubes are filled to more than 65% (using a moderately sensitive thromboplastin reagent), and to more than 90% (using a highly sensitive thromboplastin reagent). Prolonged APTT values can be observed only in specimens filled to less than 90% of capacity (70). By using 3.8% citrate, a statistically significant difference in the results of PT

and APTT assays can be observed in samples filled less than 80% and 90%, respectively (71). The effect was less pronounced when samples were drawn into 3.2% sodium citrate, as statistically significant differences were reached in PT and APTT results from 3.2% citrate tubes with fill volumes less than 60% and 70%. Pediatric blood collection tubes should be filled at least 90% full to ensure accurate PT test results (72). The current NCCLS recommends that coagulation samples should be discarded if the evacuated tube contains <90% of the expected fill volume (63). This is probably the most appropriate indication that should be provided to phlebotomists, who are not necessarily informed about the effect of final concentration of the anticoagulant in the test tube, and neither on the thromboplastin sensitivity of the laboratory assay. Unfortunately, there is little evidence so far on the minimum filling volume for samples collected in EDTA and heparin containing tubes. However, when the concentration of lithium heparin in the tube is increased to three and five times normal (reflecting incomplete tube filling), alanine aminotransferase, amylase, aspartate aminotransferase, lipase, and potassium all exhibit some changes when compared with serum (73). Additionally, small blood volumes collected by heparinized sampling devices in pediatric samples might lead to excess heparin that may significantly affect sodium determinations and yields false reports of critical hyponatremia (74). These data suggest that all the anticoagulant tubes should be completely filled to prevent spurious intraindividual variations in serial specimen analysis.

Pediatric and capillary tubes are now available for collecting minimum amounts of blood in newborns, animals or patients with difficult venous accesses and they have the contextual potential to decrease collection volumes without compromising the ability of the laboratory to report a reliable and timely result (75). Capillary tubes use capillary action to draw the blood into the microtube, thereby eliminating the tendency for scooping. When filled end-to-end, the length of the capillary tube defines a specific amount of collected blood. As some of these capillary tubes can also be coated with anticoagulants and the blood mixes immediately with them while being collected, they are also suited for routine hematology and coagulation testing. As hemolysis is negligible and the difference over traditional vacuum tubes in results of routine laboratory testing does not reach clinical significance, the use of either capillary or pediatric tubes is suitable for the collection of blood for many of the tests commonly ordered (72,76-79), though a higher percentage of rejected specimens can still be recorded for microcollection tubes than for other containers (14).

Serum or plasma separator tube facilities were introduced nearly 30 years ago and are now widely used in clinical laboratories for routine blood collection, as they provide excellent agreement of test results when compared with non-gel tubes (80). These tubes have gained widespread acceptance due to the advantage of the bar-

rier gel that facilitates rapid separation of serum or plasma from cellular constituents of blood, thus preventing artifacts from either hemolysis or a prolonged cell-plasma interface. However, besides gel stability and analyte incompatibilities, there are some other limitations associated with gel tubes (81). Regardless of the presence of separators, a four-to-six times gentle inversion of the tube is necessary for most tubes containing anticoagulants or clot activators, as this process creates a more homogeneous mixture between blood and additives, allowing a complete anticoagulation or clotting of the sample (62). It was recently reported that elevated potassium values might be encountered in gel tubes with additives. This is likely to occur from improper mixing of clot-activator gel tubes (82) or delayed centrifugation of heparin gel tubes (83). That is because blood cells, especially platelets, release potassium as the specimen clots. Therefore, in the presence of incomplete anticoagulation or clotting of the specimen, the potassium concentration is likely to increase in a time-dependent fashion, especially in samples with substantially increased platelets and white blood cell counts (82,83).

## IDENTIFICATION AND PREVENTION OF ERRORS DURING BLOOD COLLECTION

Although the widespread implementation of automation technology and robotics within clinical laboratories will further decrease the impact of the pre-analytic phase on the reliability of laboratory testing, it is not expected to exert such a substantial influence on phlebotomy errors. Therefore, alternative policies should be acknowledged. Three complementary strategies must be advocated to reduce the burden of phlebotomy errors: (I) implementation of reliable strategies for error identification, an essential prerequisite to identify the phlebotomy activities most inclined to errors, (II) the adoption of effective preventive measures and educational policies, (III) implementation of extra-analytic quality indicators which should enable constant monitoring and improvement of this crucial process of the laboratory workout.

### Error tracking and identification

Regardless of the source, the magnitude of medical errors documented in the 1999 Institute of Medicine report "To Err Is Human", highlights the need for establishing and implementing state-based mandatory reporting systems for medical errors (84). Thus, improving laboratory performance requires accurate procedures for identifying the processes which are more susceptible to errors or uncertainties (85). Although a general hierarchy of errors can be established on the current literature, the extreme heterogeneity of the phlebotomy facilities worldwide calls for translation of this process to a local basis. This policy involves an effective system to systematically identify, track and monitor laboratory errors



within the local setting. Systems focused on analytic errors, which represent only a minor percentage in the total testing process, will not serve this purpose (5,6). Thus, effective approaches will have to be based on highly sensitive systems, able to unmask errors within the pre-analytical phase. The main problem to overcome is that it is still extremely difficult for the laboratory staff to identify all pre-analytic errors, as most of them will neither produce detectable abnormal results nor raise questions for the user (5). Error identification on the basis of either complaints or fortuitous detection might lead to a substantial underestimation of the problem. Development and implementation of rigorous policies of process analysis to identify the error risk related to different procedures is likely to produce the best results and will also permit considerations on the indexing of specific responsibilities and errors to either patient or tests performed (5). In fact, if incidents are simply reported in absolute terms without relating to the activity, it is not possible to interpret whether performance is adequate by any of the means for comparison (86). In general, the most suitable approaches encompass accurate error detection before or within the phases of the testing process (e.g. hemolytic, icteric, lipemic serum, deviations from previously determined total protein concentration or other suspect deviations), harmonizing the categories of relevance of the effect on patient outcome. The specimen rejection rate, one of the most effective quality indicators of specimen acceptability, might also be profitable. Thus, institution-specific error detecting systems should be identified, applied, monitored and targeted for improvement efforts. To be really effective, these systems must employ efficient data collection methods, techniques for analysis, and feedback mechanisms; action thresholds should be set sufficiently low to assure that continuous improvement is effected (14).

### **Preventive measures and educational policies**

The practice of phlebotomy generally refers to the collection of venous blood. Accordingly, phlebotomists are health care professionals who have been trained to collect blood specimens, who usually work under the supervision of physicians, nurses, medical technologists or laboratory personnel. Phlebotomists should be considered as an integral part of the health care system, as their function is to provide laboratory specimens suitable for laboratory testing. Backgrounds, educational requirements and training policies as well as certification facilities for phlebotomists are rather heterogeneous worldwide, ranging from mere practical skills to interpretative abilities. Thus, phlebotomists might range from employees with no medical or laboratory background to certified phlebotomy technicians and medical technologists, who have distinct backgrounds on the theory and practice of specimen collection and handling, along with notions on basic principles of laboratory testing.

Regardless of the local requirements, healthcare workers responsible for specific tasks, such as phlebotomy, must be properly educated and motivated to perform those tasks with as few errors as possible, as the key to reduce medical errors is to focus on improving the systems of delivering care and not to blame individuals. Restrictive acceptance policies and severe intolerance criteria for inappropriate specimens might turn out to be temporarily useful, though proactive efforts to intervene further upstream the arrival of the specimen in the laboratory might grant major benefits on the long run (87). Therefore, written policies and protocols detailing the management and the gradation of responsibilities and providing contingencies when those responsibilities are not met are essential. The main objectives of training should encompass those aspects most frequently encountered while performing venipunctures, including notions of anatomy and physiology (location of veins, nerves, tendons and arteries), characteristics of devices (needles, syringes, tourniquets, disinfectants, evacuated tube systems), phlebotomy techniques, employability skills and emergency procedures. Moreover, basic notions of psychology, mostly aimed at providing relational, reassuring skills directed either to adults or to children should be given as part of the training. The background of a phlebotomist should also encompass participation and possibly coordination of health fairs and blood drive events, participation in continuing education courses. Before entering the work force, phlebotomists should also achieve a minimum clinical curriculum, based on repeated practical experience, which should allow them to accomplish the entire phlebotomy process at the best. Finally, phlebotomists must take part in continuing education programs and supplemental training for personal and professional enhancement, or to cross-train into another related allied health care profession.

In the United Kingdom (88) as well as in most European countries, no formal certification is required to perform venipuncture, only training, often in the field. In New Zealand, phlebotomists must have a high school degree and First Aid Certificate, with all other skills being taught on the job (89). Australian phlebotomists must be registered nurses and have at least two years experience in this profession. In the United States, requirements vary by state, with some requiring certification (90). Thus, while a degree for phlebotomists is not always required, most countries and the U.S. are working towards more standardized training, including certification of health care professionals directly involved in blood drawing responsibilities, on the basis of formal phlebotomy training programs which include frontal didactic activities and field practice (90). Accordingly, some local institutions and organizations, such as the U.S. National Healthcareer Association (NHA), the American Society of Phlebotomy Technicians (ASPT), the U.S. National Phlebotomy Association (NPA), the American Society for Clinical Pathology (ASCP), the American Medical Technologists (AMT), the American

Certification Agency (ACA), the U.S. National Accrediting Agency for Clinical Laboratory Sciences (NAACLS) and the U.K. National Association of Phlebotomists (NAP), among others, offer certification or degree facilities which will finally earn the title of Certified Phlebotomy Technician (CPT) or Registered Phlebotomy Technician (RPT), which might also be internationally recognized. As even skilled phlebotomists can run into difficulties, certification programs might be very useful to increase the rate of successful phlebotomies, ensuring greater safeness for both patient and operator, but also preventing erroneous laboratory results caused by improper collection procedures (91). Since its formation, the Coalition for Phlebotomy Personnel Standards has spent much effort to introduce professional standards for phlebotomists and other specimen-collection personnel in several U.S. states (92). In this perspective, the Coalition should be regarded as a prior and essential task to improve the quality within the entire laboratory testing process. This is a first qualified step to pursue valuable objectives but, unfortunately, similar education tools did not encounter much success so far, nor were they acknowledged by the national health systems of most Western countries (93). Thus, although there are only few state licensures for phlebotomists as yet, certification is expected to gain easy success, as certified phlebotomists will have greater chances for employment and better salary opportunities.

### Extra-analytic quality indicators

The numerous publications that have emerged on quality management in recent years emphasize the importance of quality assessment schemes for assessing and eventually improving performance (94). Although scheme design and application are rather heterogeneous among quality assessment programs, most of them still lack extra-analytic indicators which should reliably monitor key processes of the pre-analytic phase, such as sample collection (86,95). The Clinical Laboratory Improvement Amendments (CLIA) have recently acknowledged that quality management programs should comply with evaluation of all the steps comprising the total testing process (95). Recognition of reliable pre-analytic and phlebotomy performance measures is an ongoing process, which is expected to encounter much more difficulties than the analytical phase, where quality indicators have been well defined (imprecision, systematic error and inaccuracy) (97). Nevertheless, definition and adoption of extra-analytic indicators and related quality specifications are pivotal steps for purposes of further quality improvements (39).

Extra-analytic requirements, performance goals and continuous monitoring of quality indicators, including acceptability of laboratory specimens, afford an opportunity to document the influence of longitudinal tracking on quality improvement. Once reliable error detection

systems are established, service providers can continuously monitor performance to verify the degree to which phlebotomists comply with required procedures and with which services their intended outcomes are achieved (98). Thus, it is essential that systems designed to eliminate errors include elements of redundancy located downstream in the process (99). Error rates in laboratory practices are collected routinely for a variety of tests worldwide, but a list of critical performance indicators has not yet been accomplished. Appropriately chosen indicators have the potential to reflect the efficiency of a particular process or outcome and should hence be considered a tool for quality assessment once sensitive baselines for error identification are set. A suitable approach is to select a number of reliable performance measures or key continuous indicators, which concern patient identification (patient misidentification, wristband errors, implausible changes in cheap and fairly constant personal parameters, e.g. cholesterol and alkaline phosphatase) and specimen acceptability (incidents in sample collection and transport, insufficient sample volume, inappropriate collection container, hemolyzed, lipemic, icteric or clotted specimens, sample lost/not received, inadequate ratio volume sample/anti-coagulant) (86). All laboratories should consider implementing these performance indicators and standardizing their own scientific designs, data analysis, and error reduction strategies accordingly (6,97). As quality is not static, measures in which there is a broad range of demonstrable performance initially are most optimal for subsequent improvement using continuous monitoring. Key continuous indicators, as those chosen on the basis of a decade's experience in the CAP Q-Probes quality improvement program, might represent a reliable paradigm for strategies designed to improve both performance and resource allocation (100). There are already ongoing experiences on this subject. Some Italian laboratories are currently promoting a network of excellence, designed to investigate markers of effectiveness of laboratory services and to share common experiences of using them in clinical practice (98). Preliminary data are available on indicators of quality in all phases of the total testing process, from appropriateness of test requests to data interpretation, and hence including indicators of several pre-analytic causes of specimen rejection. This first experience might stimulate further debate in the scientific community, encouraging more clinical laboratories to use the same indicators to improve clinical effectiveness and outcomes within the healthcare service. Indeed, extra-analytic quality indicators would also apply to physicians' offices and various other locations that perform low-risk tests with no routine regulatory oversight, where a high personnel turnover rate, lack of understanding of good laboratory practices and inadequate training might lead to serious quality concerns about practices that could lead to errors in testing and poor patient outcome (101). In this respect, the international normal ISO 15189 has recently set a maximum standard, reviewing preanalytic issues that should

be documented, controlled and defined. Nevertheless, some connatural pitfalls and practical problems in carrying out witness audits of the ISO 15189 were recently acknowledged. In particular, whereas the preanalytic phase can be at least in theory controlled completely in and by a centralized hospital laboratory, for example by the use of "sample-collection teams", the problems in preparation and transporting of samples from a peripheral practice to a decentralized analytical laboratory cannot be fully controlled at reasonable costs or efforts (102).

### CONCLUSIONS

Clinical laboratories have undergone radical changes due to technological progress and economic pressure (98). However, errors will always plague the results of laboratory testing, regardless of the continuous efforts for identification and prevention (84,86). Since the 1960s there is a rather long history of quality requirements in laboratory medicine (103). Many of the developed standards for quality improvement have pursued the analytical phase. However, there is increasing awareness that clinical laboratory quality systems entail vigilance of the entire production process, including phases involving non-laboratory personnel who manage a large part of the variability that influences the reliability of results. Guidelines for collecting samples and for evaluating submitted specimens are essential, because acceptance of improper specimens for analysis may lead to erroneous information that could affect patient care; but only by monitoring the rejected specimens on a regular basis and identifying factors associated with the rejection can we avoid errors and promote continuous quality improvement of the laboratory service (5,87). On this basis, direct control of the extra-analytical quality is expected to emerge as a major task for the laboratory community in the near future. The remedy is at the levels of identification and investigation of the systemic problems leading to the errors, going to the root of causes to help design solutions, designing effective strategies for prevention, implementation of educational policies, and corrective actions based on the designed strategy, continuous outcome measurement and monitoring (104).

Although whole process tracking and error identification are features of laboratory medicine, a significant part of the variables that influence the quality of the testing process still escape direct control or supervision by the laboratory. In particular, the lack of a conceptual framework on phlebotomy is a noticeable deficiency. Thus, how much should laboratory professionals extend their influence outside of the laboratory walls? This is much more a political rather than a practical issue that might offer advantageous revenues not only to the laboratory community but to health care providers. While health care administrators often struggle with this issue, laboratory professionals might be in the ideal position to

sustain knowledgeable information, training and education of phlebotomists, providing proficient assistance to overcome most pre-analytic problems and spreading operative guidelines that should encompass a clear description of the correct procedures for specimen collection and handling (93). Without enforcement of these principles, remarkable purposes of global quality improvement of commercial laboratories and hospital systems might be unproductive and lead to wasted efforts, increase health expenditure and put patients at the risk of being misdiagnosed, suffering medication errors, or of being otherwise mismanaged.

It is undeniable that the most effective policies might be targeted at reducing the opportunities for making errors throughout the whole testing process. In Germany, for example, a training program available on CD-Rom and called DIAPRO was started. The program contains texts and examples to train future medical professionals in preventing errors during phlebotomy. Moreover, the French nursing organization routinely plans courses and lectures on sampling and phlebotomy in the French speaking area of Europe and the Spanish Society of Clinical Biochemistry and Molecular Biology has already included pre-analytic quality criteria into their quality assurance schemes (105). Further indications regarding amount, type of specimens and stability data during storage and transport of samples can be obtained from a booklet available in seven languages issued by Working Group on Preanalytical Quality supported by the German Society for Clinical Chemistry and the German Society for Laboratory Medicine and approved by the Forum of European Societies of Clinical Chemistry (FESCC) (106). Abating the number of unnecessary tests and limiting the number of steps in which specimens are delivered to laboratories, tests are performed, and results provided to the requesting physician might be additional but not alternative objectives, along with other pre-analytic issues not immediately associated with the phlebotomist's activity, such as the appropriate utilization of the laboratory, the accurate collection of essential clinical information about the patient (age, sex, pathologies, drug therapies, body position, feeding habits and patient preparation), circadian rhythm of several analytes, thoughtful acknowledgement of the main sources of biological variability (physical exercise, diet, stress), sample transport from decentralized phlebotomy facilities, and storage (85,87,99).

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