Opinion Paper

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Preanalytical quality improvement: in quality we trust

Abstract

Total quality in laboratory medicine should be defined as the guarantee that each activity throughout the total testing process is correctly performed, providing valuable medical decision-making and effective patient care. In the past decades, a 10-fold reduction in the analytical error rate has been achieved thanks to improvements in both reliability and standardization of analytical techniques, reagents, and instrumentation. Notable advances in information technology, quality control and quality assurance methods have also assured a valuable contribution for reducing diagnostic errors. Nevertheless, several lines of evidence still suggest that most errors in laboratory diagnostics fall outside the analytical phase, and the pre- and postanalytical steps have been found to be much more vulnerable. This collective paper, which is the logical continuum of the former already published in this journal 2 years ago, provides additional contribution to risk management in the preanalytical phase and is a synopsis of the lectures of the 2nd European Federation of Clinical Chemistry and Laboratory Medicine (EFLM)-Becton Dickinson (BD) European Conference on Preanalytical Phase meeting entitled "Preanalytical quality improvement: in quality we trust" (Zagreb, Croatia, 1–2 March 2013). The leading topics that will be discussed include quality indicators for preanalytical phase, phlebotomy practices for collection of blood gas analysis and pediatric samples, lipemia and blood collection tube interferences, preanalytical requirements of urinalysis, molecular biology hemostasis and platelet testing, as well as indications on best practices for safe blood collection. Auditing of the preanalytical phase by ISO assessors and external quality assessment for preanalytical phase are also discussed.

Keywords: errors; laboratory medicine; preanalytical phase; quality; standardization.

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Introduction

With the dark shadows of one of the biggest financial crises ever since the second world war still spreading throughout

most industrialized and emerging countries, policy-makers and national governments are urgently realizing spending reviews and other economic frameworks to prevent unnecessary waists and limit the downward spiral of internal economies. In several countries the dramatic consequences of this awkward scenario have also inflated and eroded resources of national healthcare systems, which are now struggling to maintain unaltered the access to care, contain spiraling healthcare costs or maintain sustainable gains in coverage. It may seem paradoxical to most, but in several countries the substantial reduction of healthcare funding realized within pressing health care reforms not only has strongly infected anticompetitive practices of healthcare organizations, but also those healthcare areas that are traditionally virtuous for their highly accountable and easily monitored budgets, thus including in vitro diagnostic testing [1, 2]. As a consequence, clinical laboratories are now squeezed between a rock and a hard place, where the need to preserve a high degree of quality is weighted against a lower economic support by central governments.

There is a common misconception about quality of care, wherein healthcare is frequently assimilated with other "industries" as a product or service that helps somebody and enjoys a good and sustainable market. The quality of care is, however, much more than a simple and academic notion, since it has now became crucial for patient wellbeing and for economical survival of the healthcare itself [3]. More credibly, quality of care consists of "… the degree to which health services for individuals and populations increase the likelihood of desired health outcomes, are consistent with current professional knowledge, and meet the expectations of healthcare users" [4]. The field of laboratory diagnostics has been a forerunner in pursuing total quality, inasmuch as safeness and excellence throughout the total testing process have been firmly established more than a century ago [5], and continually reaffirmed with publication of original studies, case reports, editorials, critical reviews and even meta-analyses, that have swollen the pages of this journal since its birth, 50 years ago [6].

Quality in laboratory medicine should be defined as the guarantee that each single step throughout the total testing process is correctly performed, thus assuring valuable medical decision-making and effective patient care [7]. In the past decades, a 10-fold reduction in the analytical error rate has been achieved thanks to improvements in the reliability and standardization of analytical techniques, reagents, and instrumentation [8]. Notable advances in information technology, quality control and quality assurance methods have also assured a valuable contribution for reducing uncertainties, so that the overall rate of laboratory errors is now established at nearly 4.6 sigma, improved by nearly 0.15 sigma (or

otherwise 1600 ppm) in the past 10 years and, much lower than the chance of losing your luggage in the airport (i.e., 4.0 sigma) or suffering from a diagnostic error in radiology (i.e., 3.24 sigma) (Figure 1) [9]. However, whilst current quality indicators in laboratory medicine still tend to focus on the performance and efficiency of analytical processes, several lines of evidence suggest that most errors in the total testing process actually fall outside the analytical phase, and the pre- and postanalytical steps have been found to be much more vulnerable [10–13]. The minor attention paid to extra laboratory factors is thus in stark contrast with the body of evidence pointing to the multitude of errors that continue to occur in the preanalytical phase. This article, which is the logical continuum of the former already published in this journal 2 years ago, provides additional contribution to risk management in the preanalytical phase and is a synopsis of the lectures of the 2nd European Federation of Clinical Chemistry and Laboratory Medicine (EFLM)-Becton Dickinson (BD) European Conference on Preanalytical Phase meeting entitled "Preanalytical quality improvement: in quality we trust" (Zagreb, 1–2 March 2013) (http://www.pre-analytical-phase.org/node/1). The leading topics that will be discussed include quality indicators for the preanalytical phase, insights about phlebotomy practices including collection of suitable samples for blood gas analysis and pediatric testing, lipemia and blood collection tube interferences on clinical chemistry assays, preanalytical requirements of urinalysis, molecular biology hemostasis and platelet testing, as well as indications on how safe sharps and other best practices should be implemented and monitored. A general overview about auditing of the preanalytical phase by International Organization for Standardization (ISO)
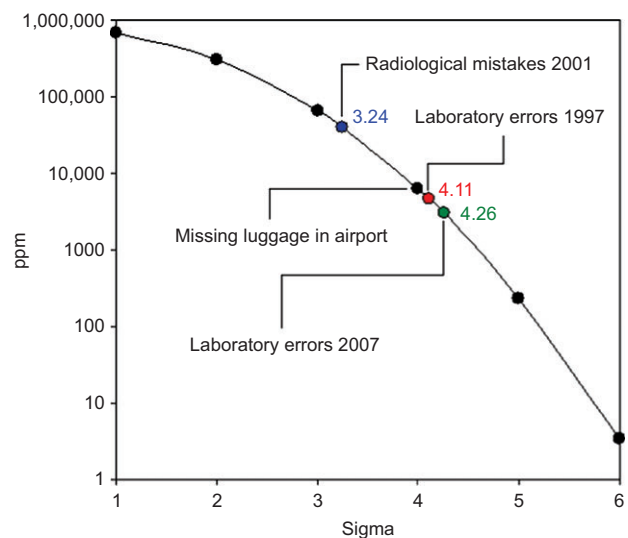

Figure 1 Six sigma metrics of laboratory errors.

assessors and external quality assessment for preanalytical phase will also be provided.

The EFLM working group on the preanalytical phase

It is a common perception that excellence in laboratory diagnostic is often perceived as a synonymous of analytical quality. The newly established EFLM working group WG-Preanalytical phase is intended to fill a gap in the current international scientific activity about total quality in laboratory diagnostics. With already existent WGs, either supported by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) or EFLM, being focused on the analytical quality or on quality indicators (see below), the aims of the EFLM working group WG-Preanalytical phase are to assess excellence in current preanalytical practices, identify some of the most critical elements and make recommendations to reduce the impact of the preanalytical phase in laboratory medicine for the most critical segments of this framework [12]. Several educational and scientific activities are already ongoing. An international, educational meeting supported by the EFLM has already been successfully organized in Parma, Italy in 2011, while the second edition will take place in Zagreb, Croatia in 2013. The focus of both conferences is the management of quality of the preanalytical laboratory practices, by designing attractive scientific programs with interactive discussions and e-voting sessions to enable the exchange of ideas and knowledge related to some most common issues and everyday problems. Another important initiative is a European survey concerning phlebotomy practices, containing 16 questions, which will help identify by whom phlebotomy is done and what level of education is required for this specific task. It is our intendment that the outcome of this survey will contribute to define reference (best) practices in this field, as well as issue recommendations or guidelines about this essential but often neglected laboratory practice.

Quality indicators in the preanalytical phase

According to the approach of the Institute of Medicine (IOM) to quality in healthcare, the identification of reliable quality indicators (QIs) is a crucial step in enabling users to quantify the quality of a selected aspect of care, by comparing it against a defined criterion. A quality indicator is thus

"an objective measure that potentially evaluates all critical care domains as defined by the IOM (patient safety, effectiveness, equity, patient-centeredness, timeliness and efficiency), that is based on evidence associated with those domains, and can be implemented in a consistent and comparable manner across settings and over time". Therefore, when assessing the quality of laboratory services by QIs, it is important to ensure systematic and consistent data collection and analysis by using a comprehensive set of indicators that address all stages of the total testing process, and focus on the areas with an important impact on patient care and health outcomes. The need to harmonize proposed QIs has also been emphasized, as several projects have been launched in different countries including Spain, Brazil, Australia, Croatia, China and the UK, but with the use of different approaches and quality measures [14]. In 2008, the IFCC launched a working group named "Laboratory errors and patient safety", its primary goal being the identification and evaluation of reliable QIs and related quality specifications for addressing all the stages of the total testing process [15, 16]. The prerequisites for selected QIs were: 1) relevance and applicability to a wide range of clinical laboratories at an international level; 2) scientific soundness, with a focus on areas of great importance for quality in laboratory medicine; 3) feasibility, both regarding the data availability and the definition of thresholds for acceptable performance; and 4) timeliness and possible use as a measure or surrogate of laboratory improvement. Up to 56 QIs have been identified, 34 of which are in the preanalytical, seven are in the intra- and 15 are in the postanalytical phase. The aims of, and steps undertaken in the IFCC-WG program have already been described and preliminary results presented for better addressing future steps of the project particularly as regards pre- and postanalytical indicators.

Consequences of blood collection errors

The value of laboratory responsibilities to patient-centered care and safety is becoming increasingly apparent, especially as new sophisticated laboratory testing becomes available, particularly complex genetic tests. The preanalytical phase, including each step in venipuncture is exceptionally crucial because most of the preanalytical variables and factors can be controlled by the health-care professional collecting biological samples [17–19]. As every step in the formulation of test results must be of superlative quality, major focus should be redirected toward quality assurance aspects of patient preparation,

safety, specimen collection and specimen transportation to avoid preanalytical errors in laboratory testing. The variables found when dealing with patients and laboratory situations require professional problem-solving skills.

Since phlebotomy is the most commonly performed medical procedure worldwide, the administrative directors, pathologists, supervisors and phlebotomists need to take the time to routinely review the high-risk procedures that can lead to adverse problems and incidents affecting patient safety [20]. Based upon the generated list, the controllable (preventable) vs. non-controllable variables in preanalytical procedures need to be assessed as to the ones that have impact on preanalytical errors and patient safety. Also, inviting input from the patients and their families is becoming a communicative source for improving the safety and quality of the laboratory testing.

Some major physiological variables that are controllable and can affect the results of laboratory analysis include diet, physical exercise, circadian rhythm, posture or prolonged bed rest, travel and other lifestyle factors. Healthcare professionals involved in blood collection must be educated to become cognizant of the communication skills that should be used prior to blood collection to oversee the controllable variables, including fainting and patients who have had a mastectomy [21]. The healthcare professional must be proactive in asking the patient who is to have his/her blood collected questions regarding these medical conditions and have the knowledge base to move forward in a safe and risk reduced blood collection procedure.

Patient safety related to patient identification is an ongoing controllable challenge in all types of blood collection procedures. Also, using the correct blood collection equipment in venipuncture and skin puncture is an ongoing preanalytical challenge and requires continuous educational updates as equipment is changed in the healthcare institutions. Controllable variables, including tourniquet and fist pressure, are constant issues that must be overseen in appropriate blood collection. Hemolyzed blood is the most common reason for rejecting specimens in the laboratory and, therefore, in-depth knowledge on how to properly enter the vein with the correct blood collection equipment to avoid hemolysis in the collected specimens is a must for the phlebotomist. Improper blood collection tubes lead to numerous preanalytical errors and can adversely affect patient laboratory results and safety. Risk reduction in using blood collection equipment with congruent pieces is an everyday challenge for healthcare institutions in attempting to maintain their staff with the latest knowledge base on these issues. These are examples of the many controllable variables that can be overseen to assist in better blood analyte results, less patient errors, and improved patient safety.

Blood gas collection

Arterial blood gas (ABG), which typically entails the puncturing of a superficial artery for drawing arterial blood by means of a thin needle and small syringe, is mainly performed for gathering useful information about blood gases. It is thereby mainly aimed at measuring the arterial oxygen tension (PaO_2), carbon dioxide tension ($PaCO_2$), and pH, along with other useful parameters such as oxyhemoglobin saturation (SaO_2), partial pressure of carbon dioxide (CO_2), bicarbonate and blood lactate. As for other in vitro diagnostic tests, ABG may be vulnerable to errors throughout the total testing process, especially in the preanalytical phase [10–12]. The peculiar biological matrix of this test that is the use of whole heparinized blood, makes however rather challenging the identification of some preanalytical errors, especially those attributable to interference from spurious hemolysis, lipemia and turbidity. Nevertheless, recent studies suggest that the chance of processing unsuitable blood may be noting but meaningless in this setting. In an investigation of all samples referred for ABG in the laboratory of the large Academic Hospital of Parma, which were systematically centrifuged after testing had been completed, Lippi et al. found a rate of visible hemolysis of 1.2% [22]. In a further study, Salvagno et al. assessed the serum indices after centrifugation of all routine and stat samples referred for ABG analysis to the clinical laboratory of the Academic Hospital of Verona over a 2-month period [23]. Interestingly, 28% of these displayed at least one serum index exceeding the cut-off (27% from the emergency department and 29% from clinical wards). More specifically, 4% of samples displayed a hemolysis index over 60, 11% a lipemic index over 30, and 13% an icteric index over 2. The rate of hemolyzed specimens from the emergency department was exactly double that of clinical wards (6% vs. 3%), whereas the rate of other types of unsuitable specimens was globally comparable (i.e., 9% vs. 12%, and 12% vs. 14% for lipemic and icteric specimens, respectively). This data attest that preanalytical non-conformance may be frequent in ABG analysis, at rates comparable and even higher than those recorded for other in vitro diagnostic tests. Whenever highly deviating test results are produced or when there are reasonable doubts about the quality of the ABG specimen, it may henceforth be advisable to set a rapid centrifugation of the sample followed by assessment of the serum indices on the plasma. This can be regarded as a reliable approach to identify potentially unsuitable samples and prevent unreliable test results from being released.

Pediatric blood collection

Children should not be considered as little adults or miniatures, and their unique physical and mental development, growth, nutrition and diseases should not be overlooked in all medical fields. Pediatric care is provided in various environments, including offices of primary care physicians, pediatricians, general practitioners, public health clinics, acute care facilities, general hospitals, children's hospitals, as well as academic medical centers. Laboratory diagnostics in outpatient settings differ from in-hospital testing. However, laboratory results are affected in all pediatric settings, and predominantly by preanalytical variables. Patient age can hence be considered one of the most important preanalytical variables [24]. Each step of blood collection in selected pediatric age group such as the preparation of the child before the procedure with or without the help of parents, preparation of the blood collection site, equipment for specimen collection, the specimen collection itself, and handling and storage of the specimen is thereby unique.

The performance of venipuncture in both infants and children requires special training and skill, particularly for sites such as scalp and jugular veins and umbilical artery catheters. In infants, <5% of the total blood volume should be removed in a single draw, but several blood draws per day may be requested in acutely ill inpatients [21, 25]. The most suitable mean to reduce the risk of iatrogenic anemia and transfusion is obviously the reduction of sample volume. Skin puncture procedure in laboratory testing is traditionally more frequent in children than in adults, the heel and fingertip being the more common sites. The University Children's Hospital in Bratislava offers a wide range of inpatient and outpatient services. The hospital is a 397-bed pediatric facility, consisting of 11 clinics and eight departments, with centers for cardiology, pediatric dialysis, bone marrow and liver transplantation, and inherited metabolic diseases. The study performed in the hospital hematology laboratory contributed to implementation of an innovative blood collection tube Becton Dickinson (BD) Microtainer® MAP Microtube for Automated Process (MAP) which is intended for the collection, anticoagulation, transport and storage of skin puncture specimen for routine hematology testing on automated hematology systems, and is targeted for pediatric, geriatric, oncology, neonatal intensive care unit patients, as well as for the general population in selected circumstances [26].

The current procedures for blood collection should be safe for patients during various periods of infancy, childhood and adolescence, and at the same time it should provide quality specimen and sufficient volume for laboratory testing. On one side detailed guidelines, special equipment and devices, qualified and experienced health workers are needed, on the other manufacturing and implementation in the laboratory testing practice of new types of pediatric-sized tubes and advanced automated laboratory instruments with minimized specimen volumes and dead volume lower than 50 μL may be required for achieving further improvements.

Blood collection tube interference on clinical chemistry assays

Substantial changes in the tubes that are used for collection of blood for most laboratory tests have occurred over the past two decades. Two of the most popular changes include: 1) a polymer gel in the bottom of the evacuated blood collection tubes; and 2) replacement of plastic for glass as the primary tube component. These changes provided a number of practical operational advantages, such as reduced centrifugation time, ability to use primary collection tubes for testing, increased sample stability in collection tubes, decreased breakage hazard, decreased weight, and suitability for disposal by incineration [27]. Blood collection tubes are, however, much more complex devices than is commonly appreciated by most laboratory professionals. Commercial tubes have multiple components that contribute to the optimal separation of serum or plasma for laboratory analysis. For example, in glass blood collection tubes, the glass interior surface itself plays a key role in the activation of blood coagulation [28]. Recent use of plastic, as the principal material of collection tubes, has required the addition of silica particles or other clot activators for optimal formation of serum. These particles may be coated with compounds, such as polyvinylpyrrolidone (PVP), to assist the adherence of the particles to tube walls and facilitate the rapid dispersion of silica into the blood specimen. The interior surface of blood collection tubes is also usually coated with a surfactant to minimize the adherence of blood cells to the tube, which reduces hemolysis and better distributes the clot activator (e.g., silica) along the tube wall. Without a surfactant, the hemolysis of red blood cells after incomplete separation from serum will alter the serum composition over time. Stoppers of tubes also require a coating with a lubricant to improve their ease of removal and to maintain the vacuum. Separator gels are also a common component of blood collection tubes, and they serve as a barrier between serum and clot or plasma and cells, respectively, after centrifugation of tubes. A well-recognized drawback

of serum/plasma separator tubes is the potential for the separator gel to absorb hydrophobic compounds such as some drugs [29]. Another problem is that the gel itself is unstable under extreme temperature conditions and can produce an oily film in serum/plasma, which in turn can obstruct instrument probes with subsequent downtime.

In a previous study that has been performed on the Diagnostic Products Corporation Immulite 2000/2500 analyzer by plastic Becton-Dickinson (BD) blood collection tubes [30], an interference likely attributable to a tube additive, Silwet L-720 (also shared by other plastic BD tubes) was observed. In response to the immunoassay interference, the manufacturer has reformulated the blood collection tubes to reduce the amount of tube additives [31].

Other than increased vigilance when inspecting laboratory results and improving the feedback between the clinical laboratory and clinicians, there is not much that clinical laboratories can do to readily detect blood collection tube problems. It is impractical for clinical laboratories to repeat a tube evaluation study with each new lot of tubes, but laboratories should consider comparing results from separate lots of the same tube type to detect any lot-to-lot variations when first evaluating a new tube. Similarly, it is impractical for tube manufacturers to test their tubes on all the various assay platforms, but they should ensure consistency in the amount and quality of any tube additives throughout the whole manufacturing process. Diagnostics companies may also help identify future tube problems, by providing detailed information not only on the tube type but the commercial source of the tubes that they use when determining the reference interval for any new assay. Any reference interval study done by diagnostics companies on previously developed assays with older tubes that are no longer widely used, such as glass tubes, should ideally be repeated with tube types that are currently used by their customers. Thus, since the quality of patient care depends on the quality of all the information that a physician uses in making treatment decisions, blood collection tubes should be manufactured to an extremely high standard like other medical devices.

Lipemia

Lipemia is traditionally defined as serum or plasma turbidity caused by increased lipoprotein (especially triglycerides-rich lipoproteins) concentration. The prevalence of lipemia is lower than that of other unsuitable specimens such as those clotted or hemolyzed, but still appears in approximately 1% of samples. Besides pathophysiological

conditions, preanalytical laboratory errors account for a large proportion of lipemic samples (i.e., improper time of sampling after a meal or after intravenous infusion of triglycerides-rich emulsions for parenteral nutrition) and, as such, can be prevented [11].

There are several mechanisms causing lipemia interference in laboratory testing. Lipemia interferes with nearly all photometric measurements by light scattering and absorption. The extent of interference is related to lipoprotein sample size and number of particles. In electrophoretic and chromatographic methods, increased proportion of lipoproteins may cause additional peaks or background noise. When measuring electrolyte concentration by flame photometry and indirect potentiometry, falsely decreased concentrations are observed due to reduced volume of sample water phase. After centrifugation, lipemic samples are not homogenous, which results with falsely decreased concentrations of water soluble constituents in the upper lipid layer [32].

Although most of the current laboratory instrumentations offer automated detection of turbidity degree, some laboratories still rely on optical identification. Besides being highly subjective and arbitrary, this approach is unsuitable when a large number of samples have to be inspected [33]. Analyzers may detect the degree of lipemia by measuring sample specter on several wavelengths. However, there is a large variability across systems, both in wavelengths used, and also in the ways of finally expressing results. Some studies have also been published, which described falsely elevated lipemic indexes in clear samples, due to increased concentration of paraproteins or some interfering components (methylene dye). Thus, samples with unusually high lipemic indexes should also be systematically inspected afterwards.

Several protocols of eliminating lipemia prior to laboratory testing have been proposed. According to the Clinical and Laboratory Standards Institute (CLSI) recommendations, ultracentrifugation should be considered as the preferred approach. Since this kind of equipment is unavailable in most laboratories due to high costs, high speed microcentifuge can however be considered as efficient when lipemia is predominantly due to chilomicrons [34]. Several other methods are also based on the physical removal of the lipoprotein layer (extraction with hydrophobic solvents or precipitation). Nevertheless, attention should be taken when assessing the concentration of hydrophobic components in these samples because as a result of lipoprotein removal, their concentration is then falsely decreased in the aqueous phase.

There are also several problems that should be considered when dealing with lipemic samples. There is no

adequate simulation protocol for testing lipemia. Most laboratories spike samples with triglyceride rich infusions (e.g., Intralipid). It has however been described that Intralipid-induced lipemia and native lipemia of the same extent (measured by the lipemic index) do not produce the same bias on measured results [35]. The manufacturer's recommendations about lipemia interferences are not unified, and in most cases describe only the effect of added Intralipid solution. Therefore, each laboratory should check these recommendations on samples with native lipemia and have detailed protocols for identifying, eliminating and reporting results from lipemic samples.

Adherence to guidelines

Medical guidelines are evidence-based and include consensus of best practice in healthcare. Healthcare staff are obliged to know relevant medical guidelines and decide on their application for individual patients. Adherence to guidelines standardizes medical care, raises care quality and reduces patient risks. Laboratory results following venous blood sample collection and analysis are important in the clinical diagnosis and treatment of patients, so ordering of analyses as well as blood drawing procedures should always adhere to medical guidelines. However, venous blood sample collection guidelines are not always followed [36, 37], and so interventions may be needed to reduce patient safety risks.

The low preanalytical error rates noted by hospital laboratories calls for large databases as well as appropriate techniques for the detection of errors and their consequent reduction. Comparisons of error rates and the effect of interventions have therefore not been possible to study at individual hospital wards or physicians' offices. To be able to assess frequent "near-misses" (i.e., mistakes that may lead to adverse events) during blood drawing would allow for comparison to guidelines, benchmarking of preanalytical practices between wards and physicians' offices and to follow the effect of corrective educational interventions than the current assessment of underreported incidents or registered rare adverse events. We therefore developed and validated a self-reported questionnaire to assess frequently occurring, error-prone everyday blood drawing tasks at the hospital ward/physicians' office level [38]. To reflect varying preanalytical conditions at this healthcare level, we also used the high frequency of low-level hemolyzed venous blood serum samples instead of the low frequency of high-level hemolyzed samples, which is commonly used for rejection of specimens to avoid analytical interference [39]. To assess primary healthcare physicians ordering of analyses we compared the ordering frequency of analyses to medical guidelines for a number of diseases.

The venous blood sample collection questionnaire surveys showed, e.g., that hospital ward staff deviated from guidelines as 20% stated that they sometimes labeled test tubes after sampling away from the patient and 10% did not always compare patient identification with the test request. Of physicians' office blood drawing staff, only 54% always performed patient identification according to guidelines, 6% stated they always allow patients to rest the recommended time prior to sampling, and 12% to release venous stasis as soon as possible during sampling. Samples collected in the physician's office with the highest prevalence of (low-grade) hemolysis were 6.1 times (95% CI 4.0–9.2) more often hemolyzed as compared with the physician's office with the lowest prevalence. Rural physician's office serum samples were hemolyzed 1.7 times more often compared to the urban physicians' office samples [39]. The national diabetes guidelines regarding the frequency of glycated albumin and urinary albumin analysis ordering were followed by physicians in 12 and 10 physicians' office out of 32 in the County Council, respectively.

Repeated questionnaire surveys and monitoring of sample low-level hemolysis of hospital ward and physicians' offices staff practices would highlight specific problems and make it possible to follow the effect of corrective actions. We performed a large-scale short educational intervention on all (approx. 2500) County Council staff, but venous blood sample collection handling practices were not improved (low-level sample hemolysis remained unchanged) [40], whereas patient identification, test tube labeling and information search procedures improved (questionnaire survey – manuscript) when comparing 11 physicians' offices before and after intervention.

Given the instruments to assess the frequent preanalytical "near-misses" at all healthcare levels, the challenge to the laboratory medicine profession is now to join in finding effective educational methods to increase healthcare staff adherence to medical guidelines.

Preanalytical requirements of urinalysis

Sediment analysis has been the gold standard in urinalysis, whereas automation (i.e., automated microscopy, flow cytometry) has improved accuracy. Since urine samples

are collected by patients, urinalysis is highly susceptible to preanalytical problems, and focusing on these issues is essential to improve reliability of testing since preanalytical demands will be stricter as a test tends to provide more reliable results. Informing patients involves more than only explaining the practical aspects of sampling, as the influence of biological factors (e.g., exercising, contamination) should be particularly stressed. Illustrated instructions can be provided, while procedures should take into account patient characteristics, e.g., the presence of a catheter. Midstream (clean-catch) portions of first morning urine samples collected in a sterile container are recommended. Overnight bacterial growth in the bladder is possible, and bacteriuria can influence the formed elements. Higher reproducibility is achieved by using second morning urine. Red blood cell morphology remains a separate analysis; correct assessment depends on pH and osmolality. Washing the intimate parts minimizes contamination, but the use of soap or antiseptics is dissuaded due to its influence on germs [41].

Container design should enable easy sampling and ensure optimal transport. Requirements can be added in the function of procedures (e.g., amber-colored containers for light sensitive analytes). Besides the use of a primary container, it is advised to aliquot the original sample for chemical, microbiology and morphology analyses. Vacuum systems allow sample aspiration into secondary containers, but can only be used for chemical analysis, since the pressure difference during aspiration causes disintegration of casts. In low conductivity samples, red blood cell count is lower in vacuum tubes. Although it is not good practice, there can be a tendency to dip strips in the container. It is recommended to transfer urine to the strip instead of immersing the strip into the sample [41].

Increased time lag between sampling and analysis, lack of temperature control and use of non-preserved samples not analyzed within 2 h can decrease sample quality. Alkaline pH, low density and low osmolality promote lysis. Stabilizers prevent metabolic changes and bacterial growth. Ethanol and polyethylene glycol are used to preserve cells. Containers prefilled with boric acid, formic acid or other stabilizers are also used [42]. Red blood cells are difficult to stabilize, in contrast to white blood cells, casts and epithelial cells. Adding formaldehyde results in bad preservation of red blood cells. Preservatives can also inhibit protein degradation. The 24 h collection represents the reference method for stable analyte quantification (protein:creatinine ratio is an alternative). No preservatives are necessary if strip testing is performed within 24 h and the sample is refrigerated. However, when refrigeration is unfeasible, preservatives can thus be added, the choice of which depends on the analyses, since some enzymatic reactions may be negatively affected. Boric acid keeps the pH acid, but may affect strip reactions; its addition impairs correct pH measurements. Preservative containing tubes preserve strip results for 6–24 h (exceptions are glucose and nitrite). Particles should be examined within 1 h (at room temperature) or 4 h (refrigerated), to prevent lysis. Refrigeration causes precipitation of urates and phosphates. Particle lysis is pronounced with higher pH and lower density. Long standing samples show a pH increase. *Proteus* sp. produce urease, resulting in an increase of pH. Undissolved powder causes a background signal in flow cytometric red blood cell counting. There is no preservative that can be used to stabilize urine for strip and particle analysis [41].

Preanalytical phase for molecular biology testing

Molecular biology testing and proteomic techniques are rapidly evolving methods in clinical laboratories and could move the diagnostic procedures on a higher level than classical methods. As other fields of IVDs, the high diagnostic potential must be accompanied by higher quality assurance. Analytical technologies for molecular biology and proteomic analysis are on very high level, but the preanalytical phase is still not clear enough, nor specified. The biggest problem is the high degree of heterogeneity of methods as well as samples used.

As regards DNA, formalin-fixed paraffin-embedded samples may be stored for years, since both DNA and RNA are fairly stable. The pre-fixing steps are established for many years and are not likely to be changed. The biggest problem is the extraction of nucleic acids, as they are often degraded, cross-linked to proteins and modified. Automated extraction is available and should henceforth be preferred [43]. Extracted material is usually well-suited for polymerase chain reaction (PCR), sequencing faces to DNA breaks and other DNA modifications [44].

As regards amniotic fluid and chorionic villus, the samples should be handled very carefully as they are usually irreplaceable. Contamination of amniotic fluid by maternal sources could be decreased by the cultivation of amniotic fluid cells, but it does not help in chorionic villus samples. Standard procedures, published by American College of Medical Genetic must be followed (e.g., in a recent survey of 35 laboratories in the US, 60% tested the appearance of maternal cells, only) [45].

The assessment of free DNA and/or RNA in plasma is increasingly used in laboratory diagnostics and is

probably one of the most appealing perspective areas in molecular biology testing. The differences between plasma and serum are substantial, plasma being considered the most suited material. The concentration of DNA in serum is usually higher due to the release of cell-free DNA from leukocytes during clotting, which has implications mostly in testing cell-free DNA in cancer, trauma and transplant patients [46]. Fetal DNA in maternal plasma is considered to have the same concentration as in maternal serum. Careful centrifugation, followed by the additional step of microcentrifugation or filtration is effective in producing cell-free plasma. Extraction by phenol-chloroform-isoamylalcohol seems to be preferable. However, the stability of sample differs and for short-term storage (i.e., up to 8 h) EDTA plasma may be used, but for longer storage the use of special sample tubes (e.g., PAX gene™) is advisable. The typical concentration of free DNA in plasma in healthy persons varies from 0 to 100 mg/mL of blood (on average 30 ng/mL), the vast majority being as double-stranded DNA. Although the clearance mechanisms have not been fully elucidated, free fetal DNA in maternal blood is cleared rapidly after birth, with an usual half-life of approximately 16 min.

MicroRNA are also receiving growing interest. At variance with DNA, its plasma concentration is higher than in serum, but the removal of cellular and subcellular components is effective to reduce the plasma concentration to values similar to the serum. MicroRNA are stable at room temperature for up to 24 h, refrigerated or frozen for up to 72 h. Hemolysis has different influence on different microRNA, with the concentration of some of them being increased in hemolyzed specimens.

The peptidome analysis of human urine is challenging, since urine has many potential variables, including sampling, storage, freezing conditions, freeze/thaw cycles pH, urine salt and proteins, blood and bacterial presence. Separation of proteins by magnetic beads, followed by MALDI-TOF mass spectrometry is henceforth recommended.

In conclusion, no consensus has been reached so far on the best preanalytical practices in the field of molecular biology and standardization of all preanalytical activities in molecular biology and proteomic analyses is thereby deeply advisable and needed.

Preanalytical phase for hemostasis and platelet function testing

A number of preanalytical variables have been shown to affect the results of many laboratory tests including those related to hemostasis and thrombosis [28, 47–51]. One important preanalytical variable affecting some clotting tests as well as platelets is hemolysis [52]. Some coagulation analyzers are already available with automated preanalytical checks. The CS2100i (Sysmex Corporation), e.g., has novel features related to processing samples which contain interfering substances including hemoglobin. For all clotting endpoints transmitted light is monitored at five different wavelengths (340, 405, 575, 660 and 880 nm). The instrument automatically checks test plasma for the presence of hemolysis (as well as icterus and lipemia) and flags results to alert the operator. We decided to investigate the effect of hemolysis on routine coagulation tests. Samples with visible hemolysis that failed the empirical acceptance criteria were retained (n=59), and analyzed alongside matched samples from the same patients which were free of interference and had been collected within 4 h of the former. We measured free hemoglobin in the sample with hemolysis, which ranged from 0.5 to 9.0 g/L (mean 1.7 g/L). In relation to some individual cases, the differences were sufficient to influence patient management. For example, in four samples where the baseline D-dimer result was below the cut-off for venous thromboembolism, D-dimer was found to be falsely elevated above the diagnostic threshold in those with visible hemolysis, which could lead to further and unnecessary examinations depending on the probability scoring. Five activated partial thromboplastin times (APTTs) with one reagent were more than 4 s different between clear and hemolyzed samples, with changes in both directions. False normal APTTs were obtained in hemolyzed samples from two patients when analyzed with a second APTT reagent.

Under-filling of citrated blood samples is another critical preanalytical variable, which can affect the results of several – if not all – clotting tests. It has been reported that for 5 mL siliconized glass tubes (Vacutainer, Becton Dickinson) there is no difference between the results obtained from 100% full tubes and 60% full tubes for Prothrombin Time (PT) and 70% full for APTT [53]. Identical results were reproduced in further studies [54]. It is likely that the effects of under-filling may depend on the blood collection tube used, including composition, inner dimensions and the air space to surface area ratio. Under-filled 3 mL plastic tubes (Vacutainer Plus, Becton Dickinson) which failed the local acceptance criteria (i.e., containing <80% of target volume) were retained (n=18) and analyzed with a Sysmex CS2100 alongside matched samples from the same patients which contained 95%–100% of target fill volume and were collected within 4 h of the former. All samples had normal hematocrit values. Under-filling ranged from 56% to 79% (mean, 67%). The results of

screening tests (PT, APTT, thrombin time) were significantly longer when tubes were under-filled, frequently to the extent that patient management decisions could be affected. Fibrinogen was significantly lower in under-filled samples. Therefore, blood samples from subjects with normal hematocrit values collected into these 3 mL tubes should not be accepted for analysis if <80% full.

Platelet function testing by optical aggregometry has proved difficult to standardize and one reason is the important influence of preanalytical variables. Some guidance is available in relation to this [55], which recommends the following: collect samples after fasting and resting; use 19–21 g needle (butterfly cannulae allowed if blood flow is not restricted); do not use the first 3–5 mL collected for platelet function testing; use evacuated or syringe collection; utilize 105–109 mM trisodium citrate (preferable buffered to help maintain pH); maintain at room temperature; do not transport via pneumatic tube system; keep interval between collection and analysis preferably between 30 and 120 min, but <4 h. Platelets responses to agonists are labile following collection, so we investigated platelet responses to standard agonists in platelet rich plasma (PRP) from five normal subjects prepared from whole blood that had been stored for up to 5 h. The mean maximum aggregation responses are shown in the Table 1, and it can be seen that important deterioration in responses were beginning after 3 h storage. We conclude that for these reagents testing should be performed within 2 h of sample collection.

Auditing of the preanalytical phase – ISO assessors

Several medical laboratories in Europe are accredited according to the ISO15189 standard, although the percentage of accredited laboratories varies widely among countries, with frequency ranging from 0% to 75% [56]. There is an ongoing debate on the best way to harmonize this process, but there are also concerns regarding how thoughtfully the preanalytical phase would be covered by the accreditation process. Important issues include, e.g., appropriate test requisition, patient preparation, phlebotomy performed outside the laboratory (perhaps by a different staff), sample stability and handling of other sample materials such as saliva, cerebrospinal fluid or sampling from catheters [11]. The issues are complex and widespread, and often the accreditation process tends to focus on the capability of the laboratory production itself. Of note, preanalytical errors will of course still be prevalent in an accredited laboratory [57], although the substantial decrease in the number of significant non-conformities was observed in accredited laboratories over time, suggesting that ISO15189 does contribute to the quality improvement of accredited laboratories [58]. Moreover, even though a variety of preanalytical issues are actually covered in the ISO15189, it is often challenging to address the same topics as an ISO assessor along with all the other important aspects in the accreditation procedure.

Auditing of the preanalytical phase – practical overview

Due to the complexity of the preanalytical phase it has not been possible to standardize preanalytical processes to the same extent as those of the analytical phase, despite its importance for sample quality, and hence laboratory efficiency, or patient or healthcare worker safety [59]. To investigate preanalytical procedures and practices with a consistent method for all blood collection systems, standardized data collection forms were used to enable auditing of the preanalytical practices in hospitals in different countries. The processes of blood collection were assessed from storage of the blood collection materials throughout blood specimen collection itself, transport and processing of the samples within the laboratory and the resulting sample quality. By following the blood samples through the complete process, it was possible to link specific preanalytical attributes to sample quality deficiencies. Observation of the processes on the ward also allows assessment of behavior, which is important for the safety of healthcare workers.

The preanalytical phase has been observed for 3597 blood collection tubes over 1350 blood collection procedures. Sample quality was assessed for 8016 chemistry

Agonist	Time interval, h					
	<0.5	1	2	3	4	5
	Mean maximum aggregation, %					
Ristocetin (1.25 mg/mL)	80	78	77	68	58	50
ADP (3.0 µmol/L)	58	78	80	70	57	49
Collagen (1.0 µg/mL)	44	45	41	36	26	18
Arachadonic acid (1.5 mmol/L)	92	92	92	84	69	26

Table 1 Platelet responses to standard agonists in platelet rich plasma (PRP) form five normal subjects prepared form whole blood that had been stored for up to 5 h.

and 3532 coagulation tubes. For all clinical chemistry samples, 8.9% showed some level of hemolysis. For the hemolyzed samples where the preanalytical phase had been observed, 47% had prolonged use of tourniquet, 31% were drawn through catheters and for 38% the disinfectant was not allowed to dry before venipuncture, three possible causes of hemolysis [60]. For serum clinical chemistry samples, 5.9% showed fibrin formation postcentrifugation. Where fibrin formation was observed in serum samples, 26% had less than the manufacturers recommended clotting time and 81% had not been mixed impacting the suspension of the clot activator. Additional observations were; including incorrect identification procedure of the patient (56%), tubes labeled prior to collection (61%), coagulation tubes filled to <90% of nominal tube volume (7%), which has the potential to lead to incorrect analytical results [53]. Gloves were not worn in 37% blood collection procedures, and the activation of the needle safety device was incorrect in 19%. In conclusion there are still considerable improvements that can be made in order to improve processes and practices that are key to sample quality, and hence laboratory efficiency, or patient or healthcare worker safety. Accordingly, standardized audit methodology enables an institution to compare results between different areas of the hospital in order to understand how different practices are impacting sample quality. The prospective nature of the audits permits identification of issues within an institution based on more data than that from rejected samples and therefore affords a more complete understanding for all of those involved in the preanalytical phase. The results can be used in a targeted manner for information and training purposes, providing the direct link between the blood collection practice and the resulting sample quality issues.

External Quality Assessment for the preanalytical phase

According to the ISO 15189 5.6.4, External Quality Assessment (EQA) programs should, as far as possible, provide clinically relevant challenges that mimic patient samples and have the effect of checking the entire examination process, including pre- and postexamination procedures [61]. Although a lot of focus has been given to the preanalytical phase, claiming that it accounts for most of the laboratory errors, few initiatives have been taken to incorporate this into regular EQA. One of the reasons for this is probably that the examination of preanalytical errors are best done in local settings in specially designed studies. When it is incorporated into EQAs, it is usually done by circulating questionnaires or asking participants to register onto a web site to report their practices and errors [62]. Sometimes samples addressing preanalytical problems (e.g., hemolysis or lipemia) are incorporated in traditional EQAs, or else specific samples containing defined amounts of interfering substances may be prepared and, with results further assessed for comparability and bias across distant laboratories, even using rather different equipment [63, 64].

Cases reports: the preanalytical detective

The preanalytical phase is a complex and dynamic process differing not just from one hospital environment to the next, but within the hospital as well. Preanalytical errors often cause random errors undetectable by normal quality control methods. In order to determine the cause of these random errors, it is necessary for the laboratory professional to become a sort of "detective". Through a series of deductions and research, (s)he can identify the cause and put corrective actions in place, whenever possible.

A number of case studies illustrate this approach, e.g., why fibrin masses were created in serum samples after overnight shipment, which led to samples requiring additional processing steps? What caused elevated potassium results, which led to a patient being admitted as an emergency but whose potassium normalized upon admission? In this latter case there were several incidents, where the potassium analysis of the original specimen was confirmed by repeat analysis on an additional tube collected at the same time. Results were also confirmed and reproduced on another analyzer. There was no visible hemolysis or procedures that were likely to lead to hemolysis. In another case patients were admitted for bypass surgery and had postoperative complications. Although, their preoperative potassium was within the normal range, the postoperative concentration had risen to >7.0 mmol/L. This led the laboratory professional to question whether this was a collection or rather a laboratory error [65]. Finally, why do high levels of proteins (e.g., >135 g/L) cause sporadic inappropriate separation of plasma and serum from cells in gel separation tubes [66]?

In all of these cases, procedures and patient treatment regimes normally outside the control of the laboratory led to preanalytical errors that have impacted on sample quality, laboratory efficiency and patient care. Recommendations should hence be issued and followed to support strategies and practical policies that laboratories can implement, to reduce the impact of the preanalytical errors, and thereby increase laboratory efficiencies and reduce the potential for inappropriate patient care [67].

Conflict of interest statement

References

1. Barletta G, Zaninotto M, Faggian D, Plebani M. Shop for quality or quantity? Volumes and costs in clinical laboratories. Clin Chem Lab Med. [Epub ahead of print]. DOI: 10.1515/cclm-2012-0415.

2. Lippi G, Mattiuzzi C. Testing volume is not synonymous of cost, value and efficacy in laboratory diagnostics. Clin Chem Lab Med [Epub ahead of print]. DOI 10.1515/cclm-2012-0502.

3. Lippi G, Simundic AM, Mattiuzzi C. Overview on patient safety in healthcare and laboratory diagnostics. Biochem Med (Zagreb) 2010;20:131–43.

4. Buttell P, Hendler R, Daley J. Quality in healthcare: concepts and practice. Westport: Praeger, 2008:62–83.

5. Lippi G, Plebani M, Simundic AM. Quality in laboratory diagnostics: from theory to practice. Biochem Med (Zagreb) 2010;20:126–30.

6. Plebani M, Lippi G. Closing the brain-to-brain loop in laboratory testing. Clin Chem Lab Med 2011;49:1131–3.

7. Plebani M. The detection and prevention of errors in laboratory medicine. Ann Clin Biochem 2010;47:101–10.

8. Ceriotti F, Cappelletti P, Caputo M, Di Serio F, Messeri G, Ottomano C, et al. A risk-analysis approach to the evaluation of analytical quality. Clin Chem Lab Med 2011;50:67–71.

9. Plebani M, Lippi G. To err is human. To misdiagnose might be deadly. Clin Biochem 2010;43:1–3.

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. Lippi G, Chance JJ, Church S, Dazzi P, Fontana R, Giavarina D, et al. Preanalytical quality improvement: from dream to reality. Clin Chem Lab Med 2011;49:1113–26.

12. Simundic AM, Lippi G. Preanalytical phase – a continuous challenge for laboratory professionals. Biochem Med (Zagreb) 2012;22:145–9.

13. Plebani M, Lippi G. Improving the post-analytical phase. Clin Chem Lab Med 2010;48:435–6.

14. Plebani M, Sciacovelli L, Lippi G. Quality indicators for laboratory diagnostics: consensus is needed. Ann Clin Biochem 2011;48:479.

15. Sciacovelli L, O'Kane M, Skaik YA, Caciagli P, Pellegrini C, Da Rin G, et al. Quality indicators in laboratory medicine: from theory to practice. Preliminary data from the IFCC Working Group Project "Laboratory errors and patient safety". Clin Chem Lab Med 2011;49:835–44.

16. Plebani M. Quality indicators to detect pre-analytical errors in laboratory testing. Clin Biochem Rev 2012;33:85–8.

17. Miller H, Lifshitz MS. Pre-analysis. In: McPherson R, Pincus MR, editors. Henry's clinical diagnosis and management by laboratory methods, 21st ed. Philadelphia: WB Saunders, 2007:20–30.

18. Clinical and Laboratory Standards Institute (CLSI): The key to quality-the fundamentals for implementing a quality management system in the clinical laboratory. Wayne, PA: CLSI, 2007.

19. Plebani M. Errors in clinical laboratories or errors in laboratory medicine? Clin Chem Lab Med 2006;44:750–9.

20. Clinical and Laboratory Standards Institute (CLSI). Laboratory quality control based on risk management; approved guideline. Wayne, PA: CLSI, 2012.

21. Garza D, Becan-McBride K. Phlebotomy handbook: blood specimen collection from basic to advanced, 8th ed. New Jersey: Prentice Hall Publishers, 2010.

22. Lippi G, Ippolito L, Fontana R. Prevalence of hemolytic specimens referred for arterial blood gas analysis. Clin Chem Lab Med 2011;49:931–2.

23. Salvagno GL, Lippi G, Gelati M, Guidi GC. Hemolysis, lipaemia and icterus in specimens for arterial blood gas analysis. Clin Biochem 2012;45:372–3.

24. Coffin CM, Hamilton MS, Pysher TJ, Bach P, Ashwood E, Schweiger J, et al. Pediatric laboratory medicine. Current challenges and future opportunities. Am J Clin Pathol 2002;117:683–90.

25. Howie SR. Blood sample volumes in child health research: review of safe limits. Bull World Health Organ 2011;89:46–53.

26. Conversion to BD Microtainer® MAP Microtube for Automated Process may be beneficial even in a laboratory setting with low frequency of microcollection specimens. BD Diagnostics Preanalytical Systems, 2010. Available from: www.bd.com/vacutainer. Accessed 1 September, 2012.

27. Landt M, Wilhite TR, Smith CC. A new plastic evacuated tube with plasma separator. J Clin Lab Anal 1995;9:101–6.

28. 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.

29. Dasgupta A, Dean R, Saldana S, Kinnaman G, McLawhon RW. Absorption of therapeutical drugs by barrier gels in serum separator blood collection tubes. Volume- and time-dependent reduction in total and free drug concentrations, Am J Clin Pathol 1994;101:456–61.

30. Bowen RA, Chan YC, Cohen J, Rehak NN, Hortin GL, Csako G, et al. Effect of blood collection tubes on total triiodothyronine and other laboratory assays. Clin Chem 2005;2:424–33.

31. Bowen RA, Chan Y, Ruddel ME, Hortin GL, Csako G, Demosky SJ Jr, et al. Immunoassay interference by a commonly used blood collection tube additive, the organosolicone surfactant silwet L-720. Clin Chem 2005;51:1874–82.

32. Calmarza P, Cordero J. Lipemia interferences in routine clinical biochemical tests. Biochem Med (Zagreb) 2011;21:160–6.

33. Simundic AM, Nikolac N, Ivankovic V, Ferenec-Ruzic D, Magdic B, Kvaternik M, et al. Comparison of visual versus automated detection of lipemic, icteric and hemolyzed specimens: can we rely on a human eye? Clin Chem Lab Med 2009;47:1361–5.

34. Dimeski G, Jones BW. Lipaemic samples: effective process for lipid reduction using high speed centrifugation compared with ultracentrifugation. Biochem Med (Zagreb) 2011;21:86–94.

35. Bornhorst JA, Roberts RF, Roberts WL. Assay-specific differences in lipemic interference in native and intralipid-supplemented samples. Clin Chem 2004;50:2197–201.

36. Söderberg J, Brulin C, Grankvist K, Wallin O. Preanalytical errors in primary healthcare: a questionnaire study of information search procedures, test request management and test tube labelling. Clin Chem Lab Med 2009;47:195–201.

37. Söderberg J, Wallin O, Grankvist K, Brulin C. Is the test result correct? A questionnaire study of blood collection practices in primary health care. J Eval Clin Pract 2010;16:707–11.

38. Bölenius K, Brulin C, Grankvist K, Lindkvist M, Söderberg J. A content validated questionnaire for assessment of self-reported venous blood sampling practices. BMC Res Notes 2012;5:39.

39. Söderberg J, Jonsson PA, Wallin O, Grankvist K, Hultdin J. Haemolysis index – an estimate of preanalytical quality in primary health care. Clin Chem Lab Med 2009;47:940–4.

40. 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 [Epub ahead of print]. DOI: 10.1515/cclm-2012-0159.

41. Coppens A, Speeckaert M, Delanghe J. The pre-analytical challenges in urinalysis. Acta Clin Belg 2010;65:182–9.

42. Kouri T, Vuotari L, Pohjavaara S, Laippala P. Preservation of urine for flow cytometric and visual microscopic testing. Clin Chem 2002;48:900–5.

43. Bohmann K, Hennig G, Rogel U, Poremba C, Mueller BM, Fritz P, et al. RNA extraction from archival formalin-fixed paraffin-embedded tissue: a comparison of manual, semiautomated, and fully automated purification methods. Clin Chem 2009;55:1719–27.

44. Chen W, Cai F, Zhang B, Zhong XY. Strategies of reducing input sample volume for extracting circulating cell-free nuclear DNA and mitochondrial DNA in plasma. Clin Chem Lab Med 2011;50:261–5.

45. Schrijver I, Cherny SC, Zehnder JL. Testing for maternal cell contamination in prenatal samples: a comprehensive survey of current diagnostic practices in 35 molecular diagnostic laboratories. J Mol Diagn 2007;9:394–400.

46. Wagner J. Free DNA – new potential analyte in clinical laboratory diagnostics? Biochem Med (Zagreb) 2012;22:24–38.

47. Lippi G, Franchini M, Montagnana M, Salvagno GL, Poli G, Gudi GC. Quality and reliability of routine coagulation testing: can we trust that sample? Blood Coagul Fibrinolysis 2006;17:513–9.

48. Favaloro EJ, Lippi G, Adcock DM. Preanalytcial and postanalytical variables: the leading causes of diagnostic errors in haemostasis? Semin Thromb Hemost 2008;34:612–34.

49. Adcock Funk DM, Lippi G, Favaloro EJ. Quality standards for sample processing, transportation, and storage in hemostasis testing. Semin Thromb Hemost 2012;38:576–85.

50. Plebani M, Favaloro EJ, Lippi G. Patient safety and quality in laboratory and hemostasis testing: a renewed loop? Semin Thromb Hemost 2012;38:553–8.

51. Favaloro EJ, Plebani M, Lippi G. Quality in hemostasis and thrombosis – part I. Semin Thromb Hemost 2012;38:549–52.

52. Lippi G, Musa R, Avanzini P, Aloe R, Pipitone S, Sandei F. Influence of in vitro hemolysis on hematological testing on Advia 2120. Int J Lab Hematol 2012;34:179–84.

53. Adcock DM, Kressin DC, Marlar RA. Minimum specimen volume requirements for routine coagulation testing; dependence on citrate concentration. Am J Clin Pathol 1998;109:595–9.

54. Lippi G, Salvagno G, Montagnana M, Guidi G, Favaloro EJ. The bias of routine hemostasis testing due to underfilling of primary blood tubes is not mathematically predictable. Annual Meeting of the American Association of Clinical Chemistry (AACC), Los Angeles (CA-USA), 15–19 July 2012. Clin Chem 2012;(Suppl):A17.

55. Harrison P, Mackie I, Mumford A, Briggs C, Liesner R, Winter M, et al. Guidelines for the laboratory investigation of heritable disorders of platelet function. Br J Haematol 2011:155;30–44.

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

57. Simundic AM, Nikolac N, Vukasovic I, Vrkic N. The prevalence of preanalytical errors in a Croatian ISO 15189 accredited laboratory. Clin Chem Lab Med 2010;48:1009–14.

58. Ho B, Ho E. The most common nonconformities encountered during the assessments of medical laboratories in Hong Kong using ISO 15189 as accreditation criteria. Biochem Med (Zagreb) 2012;22:247–57.

59. Bonini P, Plebani M, Ceriotti F, Rubboli F. Errors in laboratory medicine. Clin Chem 2002;48:691–8.

60. Lippi G, Blanckaert N, Bonini P, Green S, Kitchen S, Palicka V, et al. Haemolysis: an overview of the leading cause of unsuitable specimens in clinical laboratories. Clin Chem Lab Med 2008;46:764–72.

61. Ricós C, Biosca C, Ibarz M, Minchinela J, Llopis M, Perich C, et al. Quality indicators and specifications for strategic and support processes in laboratory medicine. Clin Chem Lab Med 2008;46:1189–94.

62. Carraro P, Zago T, Plebani M. Exploring the initial steps of the testing process: frequency and nature of pre-preanalytic errors. Clin Chem 2012;58:638–42.

63. Lippi G, Luca Salvagno G, Blanckaert N, Giavarina D, Green S, Kitchen S, et al. Multicenter evaluation of the hemolysis index in automated clinical chemistry systems. Clin Chem Lab Med 2009;47:934–9.

64. Plebani M, Lippi G. Hemolysis index: quality indicator or criterion for sample rejection? Clin Chem Lab Med 2009;47:899–902.

65. Johnston JB, Messina M. Erroneous laboratory values obtained from central catheters. J Intraven Nurs 19991;14:13–5.

66. Van den Ouweland, Church S. High total protein impairs appropriate gel barrier formation in BD Vacutainer blood collection tubes. Clin Chem 2007;53:364–5.

67. Lippi G, Mattiuzzi C, Plebani M. Event reporting in laboratory medicine. Is there something we are missing? MLO Med Lab Obs 2009;41:23.