Preanalytical Variables and Their Influence on the Quality of Laboratory Results

Introduction

The preanalytical phase is an important component of laboratory medicine (1,2,3,4). Under the broad umbrella of the preanalytical phase can be included specimen collection, handling and processing variables, physiological variables such as the effect of lifestyle, age, gender, pregnancy and menstruation and endogenous variables such as drugs and circulating antibodies. Some of the preanalytical variables such as specimen variables can be controlled, while a knowledge of uncontrollable variables need to be well understood in order to be able to separate their effects from disease related changes affecting laboratory results. Only in the last decade there has been an intense focus on the preanalytical phase leading to the development of recommendations and standards.

Abstract

While analytical standards have been developed by established quality control criteria, there has been a paucity in the development of standards for the preanalytical phase. Only recently recommendations have been published regarding the quality of samples including the definition of the optimal sample size, the use of anticoagulants and stabilizers, stability criteria regarding transport and storage and handling of hemolytic, lipemic and icteric samples. Technical recommendations regarding sampling, transport and identification have been developed by national and international consensus organizations. The development of a preanalytical quality manual takes on an urgency in the goal towards achieving total quality control. Every day the laboratory is confronted with data arising out of preanalytical errors. Ability to recognize such data is critical to maintaining total laboratory quality and will be illustrated with case studies.

Recommendations & Standards for the Preanalytical Phase

In recent years several recommendations and standards have been developed for the preanalytical phase (5,6,7). The working group on preanalytical variables of the German Society for Clinical Chemistry and the German Society for Laboratory Medicine proposed comprehensive recommendations on the quality of diagnostic samples (5) and more recently on the handling of hemolytic, icteric and lipemic samples (6). The content of the quality of diagnostic samples document includes information on the choice of anticoagulants to use, the definition of the optimal sample size and analyte stability in sample matrix for each analyte. The merits and demerits of plasma over serum are addressed together with recommendations of sample collection and transport time, centrifugation and storage conditions (5). The optimal sample volume needed for laboratory tests has been defined based on twice the analytical volume of serum or plasma required for laboratory tests plus the dead volume of sample cup, replicates, and secondary tubes. In general, for testing 20 analytes in clinical chemistry, 3 to 4 mL of whole blood is needed to obtain heparinized plasma, while 4 to 5 mL of clotted blood is needed to express serum. 2 to 3 mL of EDTA blood and citrated blood is sufficient to perform hematology and coagulation tests respectively. 1 mL of whole blood is sufficient to perform 3 to 4 immunoassays. For erythrocyte sedimentation rate measurements 2 to 3 mL of citrated blood is adequate. Capillary sampling for blood gases requires 50 µL of arterial blood, while for venous sampling 1 mL of heparinized blood is recommended (5). The quality of diagnostic samples document also includes a comprehensive listing of analytes and their stability in the sample matrix (5).

An in-depth discussion of definition and mechanisms of hemolysis, definition and causes of lipemia and characteristics of icteric sample are included in the document on the hemolytic, icteric and lipemic sample (6). This document also provides guidelines for handling hemolytic samples, measures to eliminate lipemia and procedures to overcome interference by bilirubin in the icteric sample, thus extending previous recommendations of the NCCLS (8).

The checklist prepared by the College of American Pathologists for Laboratory Inspection and Accreditation addresses specimen related preanalytical variables (9).

International standardization bodies such as the ISO 6710 have issued standards for type and concentrations of anticoagulants to be used for venous blood samples (7).

The National Committee for Clinical Laboratory Standards (NCCLS) in U.S.A. constantly update their guidelines on several aspects of the preanalytical phase. An updated listing of the NCCLS Standards can be found in their current catalogue available from NCCLS (8).
Establishment of a quality manual addressing preanalytical variables is a prerequisite for implementing measures to recognize and control this crucial component of laboratory quality, which cannot be detected by traditional analytical quality control procedures.

The preanalytical quality manual should address both patient and specimen variables. Thus it should address the minimum sample volume needed for a laboratory test and equations to calculate sample volume for the number of tests requested for a patient. Defining optimum sample volume is critical to safeguard the patient from excessive blood collection that would in turn lead to iatrogenic anemia. Sample identification guidelines should be explicitly spelled out in the manual.

Instructions to the patient in preparation for specimen collection including fasting overnight for at least 12 hours, refraining from exercise and stressful activity the night before and just prior to blood collection should be provided. Guidelines for the collection of other body fluids such as urine should be included in the manual.

The posture during blood sampling, the duration of tourniquet application, the time of blood collection to minimize diurnal effects and the order of specimen collection should all be addressed in the preanalytical quality manual (1, 2).

Sample processing guidelines, transportation and specimen storage conditions should be clearly delineated (2).

The quality manual should have a comprehensive listing of analytes and notation on the effect of at least commonly encountered influence and interference factors.

Finally since the quality manual will be a source book for the laboratory professional an updated bibliography of preanalytical standards and compendia on drug interferences should be included for further consultation.

Recognition of Preanalytical Variables Causing Changes in Laboratory Results

Every laboratory should have a strategy for recognizing preanalytical errors. The following case reports and isolated laboratory data are intended as an exercise in the detection of preanalytical errors.

Case #1

A 55-year-old man was hospitalized with a serum potassium of 6.9 mmol/L on a non-hemolyzed sample obtained in an outpatient clinic. All other laboratory tests were normal. During hospitalization serum potassium values ranged from 3.9 - 4.5 mmol/L (normal 3.5 - 5.0 mmol/L). It was learnt that in the outpatient clinic, blood was collected with the application of tourniquet and fist clenching, while in the hospital ward, blood was collected through an in-dwelling catheter (10). The cause of this pseudohypokalemia was due to repeated fist clenching during tourniquet application which was intended to make the veins prominent. The contraction of forearm muscles causes release of potassium since there is a reduction in intracellular negativity during the depolarization of muscle cells causing efflux of potassium (1, 10). This effect can lead to a 1-2 mmol/L increase in potassium with as much as 2.7 mmol/L increase, which was noted in a healthy subject due to fist clenching during phlebotomy (11).

Case #2

A 40 year old male was hospitalized with a serum potassium of 8.0 mmol/L obtained on a non-hemolyzed specimen. Treatment that was administered to lower serum potassium levels were apparently unsuccessful since post-therapy serum potassium concentration was 7.5 mmol/L on a non-hemolyzed specimen. By now the patient became confused, developed muscle cramps and began to vomit. The doctor now requested a stat whole blood potassium determination, which yielded a potassium concentration of 2.7 mmol/L. The doctor promptly terminated therapy administered to lower serum potassium concentration. On examination of the hematology data, the white blood count was 20 x 10^9 / liter (normal 4.5 x 11.0), and the platelet count was 480 x 10^9 / liter (normal 150 - 350).

The cause of increased serum potassium on a non-hemolyzed specimen was due to the lysis of platelets and the release of potassium during the centrifugation procedure. In whole blood, however, platelets were intact and the potassium values obtained by the ion selective electrode procedure reflected the true value. The patient actually had normal potassium when therapy was initiated based on the initial spuriously high serum potassium results.

Case #3

A 75-year-old woman who appeared to be confused was hospitalized. Strikingly abnormal results in her electrolyte profile were a serum sodium of 162 mmol/L (normal 135 - 145 mmol/L) and a chloride of 125 mmol/L (normal 100-108 mmol/L).

Three days after her hospitalization her serum sodium and chloride values had returned to the normal range, and she appeared well and alert. Upon discharge from the hospital she complained about the quality of the hospital food and especially the soups she was so fond of and were denied to her.

Actually her hypernatremia and hyperchloremia was a result of her consuming within 10 hours two bowls each of three different kinds of soups (chicken-vegetable soup, tomato soup, pork-tomato soup). Her total sodium intake was in the range of 1338-1873 mmol/L, which even after dilution by body water resulted in increasing her serum sodium and chloride concentrations to 162 and 125 mmol/L respectively. Her confused state was due to the hyperosmotic effect of sodium causing efflux of water from the brain cells (12).

Case #4

An anemic 85-year-old woman on admission had the following hematology results. Hemoglobin 10.3 g/dL (6.4 mmol/L) (normal female 12.0 - 16.0 g/dL, 7.4 - 9.9 mmol/L), WBC 9.2 x 10^9 /L (normal 4.5-11), 10 g/L platelet count 354 x 10^9 /L (normal 150- 350). One week later hemoglobin was 22.9 g/dL (14.2 mmol/L), WBC 3.7 x 10^9 /L, and platelet count 78 x 10^9 /L. Analysis was repeated three times only to obtain similar abnormal results. However, on repeating the analysis for the fourth time the following data was obtained similar to results obtained on admission: Hemoglobin 10.2 g/dL (6.3 mmol/L), WBC 8.6 x 10^9 /L, platelet count 355 x 10^9 /L. The reason for
this discrepancy was that the blood collection tube was so overfilled that the air bubble right below the stopper was unable to move to the bottom of the tube in order to effect proper mixing on the rocking mixer. By the time analysis was repeated for the fourth time from the same tube enough blood had been aspirated from the same tube to provide sufficient space for the air bubble to move and effect mixing on the rocking mixer (13).

**Case #5**

A 38-year-old female biochemist handles experimental animals in her research. She is on oral contraceptives. Her serum thyroxin concentration was 180 nmol/L (normal 58-140), Free T, 19.3 pmol/L (normal 9.0 - 24.5), TSH 15 U/L (0.5 - 5.0). A repeat TSH performed in another laboratory by a different method turned out to be normal (4.0 U/L). The spuriously increased TSH was due to the presence in the subject's serum of human antimeuse antibodies (HAMA) which can interfere in a two-site immunometric assay by either bridging the capture and indicator antibodies thus giving a false positive result or if the HAMA is in excess prevent the labeled antibody from binding to the capture antibody and analyte complex thereby yielding a false negative result. Apparently the assay that overestimated TSH did not have sufficient amounts of mouse immunoglobulins in the assay mixture to completely absorb the HAMA (1).

**Case #6**

A 59-year-old woman treated with a cholinesterase inhibitor had the following electrolyte profile. Sodium 140 mmol/L, potassium 4.2 mmol/L, chloride 114 mmol/L and bicarbonate 34 mmol/L. It turned out to be normal (22-26). The anion gap was negative which is theoretically impossible. On further investigation we learn that the cholinesterase inhibitor administered to the patient is pyridostigmine bromide (3-hydroxy-1-methylpyridinium bromide dimethyl carbamate). Since the ion-selective electrode that was used to measure chloride is equally sensitive to bromide, chloride was overestimated resulting in a negative anion gap (14).

**Case #7**

A 60-year-old man with thinning hair is taking a drug to increase hair growth. Prior to treatment with this drug his prostate specific antigen (PSA) was 10 µg/L (normal 0.0 - 4.0) later, his PSA was 4.5 m g/L. All other laboratory tests were normal. The drug he was taking (finasteride) interferes with the conversion of testosterone to dihydrotestosterone, and older men with benign prostatic hyperplasia (BPH) are reported to experience a 50% drop in PSA (15).

We will conclude this paper by highlighting three most common and glaring preanalytical pitfalls. First, the electrolyte profile in a patient with glucose in excess of 55.5 mmol/L (1000 mg/dL) normal 3.9 - 6.1 mmol/L (70-110 mg/dL): Sodium 81 mmol/L, potassium 2.3 mmol/L, Chloride 48 mmol/L, bicarbonate 18 mmol/L. It turned out that the patient was receiving a glucose infusion and blood was collected from the same arm that was receiving the infusion causing a dilutional effect on the electrolyte values. Abnormal laboratory findings in a 43 year old male: Alkaline phosphatase 5 U/L (0.08 µkat/L) (normal 45-115 u/L, 0.75-1.92 µkat/L), calcium 0.5 mmol/L (2.0 mg/dL) (normal 2.1 - 2.6 mmol/L, 8.5 - 10.5 mg/dL) and potassium 22.0 mmol/L on a non-hemolyzed sample. On further investigation it was found that the plasma was obtained from blood collected in a tri potassium EDTA tube. EDTA chelated magnesium and zinc required for the activity of alkaline phosphatase, hence the alkaline phosphatase activity was low. EDTA also chelated calcium leading to its gross underestimation. Potassium in EDTA was responsible for dramatically elevating the potassium concentration to a physiologically impossible level.

Finally a specimen analyzed after a weekend storage in the refrigerator had a serum sodium 116 mmol/L, potassium (non-hemolyzed) 27.0 mmol/L, chloride 10.2 mmol/L, bicarbonate 26 mmol/L, and glucose 2.7 mmol/L (48 mg/dL). The specimen had been stored in the refrigerator as clotted blood leading to the inhibition of sodium-potassium ATPase pump leading to efflux of potassium from the cells and the influx of sodium into the cells. Even in the refrigerator glucose continued to be metabolized by the cells contributing to the spuriously low glucose value.

**Summary**

Preanalytical phase is an important component of total laboratory quality. Current efforts towards the standardization of preanalytical phase has increased the awareness of the effect of this critical component on laboratory results. With this awareness and the introduction of strategies to recognize preanalytical errors the goal of achieving total laboratory quality is finally within our grasp.

**References**


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