EFLM Paper

Giuseppe Lippi*, Fay Betsou, Janne Cadamuro, Michael Cornes, Michael Fleischhacker, Palle Fruekilde, Michael Neumaier, Mads Nybo, Andrea Padoan, Mario Plebani, Laura Sciacovelli, Pieter Vermeersch, Alexander von Meyer and Ana-Maria Simundic, on behalf of the Working Group for Preanalytical Phase (WG-PRE), European Federation of Clinical Chemistry and Laboratory Medicine (EFLM)

Preanalytical challenges – time for solutions

https://doi.org/10.1515/cclm-2018-1334
Received December 17, 2018; accepted January 8, 2019; previously published online February 2, 2019

Abstract: The European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for the Preanalytical Phase (WG-PRE) was originally established in 2013, with the main aims of (i) promoting the importance of quality in the preanalytical phase of the testing process, (ii) establishing best practices and providing guidance for critical activities in the preanalytical phase, (iii) developing and disseminating European surveys for exploring practices concerning preanalytical issues, (iv) organizing meetings, workshops, webinars or specific training courses on preanalytical issues. As education is a core activity of the WG-PRE, a series of European conferences have been organized every second year across Europe. This collective article summarizes the leading concepts expressed during the lectures of the fifth EFLM Preanalytical Conference "Preanalytical Challenges – Time for solutions", held in Zagreb, 22–23 March, 2019. The topics covered include sample stability, preanalytical challenges in hematology testing, feces analysis, bio-banking, liquid profiling, mass spectrometry, next generation sequencing, laboratory automation, the importance of knowing and measuring the exact sampling time, technology aids in managing inappropriate utilization of laboratory resources, management of hemolyzed samples and preanalytical quality indicators.

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

***Corresponding author: Prof. Giuseppe Lippi,** Section of Clinical Biochemistry, University Hospital of Verona, Piazzale L.A. Scuro 10, 37134 Verona, Italy, Phone: +0039-045-8124308, E-mail: giuseppe.lippi@univr.it. https://orcid.org/0000-0001-9523-9054
Fay Betsou: Integrated Biobank of Luxembourg, Luxembourg, Luxembourg
Janne Cadamuro: Department of Laboratory Medicine, Paracelsus Medical University, Salzburg, Austria
Michael Cornes: Worcestershire Acute Hospitals NHS Trust, Worcester, UK
Michael Fleischhacker: DRK Kliniken Berlin Mitte, Clinic for Internal Medicine – Department of Pneumology, Berlin, Germany
Palle Fruekilde and Mads Nybo: Department for Clinical Biochemistry and Pharmacology, Odense University Hospital, Odense, Denmark
Michael Neumaier: Institute for Clinical Chemistry, Medical Faculty Mannheim of Heidelberg University, Mannheim, Germany
Andrea Padoan, Mario Plebani and Laura Sciacovelli: Department of Laboratory Medicine, University Hospital of Padova, Padova, Italy. https://orcid.org/0000-0003-1284-7885 (A. Padoan); https://orcid.org/0000-0002-0270-1711 (M. Plebani); https://orcid.org/0000-0003-3156-1399 (L. Sciacovelli)
Pieter Vermeersch: Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium
Alexander von Meyer: Kliniken Nordoberpfalz and Klinikum St. Marien, Weiden and Amberg, Germany
Ana-Maria Simundic: Department of Medical Laboratory Diagnostics, Clinical Hospital "Sveti Duh", Zagreb, Croatia

Introduction

The European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for the Preanalytical Phase (WG-PRE) was originally established in 2013, with the main aims of (i) promoting the importance of quality in the preanalytical phase of the testing process, (ii) establishing best practices and providing guidance for critical activities in the preanalytical phase, (iii) developing and disseminating European surveys for exploring practices concerning preanalytical issues, (iv) organizing meetings, workshops, webinars or specific training courses on preanalytical issues (Table 1) [1]. The WG-PRE has already achieved many important goals related to its terms of reference and will continued to do so in the future, with the purpose of improving the overall culture of quality in preanalytical phase across Europe and beyond, a goal than could also be achieved by collaborating with other extra-European federations or national associations [2].

Table 1: Terms of reference of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for the Preanalytical Phase (WG-PRE).

– Promoting the importance of quality in the preanalytical phase of the testing process
– Establishing best practices and providing guidance for critical activities in the preanalytical phase
– Developing and disseminating European surveys for exploring practices concerning preanalytical issues
– Organizing meetings, workshops, webinars or specific training courses on preanalytical issues

Education, one of the core activities of the WG-PRE, has been mostly pursued by organizing a series of European conferences every second year across Europe. Four conferences have already been organized in Parma in 2011 [3], in Zagreb in 2013 [4], in Porto in 2015 [5] and in Amsterdam in 2017 [6]. These meetings, which have provided a contribution for improving the quality in the preanalytical phase, have been the largest such conferences across Europe, bringing together over 600 participants at the last occasion. The program of these conferences has been tailored by the Scientific Committee to provide updated knowledge in preanalytics and developing an open forum for interactive discussions and professional improvement.

This fifth collective article is hence the latest of the opinion papers published by the EFLM WG-PRE following from the previous Preanalytical Conferences, and summarizes the leading concepts and issues expressed during the lectures of the fifth EFLM Preanalytical Conference "Preanalytical Challenges – Time for solutions", held in Zagreb, 22–23 March, 2019. The topics covered included sample stability, preanalytical challenges in hematology testing, feces analysis, bio-banking, liquid profiling, mass spectrometry, next generation sequencing and laboratory automation, the importance of knowing and measuring the exact sampling time, technology aids in managing inappropriate utilization of laboratory resources, management of hemolyzed samples and preanalytical quality indicators.

Preanalytical challenges in laboratory automation

Total laboratory automation (TLA) has recently expanded dramatically in many laboratories and has a wide variety of advantages in a high-volume laboratory with 24/7 activity [7]. The automation solution varies from automated equipment for the majority of analyses, to cover the inclusion of an automated sample reception unit and/or track solution delivering the samples to the analyzers and, finally, to automated transportation facility (e.g. tube transportation or a vehicle) that delivers samples directly to reception units. Undoubtedly, automation has significantly improved efficiency and shortened turnaround times (TAT) [8] and has also reduced the hands-on time and thereby the number of (possible) human errors. Nevertheless, there are still many preanalytical caveats that laboratory professionals must address [9]. The more automated the system becomes, the harder it is to unravel errors and perhaps even to discover them in the first place. In the worst case, nothing is noticed before a substantial number of patients are potentially harmed. Further efforts should therefore be made to focus on preanalytical issues within the TLA and facilitating future decision-making in an increasingly automated laboratory environment. No doubt, much will still depend on the specialist knowledge of a laboratory professional, and continuous dialog with clinicians on a number of matters will perhaps be even more important in a fully-automated laboratory to understand and, if necessary, improve test algorithms, reflex testing, as well as to assure deliverance of laboratory test results to the right clinician as expeditiously as possible.

The importance of knowing the exact sampling time and ways to measure it

Several pre- and postanalytical quality indicators in laboratory medicine are strongly dependent on the time the sample is collected. The duration of sample transport is a leading quality indicator, defined by standards such as the International Organization for Standardization (ISO) 15189:2012. As analytical stability for most laboratory tests is time- and temperature-dependent, sampling time information is crucial for qualifying a sample as suitable for being tested. Moreover, sampling time information is indispensable when interpreting test results for therapeutic drug monitoring, hormones and other parameters exhibiting circadian variation [10–12].

Despite its unquestionable importance for accurate analytical and post-analytical sample handling, sampling time information is often missing. Although the retrieval and documentation of correct sampling times may be a major challenge for many medical laboratories, some

facilities have already helped solve this issue. Depending on the local healthcare environment, the problem can be addressed in different ways. In some situations, information technology (IT) solutions may be the most appropriate approach, whilst a more pragmatic and less technical approach might be more sensible in other situations. In any case, human and financial resources need to be defined and allocated before implementing systems or processes.

Several different approaches aiming to solve the problem of retrieving a correct sampling time are currently being developed or are already in use. In order to provide high quality analytics and interpretation of laboratory tests, laboratories need to find a suitable approach for retrieving correct sampling times, fitting properly to their local environment.

Technology aids in optimizing utilization of laboratory resources

Although laboratory testing shall be used for the right patient, using the right test at the right time and with accurate data interpretation, clinicians or nurses do not often fulfill a reasonable approach when ordering tests, especially for inpatients [13]. This may frequently lead to over- or under-utilization of laboratory resources, thus potentially jeopardizing patient health. The reasons for an inappropriate use of laboratory tests include broad laboratory ordering profiles, defensive medicine, insufficient education, availability-triggered demand, among others [14]. Test ordering is hence a framework where laboratory professionals shall provide their medical expertise, assisting the selection of the right test and the accurate interpretation of results, thus more efficiently managing the demand of laboratory resources. This objective can be accomplished by educational interventions or using digital tools integrated in the laboratory information system (LIS). As the overall number of laboratory professionals is typically limited in most healthcare settings, the latter option seems more efficient. Demand management tools, which have proven to be effective, include laboratory diagnostic algorithms, gate-keeping strategies such as re-testing intervals, harmonization and re-evaluation of ordering profiles among others [15]. As a reasonable premise to all efforts made to improve the appropriateness of laboratory test usage, strategies need to be developed in close collaboration with clinicians, based on current evidence and revised/updated on a regular basis. In the future, laboratory professionals shall need to engage far more outside of the analytical part of the testing process, thus providing their vast expertise to benefit patient outcome.

Preanalytical requirements in hematology

Laboratory hematology is an essential part of diagnostic reasoning and managed care of most, when not all, hematologic diseases [16]. As many other areas of laboratory medicine, total quality in hemostasis testing is an essential premise for obtaining reliable and clinically usable data. The preanalytical issues related to laboratory hematology are frequently similar to those of other areas of diagnostic testing, and hence include accurate patient identification, as well as appropriate procedures for sample collection, handling, transportation and storage [16]. Unlike clinical chemistry, immunochemistry and hemostasis testing, however, laboratory hematology has a unique trait, represented by the need to irreversibly inhibit blood coagulation, and hence maintain the sample indefinitely anticoagulated for blood cells enumeration, sizing and differentiation. This can be achieved by using the specific additive ethylenediaminetetraacetic acid (EDTA). The blood collection tubes for hematologic testing typically contain dipotassium EDTA (K_2-EDTA) in a powdered state, coated onto the tube walls. The EDTA mainly acts by irreversibly chelating bivalent ions, especially ionized calcium (Ca^{2+}), which is essential for the appropriate development of blood coagulation, through the establishment of a bridge between negatively charged phospholipids and the gamma-glutamic acid moiety of clotting factors [17]. This would hence require a thorough interaction between K_2-EDTA and blood during tube mixing, to ensure that all Ca^{2+} molecules present in the blood tube are irreversibly chelated. The use of alterative anticoagulant mixtures for laboratory hematology (e.g. lithium-heparin or sodium citrate) is usually discouraged, except in specific conditions such as EDTA-dependent pseudothrombocytopenia [18]. Additional frequent causes of sample non-conformance, especially the presence of small clots or interfering substances such as cell-free hemoglobin (i.e. spurious hemolysis), bilirubin (i.e. icterus) and turbidity (i.e. lipemia), are sources of great concern in laboratory hematology, as the use of whole blood rather than serum or plasma would make their visual or spectrophotometric identification rather challenging or virtually unfeasible. This would need additional tools to be developed (e.g. clot sensors,

algorithms for analyzers, digital morphology), which may finally help to increase the overall quality in laboratory hematology.

Preanalytical issues in feces analysis

Qualitative and quantitative feces analyses are a part of routine laboratory diagnostics, although this material is vulnerable to many sources of variability, related to matrix heterogeneity, sample stability and preparation. Feces is characterized by a high intrinsic variability in density and texture, both within the same sample and among specimens collected at different time points between successive bowel movements [19]. Between-specimens heterogeneity increases in parallel with time from one bowel movement and another. Within-stool heterogeneity can be limited by collecting a representative amount of sample and by sampling multiple spots from different sites within the same specimen. The sampling technique, usually performed either by sample weighting or apposite devices (dipsticks), is another notable preanalytical aspect. Although sampling is performed by trained personnel using dipsticks, a high variability in the amount of collected sample remains (typically >20%). Manual weighting appears more accurate, but less practical for routine analysis, especially in laboratories analyzing large volumes of samples. Regarding sample conservation, it has also been recently demonstrated that fecal calprotectin (fCal) concentration may decrease after 24 h by 12% at room temperature and 13% at 4 °C, respectively [20]. Different preanalytical factors of fecal testing should hence be controlled and standard handling procedures should be followed for obtaining clinically reliable data. Finally, a compeling need has emerged for developing harmonization programs aimed at limiting misinterpretation of test results.

Recommendations for managing hemolyzed samples

Visual inspection of serum indices is highly unreliable and should be replaced by automated systems. Handling and managing hemolyzed samples may lead to reporting errors for some very critical analytes and thus affect clinician reasoning and decision, an example being when an inaccurate result is reported from a hemolyzed sample. On the other hand, patients can also be harmed by an unnecessary suppression of sample results which are unaffected by the degree of hemolysis present. Although this is often not so obvious, sample rejection and subsequent sample re-collection leads to prolonged TATs, thus depriving a patient from a timely diagnosis and treatment. Delayed diagnosis may cause serious harm to patients, jeopardizing their health and well-being. To minimize patient risk, managing samples with a certain degree of hemolysis needs to be highly standardized and preferably even automated, but at the same time evidence-based and when necessary even personalized [21, 22]. The appropriate detection and management of serum indices requires adequate internal (IQC) and external quality (EQA) control mechanisms. Monitoring day-to-day variation of HIL indices should become an essential part of a daily routine in laboratories worldwide. Commercial IQC materials have only recently become available from external suppliers. It should be noted that laboratories can also use in-house IQC materials for this purpose, as a cost-effective alternative. Hence, the EFLM WG-PRE has developed a series of recommendations [23, 24] for the efficient use of serum indices, in an attempt to balance the need to produce high quality laboratory data with a need to improve patient care and outcome.

Sample stability

Pathology results are involved in most patient pathways. It is therefore essential to ensure that laboratory results are of a high quality. However, laboratories can only produce results as accurate as the sample quality allows. Analyte stability is a key part of this, and it is essential that the time and conditions a sample was subjected to and the impact on the result are known. The vocabulary in metrology defines stability as metrological properties remaining constant in time. Biomarker quantification of stability can be defined as how much an analyte deviates from initial concentrations over time [25]. Many replicate studies are performed looking at the same analytes. This is because many studies have been performed on a low number of samples, with data that is often contradictory or incomplete and additional biases are often introduced [26]. The numbers of factors that can affect analyte stability are many. Stability studies are complex and often difficult to apply across different healthcare settings and,

for this exact reason, the EFLM WG-PRE is working on some guidance. Checklist of recommendations of what needs to be considered and documented when designing a stability study are being produced. This does not state how a study should be conducted but does state what information should be included in publications to allow transferability. This will be followed-up by a tool to establish the quality of the data from already performed stability studies. These checklists are based on STARD [27] and should drive standardization and transferability of future studies.

Preanalytical quality indicators

During the Consensus Conference on "Harmonization of Quality Indicators in Laboratory Medicine: two years later" held in Padova (Italy) on October 26, 2016, a list of quality indicators (QI) has been approved on behalf of the Working Group "Laboratory Errors and Patient Safety" (WG-LEPS) of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [28]. A priority order has also been assigned to each QI, based on its relevance (related to the critical activities being monitored) and difficulties in data collection. More specifically, 26 QIs and 53 measurements concerning key processes, along with three QIs and five measurements concerning support processes and outcome measures, have been finally identified. The higher number of QIs with priority 1 (i.e. mandatory registration) relates to activities of the preanalytical phase, thus confirming the importance of QIs for monitoring and eventually improving this particularly error-prone part of total testing process [29]. Additional information collected during the past few years highlights that (i) a relatively low number of QIs has been currently implemented, (ii) difficulties remain in assuring standardized and regular comprehensive data collection and (iii) a low level of participation has been recorded from laboratories belonging to the same country. In order to achieve participation from more laboratories, there should be further discussion on the best strategy for (i) engaging international providers of EQAs in the WG-LEPS, thus improving QI harmonization, (ii) identifying a project leader in each country for better coordinating of the participation of national laboratories to the Model of Quality Indicators (MQI) project and (iii) involving accreditation bodies, so that the MQI project could be recognized as a suitable tool for complying with ISO 15189:2012 accreditation requirements [30].

Preanalytical real-world experience with mass spectrometry

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been used for decades for specialized, anti-doping, toxicology and clinical chemistry testing due to its high selectivity, sensitivity and method adaptability of this technology [31]. It is often postulated that the full advantage of LC-MS/MS technology can only be achieved with highly specialized personnel. Although this may be true for the method-development phase, this is not necessary the case following implementation in an accredited routine clinical chemistry environment. LC-MS/MS technology is a powerful tool when used in a standardized continuous setting, with strict guidelines from sampling to result dispatch. Incorrect sampling and handling often compromises both selectivity and specificity [32]. A neglected fact is that the use of gel-containing blood collecting tubes poses a very high risk for interference, either by introducing high levels of noise or, more frequently, partly concealing the components of interest, and thus posing a risk for falsely low results. Local experiences reveal that therapeutic drug monitoring (TDM) results can be dependent on the collecting tube manufacturer. Even changes in the composition of the phase-separation reagent within the same product-line, can have huge impact on analysis performance. Additional factors such as type of sampling tube from various manufacturer pose a massive validation effort which overwhelms most laboratories, thus only one or two sample matrixes are usually validated for routine analysis [33]. Full- or semi-automated LC-MS/MS is emerging and will become more available in a few years as application menus expand. This expansion is strongly facilitated by correspondence with clinical societies as well as clinical guidelines recommending the use of LC-MS/MS methods.

Standardization of blood draw for liquid profiling

The US Food and Drug Administration (FDA) approval of the first blood-based genetic test for detecting gene mutations of epidermal growth factor receptor (EGFR) in non-small cell lung cancer (NSCLC) has been a milestone for the management of cancer patients. Thus, the genetic characterization of cell-free DNA has become a routine application for the care of NSCLC patients. Additionally, liquid profiling has been deemed useful in clinical studies

for therapy monitoring, prognostic and predictive evaluation of patients' solid tumors other than lung cancer [34]. Nevertheless, many unresolved preanalytical issues remain. It is particularly important to define the optimal method for blood drawing and sample handling before plasma preparation. The use of EDTA blood tubes is still considered the gold standard, although their application for liquid profiling is suboptimal. Blood drawn into these tubes cannot be stored (not to mention shipped to a remote laboratory) and plasma preparation should be performed without delay (maximum 4–6 h when stored at room temperature) [35]. In the last few years several companies have developed new blood draw tubes which are better suited for liquid profiling purposes. These stabilize blood cells, prevent them from lysing and thus from "contaminating" cell-free DNA/RNA with cellular nucleic acids. A more detailed description of data achieved so far and comparison of new tubes with EDTA has been recently reviewed elsewhere [36].

Managing preanalytical variables in bio-banking

The work of biobanks essentially consists of processing biological materials. The input is a collected specimen and the output is a sample to be stored for future analyses. Many biobanks are embedded in clinical diagnostic laboratories, and in this case may be called "clinical biobanks" or "clinical biobank laboratories". What is considered as the "pre-examination" or "preanalytical phase" in clinical laboratories, largely corresponds to what, here, is called "processing". An accreditation standard ISO 20387:2018 (Biotechnology – Biobanking – General requirements for biobanking) has recently been published. It specifies general requirements for competence, impartiality and consistent operation of biobanks, including quality control requirements to ensure biological material and data collections of appropriate quality. Processing methods are the core activity of biobanks and deserve dedicated quality management. The quality management of the preanalytical phase in biobanks includes some new concepts such as the validation of each processing method for reproducibility, robustness, fitness-for-purpose and stability of output specimens [37]. The development and implementation of "in-process quality control materials" is part of the continuous quality assurance, as well as participation in EQA "processing schemes" [38]. The purpose of most of the samples produced by biobanks is not their use in clinical diagnostics, but rather in research projects. Therefore, the analytical methods used for validation of processing methods are generally not clinical diagnostic assays, but techniques designed to assess the fitness-for-purpose of specimens for different categories of downstream research applications [39].

Next generation preanalytics: biomolecular quality and IT approaches

The importance of the preanalytical phase for the overall accuracy and precision of laboratory results is now increasingly being appreciated not only by the laboratory, but also by the sender. Numerous influential factors have been described ranking from indication for testing, preparation for sampling, correct sampling procedures, sample transport and finally the required steps to secure preanalytics within the laboratory prior to testing [40].

The prime criterion for sound preanalytics is the maintenance of biomolecular specimen quality. However, there is neither consensus about how to measure it, nor what suitable parameters may be for that purpose. Plasma/serum can be considered the most complex (liquid) "tissue" by far with hundreds of thousands of different analytes circulating in bodily fluids at any given time. For example, protein biomarker concentrations in the blood are known to span 12 orders of magnitude between, for example, hemoglobin and interleukin-6 [41], and have very different stabilities in clinical samples. Many metabolites cannot be measured under routine conditions due to very short half-lives [42]. In order to assess the clinical validity of a laboratory test result, two variables need to be met. Firstly, the stability of a given analyte in a biological sample needs to be known, while it must be appreciated that its rate of decay may vary under different health conditions in the patient. Secondly, the time-to-analysis needs to be known, while it must be appreciated that important environmental conditions may vary prior to testing.

How to meet ISO 15189 preanalytical requirements?

ISO15189 describes the quality management system requirements for medical laboratories [43, 44]. A recent survey of European medical laboratories by the EFLM WG-PRE found that almost half of all participants were

accredited according to ISO 15189:2012. This number has increased in the recent years, at least in part because accreditation according to ISO 15189 is mandatory in many European countries. An important difference with ISO 17025, which describes the requirements for testing and calibration laboratories, is the explicit requirement to continually improve the effectiveness of preanalytical, analytical and postanalytical processes. Somewhat surprisingly, almost 10% of participants in the recent WG-PRE survey indicated not monitoring any preanalytical quality indicators. However, the ISO 15189:2012 requires the establishment of quality indicators for monitoring and evaluating critical aspects of the preanalytical phase (4.14.7). At the same time, complaints have been raised about differing interpretations by auditors of preanalytical requirements. This suggests that guidance about implementing preanalytical requirements of ISO 15189:2012 might be useful.

Conclusions

In conclusion of this collective article "Preanalytical Challenges – Time for solutions", we wish to thank all our contributors, we sincerely hope that this document may be of interest for the readership of *Clinical Chemistry and Laboratory Medicine* and will provide meaningful support for identifying the issues and opportunities to improve the quality in the preanalytical phase.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

References

1. Cornes MP, Church S, van Dongen-Lases E, Grankvist K, Guimarães JT, Ibarz M, et al. The role of European Federation of Clinical Chemistry and Laboratory Medicine Working Group for Preanalytical Phase in standardization and harmonization of the preanalytical phase in Europe. Ann Clin Biochem 2016;53:539–47.
2. Lippi G, Simundic AM. The EFLM strategy for harmonization of the preanalytical phase. Clin Chem Lab Med 2018;56:1660–6.
3. 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.
4. Lippi G, Becan-McBride K, Behúlová D, Bowen RA, Church S, Delanghe J, et al. Preanalytical quality improvement: in quality we trust. Clin Chem Lab Med 2013;51:229–41.
5. Lippi G, Banfi G, Church S, Cornes M, De Carli G, Grankvist K, et al. Preanalytical quality improvement. In pursuit of harmony, on behalf of European Federation for Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for Preanalytical Phase (WG-PRE). Clin Chem Lab Med 2015;53:357–70.
6. Lippi G, Baird GS, Banfi G, Bölenius K, Cadamuro J, Church S, et al. Improving quality in the preanalytical phase through innovation, on behalf of the European Federation for Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for Preanalytical Phase (WG-PRE). Clin Chem Lab Med 2017;55:489–500.
7. Hawker CD. Nonanalytic laboratory automation: a quarter century of progress. Clin Chem 2017;63:1074–82.
8. Lou AH, Elnenaei MO, Sadek I, Thompson S, Crocker BD, Nassar B. Evaluation of the impact of a total automation system in a large core laboratory on turnaround time. Clin Biochem 2016;49:1254–8.
9. Carraro P, Plebani M. Errors in a stat laboratory: types and frequencies 10 years later. Clin Chem 2007;53:1338–42.
10. Jensen E, Blaabjerg O, Petersen PH, Hegedüs L. Sampling time is important but may be overlooked in establishment and use of thyroid-stimulating hormone reference intervals. Clin Chem 2007;53:355–6.
11. Schumacher GE. Choosing optimal sampling times for therapeutic drug monitoring. Clin Pharm 1985;4:84–92.
12. Montagnana M, Salvagno GL, Lippi G. Circadian variation within hemostasis: an underrecognized link between biology and disease? Semin Thromb Hemost 2009;35:23–33.
13. Cadamuro J, Gaksch M, Wiedemann H, Lippi G, von Meyer A, Pertersmann A, et al. Are laboratory tests always needed? Frequency and causes of laboratory overuse in a hospital setting. Clin Biochem 2018;54:85–91.
14. Horvath A. From evidence to best practice in laboratory medicine. Clin Biochem Rev 2013;34:47–60.
15. Cadamuro J, Ibarz M, Cornes M, Nybo M, Haschke-Becher E, von Meyer A, et al. Managing inappropriate utilization of laboratory resources. Diagnosis (Berl) 2019;6:5–13.
16. Buoro S, Lippi G. Harmonization of laboratory hematology: a long and winding journey. Clin Chem Lab Med 2018;56: 1575–8.
17. Banfi G, Salvagno GL, Lippi G. The role of ethylenediamine tetraacetic acid (EDTA) as in vitro anticoagulant for diagnostic purposes. Clin Chem Lab Med 2007;45:565–76.
18. Lippi G, Plebani M. EDTA-dependent pseudothrombocytopenia: further insights and recommendations for prevention of a clinically threatening artifact. Clin Chem Lab Med 2012;50:1281–5.
19. Rapi S, Cellai F, Rubeca R. Is it possible to correctly assess the pre-analytical characteristics of faecal tests? J Lab Precis Med 2018;3:1.
20. Padoan A, D' Incà R, Scapellato ML, De Bastiani R, Caccaro R, Mescoli C, et al. Improving IBD diagnosis and monitoring by understanding preanalytical, analytical and biological fecal calprotectin variability. Clin Chem Lab Med 2018;56:1926–35.
21. Cadamuro J, Simundic AM, Ajzner E, Sandberg S. A pragmatic approach to sample acceptance and rejection. Clin Biochem 2017;50:579–81.

22. von Meyer A, Cadamuro J, Lippi G, Simundic AM. Call for more transparency in manufacturers declarations on serum indices: On behalf of the Working Group for Preanalytical Phase (WG-PRE), European Federation of Clinical Chemistry and Laboratory Medicine (EFLM). Clin Chim Acta 2018;484:328–32.
23. Lippi G, Cadamuro J, von Meyer A, Simundic AM. European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for Preanalytical Phase (WG-PRE). Practical recommendations for managing hemolyzed samples in clinical chemistry testing. Clin Chem Lab Med 2018;56:718–27.
24. Lippi G, Cadamuro J, von Meyer A, Simundic AM, European Federation of Clinical Chemistry, Laboratory Medicine (EFLM) Working Group; for Preanalytical Phase (WG-PRE). Local quality assurance of serum or plasma (HIL) indices. Clin Biochem 2018;54:112–8.
25. Gómez Rioja R, Martínez Espartosa D, Segovia M, Ibarz M, Llopis MA, Bauça JM, et al. Laboratory sample stability. Is it possible to define a consensus stability function? An example of five blood magnitudes. Clin Chem Lab Med 2018;56:1806–18.
26. Oddoze C, Lombard E, Portugal H. Stability study of 81 analytes in human whole blood, in serum and in plasma. Clin Biochem 2012;45:464–9.
27. STARD guidelines. Available from: http://www.equator-network.org/reporting-guidelines/stard/. Accessed 19 Oct 2018.
28. Sciacovelli L, Panteghini M, Lippi G, Sumarac Z, Cadamuro J, Galoro CA, et al. Defining a roadmap for harmonizing quality indicators in Laboratory Medicine: a consensus statement on behalf of the IFCC Working Group "Laboratory Error and Patient Safety" and EFLM Task and Finish Group "Performance specifications for the extra-analytical phases". Clin Chem Lab Med 2017;55:1478–88.
29. Plebani M, Sciacovelli L, Aita A, Pelloso M, Chiozza ML. Performance criteria and quality indicators for the pre-analytical phase. Clin Chem Lab Med 2015;53:943–8.
30. Plebani M, Sciacovelli L, Aita A. Quality indicators for the total testing process. Clin Lab Med 2017;37:187–205.
31. Grebe SK, Singh RJ. LC-MS/MS in the clinical laboratory – where to from here? Clin Biochem Rev 2011;32:5–31.
32. Salvagno GL, Danese E, Lippi G. Preanalytical variables for liquid chromatography-mass spectrometry (LC-MS) analysis of human blood specimens. Clin Biochem 2017;50:582–6.
33. Yin P, Lehmann R, Xu G. Effects of pre-analytical processes on blood samples used in metabolomics studies. Anal Bioanal Chem 2015;407:4879–92.
34. Heitzer E, Haque IS, Roberts CE, Speicher MR. Current and future perspectives of liquid biopsies in genomics-driven oncology. Nat Rev Genet 2018 Nov 8. doi: 10.1038/s41576-018-0071-5. [Epub ahead of print].
35. Jung K, Fleischhacker M, Rabien A. Cell-free DNA in the blood as a solid tumor biomarker–a critical appraisal of the literature. Clin Chim Acta 2010;411:1611–24.
36. Schmidt B, Reinicke D, Reindl I, Bork I, Wollschläger B, Lambrecht N, et al. Liquid biopsy – Performance of the PAXgene® blood ccfDNA tubes for the isolation and characterization of cell-free plasma DNA from tumor patients. Clin Chim Acta 2017;469:94–8.
37. Betsou F. Biospecimen processing method validation. Biopreserv Biobank 2015;13:69.
38. Gaignaux A, Ashton G, Coppola D, De Souza Y, De Wilde A, Eliason J, et al. A biospecimen proficiency testing program for biobank accreditation: four years of experience. Biopreserv Biobank 2016;14:429–39.
39. Betsou F, Bulla A, Cho SY, Clements J, Chuaqui R, Coppola D, et al. Assays for qualification and quality stratification of clinical biospecimens used in research. Biopreserv Biobank 2016;14:398–409.
40. Lundberg GD. Managing the patient-focused laboratory. Oradell, NJ: Medical Economics Co, 1975:9–42.
41. Anderson NL, Anderson NG. The human plasma proteome: history, character, and diagnostic prospects. Mol Cell Proteomics 2002;1:845–67.
42. Zimmer JS, Dyckes DF, Bernlohr DA, Murphy RC. Fatty acid binding proteins stabilize leukotriene A4: competition with arachidonic acid but not other lipoxygenase products. J Lipid Res 2004;45:2138–44.
43. Thelen MH, Huisman W. Harmonization of accreditation to ISO15189. Clin Chem Lab Med 2018;56:1637–43.
44. Antonelli G, Padoan A, Aita A, Sciacovelli L, Plebani M. Verification of examination procedures in clinical laboratory for imprecision, trueness and diagnostic accuracy according to ISO 15189:2012: a pragmatic approach. Clin Chem Lab Med 2017;55:1501–8.