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Review

Preanalytical variables for liquid chromatography-mass spectrometry (LC-MS) analysis of human blood specimens

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ABSTRACT

The use of liquid chromatography-mass spectrometry (LC-MS) for both diagnostics and research purposes is rapidly growing in clinical laboratories. As for more conventional areas of in vitro diagnostic testing, many preanalytical variables have an impact on these techniques and may hence jeopardize the quality of tests results. The leading preanalytical variables include patient preparation, the nature of the blood collection tubes and additives, interference from spurious hemolysis, sample handling and management, composition of blood tubes, contamination, as well as storage conditions. Therefore, the aim of this article is provide a narrative overview about the leading preanalytical issues which may ultimately influence LC-MS testing of human blood samples, and provide tentative indications, as for current evidence, about optimal preanalytical management of blood samples for proteomics and metabolomics studies. These general recommendations entail pre-storage centrifugation, use of appropriate tubes and additives, addition of bacteriostatic preservatives, enrichment and purification of samples, elimination of unsuitable specimens, rapid analysis or immediate storage at −70 °C, and avoidance of analyzing frozen-thawed specimens.

1. Introduction

The ever growing diffusion of accreditation programs in clinical laboratories necessitates laboratory professionals to demonstrate that each activity of the total testing process is carried out according to the highest possible quality standards [1].

Laboratory diagnostics typically develops through three main phases (i.e., preanalytical, analytical and post-analytical), and each of them is variably vulnerable to uncertainties and errors [2]. Several lines of evidence now attest that the vast majority of laboratory mistakes occur in the still manually-intensive activities of the preanalytical phase. This is mostly attributable to the lack of ideal or standardized procedures for patient preparation, to the use of unsuitable collection devices (i.e., needles, blood collection tubes), prolonged venous stasis during venipuncture, inappropriate sample management immediately after collection, long time needed for sample separation or analysis, inappropriate conditions for short- and long-term storage of biological materials [3,4]. The challenge to systematically monitor, and thereby control, all the various preanalytical variables is the main reason underneath the high prevalence of errors in this crucial step of the total testing process, so making it difficult to implement all necessary improvements, especially when most of such variables (e.g., phlebotomy and immediate sample management after venipuncture) are not directly managed or supervised by the laboratory staff [5].

One of the leading aspects to reduce uncertainties throughout the testing process is the introduction of protocols and standard operating procedure (SOP), especially effective to reduce the chance of random errors which may ultimately jeopardize sample quality, reliability of tests and managed care guided by laboratory data [3–5]. There has been a long history of the exploration into preanalytical issues in many sectors of laboratory medicine such as clinical chemistry, immunochemistry, hemostasis and laboratory hematology, so that these areas are now seemingly less vulnerable to uncertainties and errors as they were in the past decades [3–5]. Despite the vast majority of routine diagnostics tests are mostly pertinent to these areas, other branches of laboratory medicine are firmly developing. Liquid chromatography–mass spectrometry (LC-MS) has gradually emerged as one of the essential technology in routine clinical laboratories, recently becoming the reference technique for a number of quantitative analyses that cannot be reliably performed using standard laboratory techniques such as clinical chemistry, immunochemistry and capillary electrophoresis [6].

The LC-MS techniques currently play a crucial role in many "-omics", biomarker and biopharmaceutical studies. Unlike immunoassays or traditional high-pressure liquid chromatography (HPLC) methods, LC-MS offers better specificity of detection, lower matrix inter-

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ferences and high detectability of molecules present in very low concentrations [7]. Therefore, this technique is becoming increasingly popular for sensitive and reproducible identification and quantification of hundreds to thousands of primary compounds and metabolites in a single biological sample. Despite needing important efforts to develop in-house techniques, another great advantage of these techniques is that in-house method can be developed and locally validated independently from diagnostic companies, so allowing to introduce new diagnostic tests soon afterwards biomarker discovery and regardless of market availability [8].

The traditional areas of application of LC–MS in clinical laboratories include, (i) confirmation of immunoassay-positive drug screens, (ii) neonatal metabolism screening, (iii) analysis of steroid hormones, (iv) therapeutic drug monitoring (TDM) of immune-suppressants, (v) development of reference methods, (vi) endocrinology and toxicology analyses, as well as (vii) "-omics" research (e.g., proteomics, metabolomics) [7,8]. The leading advantages of these techniques include the possibility to reach a much greater degree of analytical sensitivity and specificity for the assessment of many analytes, the inherent flexibility and the growing versatility, all factors which ultimately contribute to provide an efficient and timely response to emerging and innovative clinical needs [9].

As for many other areas of in vitro diagnostic testing, quality assurance throughout the total testing process of LC-MS is a necessary requisite for generating reliable data. Unlike immunochemistry, however, these separation techniques present unique challenges, which are also attributable to the fact that only a few commercial assay kits are available in the market [9]. Notably, instrument configuration for running LC-MS assays is highly heterogeneous and necessitates especially trained and skilled personnel. The fact that appropriate staff training is foremost in LC-MS testing has been emphasized in many occasions. For example, many concerns were highlighted some years ago about the reliability of 25-hydroxyvitamin D (25-OH-D) results generated by LC–tandem MS (LC-MS/MS), because some laboratories were found to produce unreliable results due to the fact that laboratory personnel was overlooking appropriate preanalytical and analytical procedures [10]. This circumstance has provided solid evidence that internal quality control programs and external quality assessment schemes are virtually unavoidable for assuring quality of data produced using these sophisticated techniques. Nevertheless, even the most expert operator, inclined to follow strict SOPs for the analytical part of testing, may encounter serious problems when procedures for sample collection and handling are vague, poorly standardized and not regularly monitored. This aspect is unquestionably magnified in the LC-MS laboratory, where sample aliquots are frequently received frozen or already deproteinized, so that the quality of sample preparation cannot be accurately checked, also leaving potential preanalytical problems virtually undetected and/or undetectable [11].

Therefore, due the unquestionable importance of many extra-analytical variables for the quality of LC-MS testing, this review article is aimed to provide a narrative overview about the influence of many preanalytical variables on reliability of test results generated by LC-MS assays using human blood samples for both diagnostics and research purposes [12].

2. Patient preparation and demographical variables

Patient preparation plays a foremost role in proteomics and metabolomics studies, so that it should be accurately outlined and standardized, especially for biomarker exploration and diagnostic applications. Basically, the most relevant aspects pertaining the impact of patient demographic and preparation on LC-MS testing include (i) the influence of age, gender and ethnic origin on the concentration of many biomarkers, (ii) circadian variation (e.g., especially important for certain hormones such as cortisol, progesterone, testosterone) [13], (iii) intake of drugs which may ultimately interfere with the metabolism of hormones and proteins, (iv) dietary habits, (v) specific intra- and inter-individual biological variability and (vi) physical exercise [14]. Unfortunately, only few studies have thoughtfully investigated the influence of patient preparation on proteomics and metabolomics. Ishikava carried out a lipidomics study in healthy adults, and concluded that gender and age are both important determinants of analytes concentration. Another important aspect emerging from this investigation is that sample selection and appropriate handling procedures are vital for the quality of biomarkers testing [15]. In an additional study on blood metabolomics, Minami et al. found significant circadian oscillations of different types of lysophosphatidylcholines, so confirming the importance of circadian variation and physiological bio-rhythm in interpreting results of LC-MS analyses [16]. In this study, circadian variation was found to be especially important for newborns profile screening aimed to identify metabolisms defects of urea cycle metabolites such as ornithine citrulline, and 4-guanidino-butyrate.

As regards dietary habits, and likewise conventional laboratory testing, an appropriate fasting period (i.e., 8–10 h) before sample collection is usually recommended for LC-MS testing [17]. This suggestion is supported by reliable evidence showing that the metabolome may undergo dynamic changes, which continue to evolve many hours after food intake [18]. Interestingly, a number of so-called "non-nutrients" (i.e., flavones, stanols, and soy-based estrogen analogues), regularly but often unconsciously assumed with the diet, may also generate substantial changes of metabolomics profiles [19].

The impact of physical exercise on results of LC-MS has also been clearly established, especially for tryptophan, carnitine and cortisol metabolism, analysis of purine pathway, amino acid oxidation, as well as for metabolites of the gastrointestinal microbiome [20]. During contraction, the muscle cells are subjected to a kaleidoscope of substantial alterations. The contracting muscle not only influences the metabolism of several organs, but also actively releases many mediators [14], and is an important trigger for secretion of proteins and hormones with paracrine or endocrine effects [21].

Table 1
Main source or preanalytical variability in liquid chromatography-mass spectrometry (LC-MS) testing.

1. Patient preparation and demographical variables
   - Age, gender and ethnic origin
   - Circadian variation
   - Intake of drugs
   - Dietary habits
   - Intra- and inter-individual biological variability
   - Physical exercise
2. Blood tubes
   - Materials
   - Additives
   - Contaminants
3. Interference from spurious hemolysis
4. Sample handling and management
5. Sample storage
6. Freeze-thaw cycles

Table 2
Suggested protocol to be introduced in all laboratories to collect samples for liquid chromatography-mass spectrometry (LC-MS) analysis.

1. Collect samples always at the same time of the day for minimizing the impact of circadian variation
2. Collect information about diet, physical activity and drugs known to interfere with LC-MS measurements
3. Use specific blood tubes for LC-MS analysis
4. Strictly follow available guidelines about sample collection
5. Comply with available guidelines for sample handling and management
6. Do not process unsuitable samples (i.e., hemolyzed specimen)
7. Identify the optimal storage conditions according to the sample matrix and the parameters to be measured
8. Avoid freezing-thawing the specimens