Review

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Preanalytical aspects on short- and long-term storage of serum and plasma

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Abstract: Following an ordered clinical chemistry plasma/serum test, ideally the venous blood specimen is adequately collected at a health care facility, then swiftly transported to and readily handled, analyzed and sometimes interpreted at a clinical chemistry laboratory followed by a report of the test result to the ordering physician to finally handle the result. However, often there are practical as well as sample quality reasons for short- or long-term storage of samples before and after analysis. If there are specific storage needs, the preanalytical handling practices are specified in the laboratory's specimen collection instructions for the ordered test analyte. Biobanking of specimens over a very long time prior to analysis includes an often neglected preanalytical challenge for preserved quality of the blood specimen and also involves administrative and additional practical handling aspects (specified in a standard operating procedure – SOP) when demands and considerations from academic, industry, research organizations and authorities are included. This short review highlights some preanalytical aspects of plasma/serum short- and long- term storage that must be considered by clinicians, laboratory staff as well as the researchers.

Keywords: biobanking; blood specimen storage; plasma; preanalysis; serum.

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Preanalysis and preanalytical errors

Preanalysis (or the preanalytical phase) includes all procedures prior to analysis and is by far the most common cause of an erroneous laboratory analysis result [1, 2]. In a routine clinical chemistry laboratory receiving mainly whole blood, serum and plasma specimens, preanalytical errors (analysis ordering, patient preparation, specimen collection, transport and temporary storage) may have an impact on the quality or quantity of the collected sample or the data relating to the sample. In routine health care, preanalytical errors may lead to missed or incorrect samples, lost traceability due to improper patient identification or sample labelling, lost specimens or lost order/data related to the collected sample. All errors lead to extra labor efforts to trace and correct them, as well as a risk of delayed or erroneous analytical results that may be detrimental to the patient [1, 3]. Preanalytical pitfalls are well known to the routine laboratory, but specimen collection and preanalytical guideline practices in health care outside the laboratory are not always followed [4, 5].

Biobanking (long-term storage of biospecimens), in addition, always includes a prior storage step (which in itself or during sample retrieval may be detrimental for sample quality) before analysis. In biobanking, and in biomarker studies on biobanked specimens, researchers or industry, for the optimal usability of the sample [6], only rarely requests the routine laboratories' expertise on preanalytical handling and analysis of the sample.

Preanalytical variability

Overall, clinical laboratories as well as biobank facilities should all aim to minimize systematic or random preanalytical, biological, analytical and postanalytical variation by following issued guideline practices, standard operating procedures (SOPs) and instructions and strive to maintain the composition and integrity of the blood specimen as it was at the time of collection. Otherwise, unwanted reported result variability will arise, making comparisons within and between individuals and study groups

difficult. This results in risk of misdiagnosis, mistreatment, misclassification of disease, loss of study power and/or increasing costs [1, 7].

Best practice, accreditation and standardization

There is thus a demand to meticulously follow standardization and guideline practices for the preanalytical and analytical processes and methods within and between clinical and research laboratories, as well as within and between research studies (Table 1). Guidelines or opinions based on evidence or best practice for patient preparation, specimen collection, transport and storage are issued by organizations. These include the European Federation for Laboratory Medicine (EFLM), the Clinical & Laboratory Standards Institute (CSLI), the World Health Organization (WHO), the National Cancer Institute (NCI) and the International Society for Biological and Environmental Repositories (ISBER) [8–11].

The responsible health care or research authority (most often issued through the clinical laboratories) has to provide general and specific preanalytical practice instructions for each analyte on the laboratory's analysis menu, organize continuous education on preanalytics and biospecimen collection and provide surveillance of instruction/guideline compliance and specimen and data quality. For surveillance, quality indicators, observational studies and questionnaires [4, 12, 13] can be used.

A responsibility of the organization is to provide stored information the caretaker or person giving informed consent for data and/or giving/donating a biospecimen for health care or biobank studies. This includes individual data register information and of stored samples, how they will/have been used and the individual's right to opt out their register data and/or stored samples of that organization [14].

Table 1: Bullet point summary of preanalytical issues on plasma/serum storage.

- There is a need for increased consideration of preanalytical conditions prior to storage
- Preanalytical practices should be standardized and follow guidelines
- Blood specimen integrity should strive to be similar to that at the time of collection
- Patient/donator consent must always be obtained
- Samples hemolysis indices should be matched in case-control studies
- Storage of samples should be optimized for ease of future retrieval and analysis
- Clinical laboratories and biobank facilities should be consulted prior to specimen storage

Collection purpose, organizations involved and controls

The purpose for collection and storage of biospecimens should always be clear. Patient's informed consent in health care is assumed to be provided if the patient appears for voluntary blood specimen collection [15]. In this case, only the health care organization including the laboratory is directly involved, very little patient data are transferred within the organization (most data stored in patient records) and the collected specimen is generally discarded after short-term storage (hours/days) following analysis.

For biobanking of biospecimens, there is a demand for a specific stated purpose of the biobank – which biospecimens to be collected and how they are to be used. Biobanking of biospecimens within the health care organization may occur, e.g. for quality improvement, related to health care quality registers or reference interval construction, etc. [16]. Regarding research studies on biobanked biospecimens, other organizations are also normally involved, most often researchers belonging to a university or specific research organization, as well as other authorities and organizations providing statistical data required for the studies and laboratories to which specimens are sent for analysis. Research and material transfer agreements between involved parties has to be established. Approval from an institutional Ethical Committee is required describing why, how and the feasibility of the study to be performed; how the patient/study participant are informed; and how patients/individuals registry data and biospecimens are to be collected and handled within and between involved organizations [17]. The patient information and consent should be wide enough to allow for unforeseen study questions and novel use of biobanked biospecimens and registry data. A renewed patient consent would not be necessary again but the approval by the Ethical Committee should be within the frame of the overall study, the information given and the approved consent. Individual data and specimens must always be traceable, but during processing between organizations, they are often hidden through pseudo-anonymization.

A tricky and often laborious challenge both in clinical laboratory work and in biobanking studies is to define and collect relevant control patients'/individuals' data and biospecimens for matching. Collecting healthy

control individuals has to be from the same population background and the same collection routines as the patient/study individuals/groups have to apply. This is important for the laboratories' construction of relevant reference intervals and is of utmost importance when different biobank study sites provide cases as well as controls in national and international cooperative studies to increase statistical power for sub-analyses or for studies of rare diseases.

Short-term storage in clinical routine

All clinical practices, from test ordering to physician receiving the test results, need to be optimized including digitalized ordering and test result reporting within a health care organization. Analysis results are stored in the laboratory information system (LIS) and retrievable for each patient. Clinical data relating to the ordered test are most often not provided except when needed for the laboratory's handling and clinical interpretation of the test. Methods and quality controls (QCs) are always traceable as demanded also through ISO-accreditation documentation. Biospecimen collection and analysis times should ideally be stored in the LIS to be able to track expired analyte stability limits but this practice seems still relatively rare and differing between nations. However, in case the specimen collection time is not documented, then the stability of individual specimens becomes more uncertain and has the practical consequence that the short-term storage duration has to be shortened. Keeping track of time until centrifugation, transportation times, and storage times is most often the task of the collecting health care providing unit and these are not documented in the routine LIS.

In the clinical biochemistry laboratory, serum/plasma specimens are occasionally temporarily stored awaiting a scheduled transport from the collection point to the laboratory, and in the routine laboratory stored awaiting batch analyses of seldom-run low-frequency analytes or stored for laboratory development and quality assurance, for instance for the establishment of reference intervals [18]. Specimens are also stored after analysis awaiting approved analysis QC runs and/or with possibilities for demands for re-runs and complimentary orders but usually discarded after a day or two.

Collected plasma/serum specimens are normally kept in their collection tubes (primary tubes) until analysis. Many ordered analytes and cellular blood components are stable in primary tubes for several hours even if not centrifuged. The specimens are, in the case of hospital, internal as well as externally collected tubes thus transported uncentrifuged in primary tubes to the laboratory where they are centrifuged and analyzed directly. In case the ordered analyte is unstable, the specimen needs to be stabilized by centrifugation, and/or centrifuged after which plasma/serum is transferred into a plain tube and transported refrigerated, or for the most unstable analytes, frozen and then transported and stored in this condition prior to analysis. For health care centers and blood specimens collected outside of the hospital, this, however, means that centrifugation, refrigeration and freezing capacities have to be provided at the specimen collection points or health care providers in most cases.

The integrity of the blood specimen analytes from the time of blood collection until analysis (with and without centrifugation) has to be validated by the laboratory. It may also be assured for each test tube type by the test tube manufacturer for each routine analysis instrument and reagent type. Another alternative is published stability data [19–22] under similar collection, storage and transportation conditions. The laboratory must define the maximum allowable instability for each analysis. The stability of each analyte under different storage conditions should be stated in the laboratory's collection guideline as information to specimen collection points so they can optimize their collection routines with preserved analyte stability. For practical reasons, the routine clinical chemistry laboratory could focus the short-term storage routines to refrigerated storage, preferably as an automated process where the samples are discarded some time after passed stability limit.

There are different definitions of analyte stability in use. Stability criteria need to be standardized [23]. Also, new approaches for no fixed time data but stability formulas are discussed [24] and would probably display reality better even if the handling is much more complicated. The short-term storage after analysis for a few days makes it possible to reanalyze or complement the analysis order provided the specimen stability time criteria are not passed. If so, usually a new sample should be collected. In the clinical situation, however, the laboratory should not dogmatically stick to a predefined stability time point for not releasing a test result. It is advisable to be pragmatic about whether to release the test result or not, depending on the clinical situation of an aged sample or other discovered preanalytical errors with the risk of a deviating test result [25].

Preanalytical quality of individual clinical routine blood specimens are preferably monitored by the time

between specimen collection and analysis for the ordered analyte, as well as automatic monitoring of each sample's indices such as the hemolysis index (HI) which determines the concentration of free hemoglobin in the plasma/serum sample [26]. A general preanalytical quality indicator of the laboratory is the number of samples rejected for analysis because of hemolysis interference with the analysis method or due to the release of cell constituents from erythrocytes and platelets [27]. Preanalytical practices of phlebotomists, transport and storage may also relatively easy to be monitored for corrective action by clinical audit and observational [4] or questionnaire studies [12].

Biobanking (long-term storage)

The reasons for creating a biobank are many and depend on the intended purpose. Most biobanks are disease-specific where biobanking (most often including plasma and/or serum) starts at diagnosis of the disease but may also be population-based on a (largely) healthy population. With mature population-based collections with large population coverage and ensuing questionnaires on lifestyle such as food intake and physical activity, the specimens can be seen as prospective samples, which can be used to explore the disease development in that population. In addition, biospecimens and questionnaire responses from the (control) population are collected simultaneously. It can be anticipated that storage of plasma/serum/blood components will increase in the near future for demands in, e.g. health care, epidemiological studies, transplantations, improved diagnostics and personalized medicine and the results used in translational research and precision medicine. These activities are enhanced by Biobanking and BioMolecular resources Research Infrastructure – European Research Infrastructure Consortium (BBMRI-ERIC) (www.bbmri-eric.eu), a pan-European research infrastructure of biobanks and biomolecular resources in order to facilitate their access and to support high quality biomolecular and medical research.

Regardless of the purpose of the biobank, the preanalytical, biological and analytical variation of the specimens must be kept to a minimum. The preanalytical variations of biobanked biospecimens are still the main source of analyte concentration variation and are often neglected in comparison to the efforts and costs spent on the collection of specimens per se and their storage and biochemical analysis, case-control matching, data retrieval and statistical analysis of the biobanked material and related study data [6]. Blood specimen collection in general and specific guidelines should be meticulously followed and active decisions taken of the need for patient preparation such as fasting, medication instructions and collection time restrictions to early morning, etc. to minimize preanalytical variation [28]. Of particular importance to maintain blood specimen integrity is the maximal time allowed for centrifugation and freezing. It is advantageous if the time to (first) freezing is as low as possible, e.g. an hour or two, meaning that centrifugation, aliquoting and freezing should take place at or very close to the blood collection site. With optimal handling of the blood specimen in this way (and preferably including control of specimen hemolysis), the sample collection criteria and sample integrity of the biobanked material will never be questioned apart from the preanalytical variation that may occur during the following specimen storage period and during sample retrieval conditions before analysis. In this way, the optimally collected and stored biospecimen can be used for all kinds of present and future serum/plasma analyses and biomarker studies.

In biobanking, whether disease-specific or not, extra care should therefore be taken for the organization, the preanalytical procedures and QC of the specimens to be collected. For plasma/serum samples, it is advisable to organize or use a dedicated collection organization of trained phlebotomists and not collect specimens broadly in the general health care organization. The overall guideline practice compliance of phlebotomists is generally low [4], so collected and stored blood specimens of low quality are of limited usability in future research. There may also appear additional problems with patient information, collection of informed consent and questionnaires and documentation when biobanking and related activities are not performed within a specific organization for the purpose.

As indicated earlier, the necessity of optimization of the preanalytical conditions in biobanking and biobank studies is often neglected. The knowledge of preanalytical practices is locally provided by the clinical chemistry laboratory and their expertise should therefore always be used in biobanking and biobanked biomarker studies. Of note, the routine laboratory does not have the biobank storage capacity and the LIS is not adapted to contain information on biobanked specimens or the specimen's location in a biobank or the retrieval of the samples from the biobank. Biobanking must therefore be centralized (e.g. as a unit at a university hospital) or decentralized to the involved researchers themselves. With the automation available at the clinical chemistry routine laboratory, it may support the collection of samples to be biobanked, sometimes aid in aliquoting the samples with a pipetting

robot and give information on the quality of the collected samples by providing their individual hemolysis, icterus and lipemia/turbidity (HIL) indices [29].

In addition, the laboratory's expertise on how to later analyze the samples should also be used. The laboratory has knowledge on the analyte stability in the biobanked samples, their retrieval, the suitable order of analysis, how to analyze matched case and control samples to minimize within- and between-batch analysis variation, influence of freeze-thaw cycles, the type (serum/plasma) and the minimal amount of sample needed for analysis. A special competence is also required on how to minimize the use of the (often limited) biobanked sample volume by multiplexing analyses and by also using the samples' dead volume required by today's large analysis platforms for analysis. Optimization of the analysis of the biobanked samples thus saves the biobanked sample volume, streamlines the analysis process, keeps the analysis variation to a minimum, increases the overall quality of the biomarker study and saves labor and money.

Sample storage formats, volumes and temperatures

The future handling of biobanked plasma/serum also has an impact on the choice of suitable sample volumes to be stored. Retrieval of the samples should be adapted so that only the volumes needed for analysis are thawed, thus avoiding re-storage of the samples and avoiding repeated freeze-thawing cycles that have to be kept track of. Suitable storage volumes are currently in the order of 50–200 µL and the volumes are easily applicable in modern analytical instruments so that the scarce material is used up and nothing or very minute amounts of the retrieved sample volume are wasted. The ability to optimize storage in small aliquot volumes is of course dependent on the local conditions of storage freezers, costs and staffing of the biobank. Biobanking may have samples stored in automated freezers and be easily retrieved by ordering in a biobank LIS which also keep track of the "biobank account" and the condition of sample assets is very time-saving and saves personnel labor. A back-up cooling system and/or the possibility to evacuate biobanked specimens to back-up freezers in case of freezer breakdown is a standard biobanking precaution.

Regardless of the storage format, it is also necessary to control the evaporation and aggregation within the biospecimens, so the test tubes or plate wells have to be well filled and the stored aliquots have to be well mixed prior to analysis. The storage format, tubes/wells used and the storage conditions should be documented according to International Society for Biological and Environmental Repositories (ISBER) recommendations [11].

Sample integrity and stability

It is important to always strive to maintain the biospecimens in a condition similar to the time of collection in order to maintain the usability of the biospecimens. Changes will always occur during storage and retrieval of the sample and as they are difficult to track, most comparisons over time can be compensated for by carefully matching study cases with controls. Major matching parameters are usually storage medium (serum/plasma type), collection (including seasonal variation) and storage time, sex and age of the donator and number of freeze-thaw cycles (if occurring). Storage time is e.g. a prominent covariate as age or gender and need to be included in epidemiological studies involving protein levels [30]. Sample hemolysis indices should also be matched due to the high contribution of hemolysis to analysis variability [27].

As pointed out, it is ideal to have the specimen stored at optimal preanalytical conditions from the start and throughout during a biobanking project/study. The stability of the analytes after long-term storage must be compared, if possible before the study commences, but this is often impossible when the period of biobanking is extended [31]. The recovery rate may vary both up and down over time and may be difficult to ascertain when matched controls are not available, and a considerable amount of sample volume should therefore be included in QC assessment. The use for internal surrogate biomarkers as a proxy for specimen quality should be avoided as those biomarkers do not ascertain the stability of the biomarkers of interest.

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