Editorial

Giuseppe Lippi and Mario Plebani

Opportunities and drawbacks of nonstandard body fluid analysis

DOI 10.1515/cclm-2016-0862

The routine workout of most clinical laboratories entails, at least for the largest part, the analysis of conventional biological matrices such as whole blood, serum, plasma, urine and feces [1]. Nevertheless, driven by new biological discoveries and recent analytical breakthroughs (e.g. low-volume aspiration, automatic assessment of sample quality), the analysis of nonstandard body fluids including saliva, semen, cerebrospinal (CSF), peritoneal (i.e. ascites), pleuric, pericardic, pancreatic, synovial, cyst, wound, drain and washout fluids is becoming very popular for the diagnosis and management of a kaleidoscope of human diseases. This trend is generating new and intriguing opportunities for the whole field of laboratory diagnostics, but also poses important challenges for reliable management of this part of non-traditional clinical chemistry and immunochemistry testing.

The most intriguing and promising application of nonstandard body fluid testing is symbolized by the analysis of cancer biomarkers in fluids other than serum, plasma or urine. The number of clinical studies that have assessed many different cancer biomarkers in nonstandard body fluids has exponentially grown in the past decades, coupled by an increasing volume of routine requests for this type of analyses placed to clinical laboratories. In an interesting article published in this issue of the journal, Trimboli et al. critically review the current scientific literature about the measurement of fine-needle aspiration (FNA) endocrine markers (e.g. thyroglobulin, calcitonin and parathyroid hormone) [2], concluding that this approach may be particularly suited for efficient and relatively cheap diagnosis of thyroid/parathyroid tumors, provided that some essential criteria are fulfilled, which namely encompass setting adequate and standardized preanalytical procedures, providing accurate interpretation criteria and appropriately addressing those cases characterized by controversial data. These important conclusions lead the way to additional thoughts on this still largely debated issue.

Beside the increasing clinical significance of nonstandard body fluid analysis using clinical chemistry and immunochemistry assays in cancer (and other diseases) diagnostics, many drawbacks are still challenging routine and widespread implementation (Figure 1). The collection and management of the biological samples prior to testing is indeed a first and unavoidable issue. In many areas of laboratory diagnostics, preanalytical activities are highly vulnerable to errors and uncertainties [3]. Nonstandard body fluid analysis makes no exception to this rule. The main problem here is the choice of the appropriate sample type, depending on the container in which the fluid is collected. Although the cytological analysis of nonstandard body fluids is usually performed in blood tubes containing ethylenediaminetetraacetic acid (EDTA; i.e. the conventional anticoagulant used for enumeration and classification of corpuscular elements), this additive is not normally validated for standard clinical chemistry (and, occasionally, for immunochemistry) testing. Therefore, despite lithium-heparin or standard serum tubes are widespread options for non-hematological testing in nonstandard body fluids, the often poor comparability of data between these two samples types, as well as among specimens collected in different brands of tubes, should be regarded as an important source of variability. The stability of many laboratory analytes in both serum and plasma has been the object of many investigations in the past 40 years [4], leading to the generation of reliable recommendations about the most appropriate procedures for sample management [5]. Nevertheless, little is known as yet about the most appropriate conditions of time and temperature for delayed analysis of proteins and other (cancer) biomarkers in nonstandard body fluids. Measurand stability is an additional concern. Many biological fluids contain enzymes and other proteins which may degrade or bind to the analyte, thus artificially enhancing or decreasing its immunoreactivity using standard laboratory techniques and ultimately producing test results which do not mirror the real concentration of the parameter in the fluid. Of particular concern is viscosity, wherein the presence of hyaluronic acid and other substances in nonstandard body fluids may cause aspiration errors or interference throughout the analytical process. Pre-treatment with hyaluronidase may be effective to overcome most of these problems [6], but yet

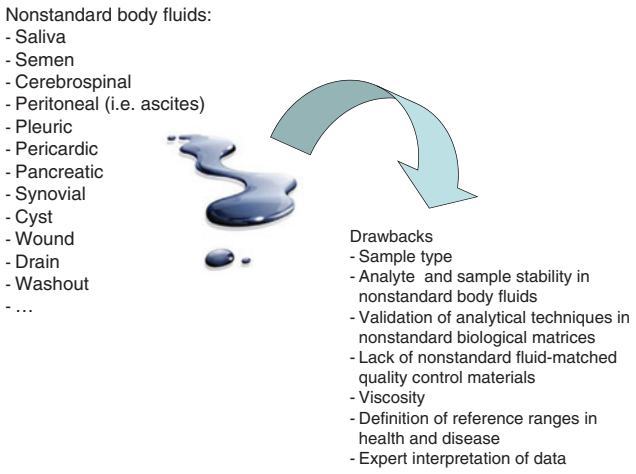

Figure 1: Leading unresolved issues in nonstandard fluid analysis.

poses some challenges about assay reliability in specimens treated with this addictive. The possible centrifugation of the specimen is another unresolved issue (e.g. centrifugation force, time, temperature, etc.). Importantly, additional preanalytical factors were found to strongly impact the quality of fecal immunochemical tests, thus reinforcing the advice of selecting appropriate collection devices and adopting specific protocols for sample collection and handling [7, 8].

The vast majority of clinical chemistry tests and immunoassays have been validated for serum and/or plasma analysis by the different manufacturers. Off-label body fluids is a concept generally referred to any type of biological fluid that is different from those reported by manufacturers of a Food and Drug Administration (FDA)-cleared assay within the so-called "Intended Use" part of the package insert [9]. Similar considerations can be made for the so-called European Community-In Vitro Diagnostics (CE-IVD) marking. Little evidence has been provided so far that results of many immunoassays are reliable using sample matrices largely differing from serum or plasma in terms of pH, proteins, ions, lipids and potential interfering substances, wherein the antigen-antibody binding conditions have been optimized for use under certain operating conditions, which are not always reproducible using different matrices. Moreover, the conventional analytical performance obtained in serum or plasma (i.e. especially referring to limit of detection, analytical sensitivity, linearity, imprecision) cannot be systematically transferred to nonstandard body fluid analysis. Therefore, validation of the assays, according to sample type and local method, is absolutely required for verifying the performance claimed by the manufacturer and before clinical practice implementation. This is also an essential requirement for laboratory accreditation according to the International Standard ISO 15189 [10]. Quality control testing is an essential part of laboratory diagnostics, entailing both the use of internal quality controls and participation to external quality assessment (EQA) schemes [11]. Unlike conventional serum or plasma analysis, analytical performance specifications and fluid-matched quality control materials are unavailable for nonconventional laboratory testing.

The lack of reliable reference ranges of many analytes in nonstandard biological fluids is probably the most critical issue. Many reasonable aspects lead us (and others) to believe that this target is virtually unreachable [12]. The main factors impairing the reliability of interpreting results of various analytes in nonstandard biological fluids include the heterogeneity of fluid production in many disease conditions (i.e. ascites, cysts, wounds and synovial infections among others), the stage of the disease, the procedure used for collecting specimens, the dilution factor and the type of the washout buffer. As it is rather unlikely that a reference range will ever be associated with results of nonstandard fluid analysis in the laboratory report, interpretive information and counseling should always accompany the data.

The Clinical and Laboratory Standards Institute (CLSI) document C49-A, devoted to provide expert guidance about the analysis of body fluids [13], raises three crucial limitations, i.e. (i) the lack of validation of commercial assays for analysis of fluids other than whole blood, serum or plasma; (ii) the need to dedicate specific laboratory resources for this type of testing; and (iii) the absence of reliable interpretative criteria (essentially, the lack of reliable reference ranges). These issues inevitably call for a major involvement of laboratory professionals in this type of testing, by means of validating commercial assays for the current "off-label" use and providing appropriate interpretive information about test results. Despite nonstandard body fluid analysis should be regarded as a promising and intriguing perspective, especially in cancer diagnostics, the many current drawbacks necessitate supranational regulatory efforts aimed to set a number of reliable criteria to help gathering the most safe and clinically usable information from this type on nonconventional testing.

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. Lippi G, Plebani M. Laboratory medicine does matter in science (and medicine)… yet many seem to ignore it. Clin Chem Lab Med 2015;53:1655–6.
2. Trimboli P, D'Aurizio F, Tozzoli R, Giovanella L. Measurement of thyroglobulin, calcitonin, and PTH in FNA washout fluids. Clin Chem Lab Med 2017;55:914–25.
3. 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.
4. Perrault R, Taylor CG, Zahradka P. The stability of select biomarkers in frozen plasma over time: an evaluation of a low-volume sample analyzer. Clin Chem Lab Med 2015;53:e97–100.
5. 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.
6. Seghezzi M, Buoro S, Manenti B, Mecca T, Ferrari R, Zappalà G, et al. Optimization of cellular analysis of synovial fluids by optical microscopy and automated count using the Sysmex XN Body Fluid Mode. Clin Chim Acta 2016;462:41–8.
7. Rubeca T, Cellai F, Confortini M, Fraser CG, Rapi S. Impact of preanalytical factors on fecal immunochemical tests: need for new strategies in comparison of methods. Int J Biol Markers 2015;30:e269–74.
8. Gnatta E, Zaninotto M, Epifani MG, Padoan A, Gjini R, Plebani M. A new sampling device for faecal immunochemical testing: haemoglobin stability is still an open issue. Clin Chem Lab Med 2014;52:1203–9.
9. Wians FH. To test or not to test? Opening pandora's box. Lab Med 2004;35:707.
10. ISO 15189:2012. Medical laboratories – requirements for quality and competence. Geneva, Switzerland: International Organization for Standardization, 2012.
11. Sandberg S, Fraser CG, Horvath AR, Jansen R, Jones G, Oosterhuis W, et al. Defining analytical performance specifications: consensus Statement from the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine. Clin Chem Lab Med 2015;53:833–5.
12. Block D. Body fluid testing in the clinical laboratory: are you ready for your next cap inspection? National Academy of Clinical Biochemistry – Scientific Shorts. Available at: https://www.aacc.org/community/national-academy-of-clinical-biochemistry/scientific-shorts/2012/body-fluid-testing-in-the-clinical-laboratory-are-you-ready-for-your-next-cap-inspection.aspx. Accessed: 19 Sep 2016.
13. Clinical and Laboratory Standards Institute (CLSI). Analysis of body fluids in clinical chemistry; Approved guideline. CLSI document C49-A, 2007.

Corresponding author: Prof. Giuseppe Lippi, Section of Clinical Biochemistry, University Hospital of Verona, P.le LA Scuro 10, 37134 Verona, Italy, E-mail: giuseppe.lippi@univr.it. http://orcid.org/0000-0001-9523-9054; and Associate Editor of *Clinical Chemistry and Laboratory Medicine*
Mario Plebani: Department of Laboratory Medicine, University Hospital of Padova, Italy. http://orcid.org/0000-0002-0270-1711; and Editor in Chief of *Clinical Chemistry and Laboratory Medicine*