The Effects of Preanalytical Variables on Some Biochemical Parameters: A Review

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Abstract

Background: Both pre-analytical variables and biological variation affect the concentration or activities of analytes in body fluids which are a reflection of the individual’s health or pathological state. Objective: The aim is to review the effects of pre-analytical variables on some biochemical parameters. Method: A review of common analytes affected in routine clinical chemistry testing by pre-analytical variables. Results: Standardization of specimen collection practices minimizes the effect of variables that causes changes in test values and thereby reduces the difficulty in interpretation of values. Conclusion: Standardization of the pre-analytical variables may not always be possible, thus one must understand the influences of the variables on the composition of body fluids and these can be easily prevented with awareness and laboratory staff education.

Keywords: Pre-analytical variables, Biochemical parameters, standardization, test values.
INTRODUCTION

The total Laboratory testing process includes three phases namely preanalytical, analytical and postanalytical. Several studies have shown that most errors occur in the preanalytical phase [1,2]. A major emphasis is currently placed on this initial phase of the total testing cycle because the preanalytical variables is believed to exert a significant influence on laboratory test results [3].

Besides the variation due to preparation of patient and blood collection such as physical activity, the fasting state, the blood collection technique [4,5], and tourniquets application time [6,7] there are additional preanalytical variables that might influence the reliability of laboratory testing. In particular variations and errors due to preparation of the specimen prior to the measurement of blood analytes such as specimen interferences. Processing of specimen and storing of specimen before the measurement causing leakage of intracellular components and producing significant biological and analytical interference [8].

Inherent in any laboratory test results on patients are influences of biological variation, inherent analytical error, preanalytical and postanalytical sources of variation and possible pathophysiological alterations. To be able to reduce the errors and for proper interpretation of test results, one must have a good understanding of these preanalytical variables.

Generally, laboratory users need only limited knowledge of the technical details of the laboratory tests. However, they should understand that the appropriate collection of patient specimens can affect results and they should therefore work with the laboratory in its effort to produce reliable test results rapidly and accurately and identifiable with the relevant patient [9]. Preanalytical variables can be grouped into four categories which include: Physiologic specimen collection, handling and interference variables [10]

In its review, we focus on the preventable preanalytical variables which include specimen type selection, blood collection, blood collection equipment and factors interfering biochemical tests.

Physiological Variables

Biological influences – Heredity, gender, age and race can affect individual lab results and these are non-controllable variables.

Age has a notable effect on reference internals; typical changes occur in serum composition at extremes of ages. In general, individuals are considered in four groups – newborn, the older child to puberty, the sexually mature adult and the elderly adult. One must always look at the age of the patient when evaluating
laboratory results. Paediatric values vary in many lab tests.

Gender can be a factor because of hormonal variations that occur with each gender. After puberty the serum activities of ALP, ALT, AST, CK are greater in men than in women. The concentrations or Albumin, Calcium and Magnesium are also higher in men than to women. Differentiation or the effects of race from those of socio-economic conditions is often difficult. Nevertheless, the serum total protein concentration is known to be higher in blacks than in whites. Carbohydrates and Lipid metabolism differ in blacks and whites [11]. Glucose tolerance is less in blacks (Table 1)

Diurnal Variation

Table 1 Pre-analytical Variables

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<td>Posture-standing sitting, lying</td>
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Adapted from [10]

Cause of error

Patient not fasting
Keeping blood overnight or refrigerating blood sample
Hemolysis of blood
Prolonged venous stasis during venesection
Taking blood from an arm with an infusion running into it
Putting blood into wrong container or tipping it from one container into another
Blood for glucose not put into fluoride
delay in analysing blood gases
Failure to keep sample cool or delay separating and freezing plasma
Palpation of prostate by rectal examination, passage of catheter enema etc in last few days
Inaccurately timed urine collection Incorrect urine or no preservative

Some possible consequences

High plasma triglyceride and glucose
High plasma k+,phosphate, LDH, AST
As above, lower plasma ALP
High plasma protein total Ca2+ and cholesterol Electrolytes and glucose concentrations similar to dilution of everything else.
Eg, EDTA or oxalate cause low plasma Ca 2+
Low blood or plasma glucose Low bicarbonate concentration low PTH, ACTH,insulin
High tartrate – labileacid phosphatase and PSA
Poorly timed 24hrs urinary excretion values,
Abnormal renal clearance values
Falsely low result e.g. urea or calcium

Table 2 Some pre-analytical Variables (extra-laboratory factors) leading to erroneous results
Many constituents of body fluids exhibit cyclical variations, throughout the day. Factors contributing to such variations include posture, activity, food ingestion, stress, daylight or darkness, and sleep or wakefulness. These cyclical variations can be quite large and therefore the drawing of the specimen must be strictly controlled. For example, the concentration of serum iron and cortisol may change by as much as 50% between 0800 and 1600. Serum potassium has been reported to decline from 5.4 mmol/L at 0800 to 4.3 mmol/L at 1400 [11].

**Exercise**

Strenuous exercise can increase the bilirubin, creatine kinase (CK), aspartaminotransferase (AST), high density lipo protein (HDL), cholesterol, lactate, lactate dehydrogenase (LD) and uric acid [4].

**Lifestyle**

Diet, caffeine, smoking and alcohol intake can have an effect on some chemical analytes. High protein diets increase levels of uric acid, urea, and ammonia in blood compared with vegetarians. Caffeine can decrease pH, increase ionic calcium and catecholamine levels. Smoking can increase glucose, triglyceride cholesterol and LDL Cholesterol. Short-term effects of ethanol include a decrease in glucose, increase in plasma lactate and an increase in uric acid and triglyceride. Moderate intake of alcohol increases the HDL cholesterol. Long term effects of alcohol include an increase in gamma glutamyl transferase (GGT), AST and ALT [12,13].

**Fasting Status**

Certain specimens are required to be fasting. They include fasting glucose and lipid profile. A non-fasting glucose will be increased compared to a fasting sample. The triglyceride and low-density lipo protein (LDL) cholesterol will be increased in a non-fasting sample compared to a fasting sample. [14]

Prolonged fasting can decrease transthyretin (prealbumin) glucose, albumin, LD, HDL, cholesterol and insulin. Dehydration causes hemo concentration, which can result in the false elevation of some chemical analytes including iron, calcium, sodium, and enzymes. An elevated haematocrit or protein can be an indication of dehydration and the patient should be rehydrated before reassessing the chemical analytes.

**Posture**

Substantial changes take place with a change from lying to the sitting position or from standing to a supine or sitting position [14].
A decrease in albumin, alkaline phosphate, ALT, bilirubin, calcium, cholesterol, total protein and triglyceride is noted when going for a standing position to a supine position. Accurate and legibly written information about the patient is essential, although electronic requesting symptoms are now available. This information includes the patient’s hospital case, number, name, date of birth. The requesting doctor must sign the form legibly.


Specimen collection variables include requisition errors, patient identification errors, tourniquet time variability improper cleansing agents, improper collection time, and intravenous or drug medication interference with the sample.

- **Tourniquet application time** - A tourniquets that is kept on too long (>3 minutes) will increase the total protein, iron, AST, bilirubin and total lipids (Table 2). A total cholesterol level may increase 5% at 2mins and 15% at 5 minutes. Repeated fist clinching can increase the potassium by 1 to 2 mmol/L. [15, 16]
- **Intravenous infusion site** - Drawing from above an intravenous infusion (iv) site should be avoided if at all possible. Drawing from below the iv site after turning off the iv for 2 to 5 minutes and discarding the first is 5ml seems to be preferable. Some laboratories may draw from above the IV as a last resort. If the IV has been shut off for 10 minutes. [17]
- **Cleansing Agents** – Providone – iodine (Betadine) used as a cleansing agent falsely elevates phosphorus, uric acid, and potassium. (Table 2)

Isopropyl (70%) alcohol should not be used for medical or legal ethanol levels. The site for venepuncture is to be cleaned with 70% ethanol. The type of collection sample, capillary or venous serum, or serum versus plasma sample can cause variances in the analyte measurement. Glucose capillary values are 1.4% higher than venous serum samples and potassium capillary samples are 0.9% higher than venous samples. Capillary samples of bilirubin calcium chloride, sodium and total protein are lower than venous serum samples (18) plasma values of potassium, phosphorus and glucose are lower than serum values. Plasma values of total protein LD, and Calcium are higher than serum values. A cholesterol, triglyceride and HDL cholesterol measured with EDTA plasma should be multiplied by 1.03 to be equivalent to a serum sample. [19, 20].

- **Anticoagulants** – most chemistry analytes are run are on serum or heparinized plasma samples depending on the analyte and methodology used to analyse it. Some anticoagulants cannot be used for certain tests. Anticoagulants containing fluoride (an inhibitor of erythrocyte glycolysis) may be used for glucose testing but will interfere with electrolyte studies by altering blood cell membrane permeability. Potassium should be estimated
on plasma from heparinised blood rather than serum – potassium released from cells especially platelets during clotting serum potassium concentrations are usually higher than those of plasma by a variable amount. Marked difference may be found in patients with leukaemia. [21]

- Gel versus Non-Gel tubes – serum or plasma separator tubes may be unacceptable for some analytes, for example, therapeutic drugs. The manufacturer of the separator tube should provide documentation of analytes that have been shown to give comparable results in serum of plasma obtained from tubes containing gels versus nongel tubes.

- Order of Draw- Clinical and Laboratory Standard Institute CLSI (formerly NCCLS) recommendations for vacutainer or syringe order of the draw of filling tubes is the following. Blood culture tubes, non additive or serum tubes, citrate or coagulation tubes, gel separator tubes, heparin, EDTA, and fluoride tubes. Filling the tubes out of this order may cause some cross contamination in the tube leading to interference in testing the analytes [22 23]

4. Handling

(I) Light and Temperature- bilirubin, vitamin B<sub>12</sub>, Vitamin A and Carotere are affected by light Temperature labile analytes include ammonia, blood gases, lactate, pyruvate. Specimens can be chilled by placing in ice water if blood gases are collected in plastic syringes and run within 20 minutes, they do not need to be iced. [24]

(ii) Specimen processing – A clot —tube specimen should be allowed to clot for 30 to 60 minutes and no longer than 2 hours before it is centrifuged and the serum separated from the clot. Analytes that increase on standing include CK, lactate, LD, phosphate and ammonia. Analytes that may decrease on standing include glucose and bicarbonate [25]

If plasma is not separated from blood cells within a few hours, the effect on plasma concentrations will be similar to that resulting from haemolysis.

The refrigeration of whole blood has the effect of raising the plasma potassium concentration probably by reducing the activity of ATPase Pump. Therefore, blood specimens must be centrifuged and the plasma separated from the cells before storing, for example overnight.

5. Interfering substances

The presence of lipemia, haemolysis and fibrin strands can be evaluated in the specimen before the analytical rum.

Haemolysis. Is one of the most frequently occurring interferences in analysis in
the clinical laboratory.

Haemolysis results from mechanical factors in specimen collection and processing.

Haemolysis interferes, with the analysis in two ways

1. Haemolysis absorbs at 431 and 555mm, if the analyte is measured spectrophotometrically at or around 430 and 555mm, the absorbance of haemoglobin can cause false elevated results [26]
2. The concentrations of various blood constituents differ in erythrocyte and plasma.

Several constituents such as potassium, LDH, acid phosphatase, AST are present in very large amounts in red cells in comparison with plasma. Consequently, haemolysed plasma gives higher values for this test. On the other hand, sodium and chloride which are present in small amounts in erythrocytes, tend to grow lower values if the serum is haemolysed [27]

It is also important to separate the serum from the clot or the plasma from the cell as soon as possible after collection to avoid the free exchange of analytes between the cell and the serum or plasma

CONCLUSION

Blood and urine samples constitute over 95% of all specimens analysed in Clinical Laboratories, the remaining 5% are cytological and surgical specimens. Blood can be considered as the major biological fluid on which quantitative analyses are performed. Consequently, specimen requirements are most rigid for blood samples. Serum is used for most of the chemical analyses. Before performing the actual analysis, one should be sure of the quality of the specimen. The analytical results on bad specimens are not only useless but also misleading and sometimes even dangerous to the patients involved. Generally speaking, analytical methodology and instrumentation do not make any adjustments or compensations for interfering factors. Therefore, it is the responsibility of the analyst to see that the samples analysed are free from any interference or deterioration.

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