

## Review Article:

# Pre-analytical phase in clinical chemistry laboratory

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## ABSTRACT

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The laboratory testing process is divided into the pre-analytical, analytical and post-analytical phases. For obtaining reliable test results, the prevention and detection of errors at all steps is required. While analytical standards have been developed by recognized quality control criteria, there is a scarcity in the development of standards for the pre-analytical phase. This phase is most prone to errors as the steps involved are directly dependent on humans and are out of direct control of the laboratory. Such errors in preanalytical stage often only become apparent in the analytical or post-analytical phase. The development of a pre-analytical quality manual is essential in achieving total quality control. Correct practices and strategies of error prevention can reduce preanalytical errors. This review focuses on prevention of pre-analytical errors that occur while collecting a specimen of blood, urine and cerebrospinal fluid. Most of these can be easily prevented with understanding and education of the personnel involved in and responsible for executing this crucial pre-analytical phase.

**Key words:** Automation, Pre-analytical Errors, Patient Care, Quality Control, Quality Improvement

Neogi SS, Mehndiratta M, Gupta S, Puri D. Pre-analytical phase in clinical chemistry laboratory. *J Clin Sci Res* 2016;5:171-8. DOI: <http://dx.doi.org/10.15380/2277-5706.JCSR.15.062>.

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## INTRODUCTION

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Pre-analytical errors are the errors that occur from the time a laboratory test is ordered by the physician until the sample is ready for analysis. Most errors in laboratory test results occur in the pre-analytical phase. Advancement in instrumental technology and automation have simplified work in terms of analytical quality in laboratory diagnostics and improved the quality of test results. The same is not true about the pre-analytical stage and it is still the stage which is most prone to errors with 46 %-71% errors encountered during the total diagnostic process.<sup>1,2</sup> Therefore, pre-analytical phase serves as an essential element of laboratory medicine where more attention is needed.<sup>3-5</sup> Another study<sup>6</sup> has reported that maximum errors occur during pre-analytical phase i.e., 61.9% while 15% were analytical,

and 23.1% were post-analytical. From a theoretical viewpoint, pre-analytical analysis may be sub-divided into a pre-preanalytical phase (where the clinician decides which test to order, based on his knowledge and experience) and the conventional pre-analytical phase, involving a series of related processes starting with patient identification, through the selection of ideal tubes, proper transportation and storage, and ending with the preparation of the samples.<sup>7</sup>

Overall, an insufficient quantity and inappropriate quality of the specimen may account for over 60% of pre-analytical errors.<sup>8</sup> Other pre-analytical variables occur due to inadequate training and understanding of the blood collection process by laboratory personnel,<sup>9</sup> patient identification and preparation errors,<sup>10,11</sup> defects in sample

Received: October 21, 2015; Revised manuscript received: March 08, 2016; Accepted: April 05, 2016.

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DOI: <http://dx.doi.org/10.15380/2277-5706.JCSR.15.062>

collection device, container, and procedure<sup>9</sup> and erroneous sample handling, separation, transport and storage.<sup>9</sup> Some sources of errors cannot be rectified and can seriously affect the reliability of test results. These can barely be identified by laboratory staff and include patient dependent variables (age, sex, physical exercise, diet, stress, and menstruation), mild or visually undetectable haemolysis, and prolonged tourniquet stasis while drawing blood. The role of the “human factor” in sample collection makes complete elimination of errors unrealistic. However, following new strategies for error prevention can lead to a substantial reduction in pre-analytical errors. These practices include: increased rate of detection of errors, use of instruments of high quality, reporting and tracking, process and risk analysis, process redesign, healthcare professional training, and improved communication among healthcare professionals.<sup>12</sup> It is therefore imperative for laboratory professionals to identify the areas of possible error, particularly with reference to samples of blood, urine and cerebrospinal fluid.

## **BLOOD SPECIMENS**

Since blood reflects the physiological status of the body systems, a lot of emphasis must be placed on the proper method of collection of blood. The common sources of errors and their consequences are mentioned below and outlined in Table 1.

### **Patient identification**

It is important to identify a patient properly so that the requisite sample is collected from the correct patient. When identifying the patient, it is necessary to provide full name, address, identification number and/or date of birth.<sup>13</sup> Hospital in-patients should wear an identification band with the above information, for verification by phlebotomist before the venipuncture. Drawing blood from the wrong person or incorrect labelling of the sample will result in incorrect results and thus may affect

the treatment protocol because of the delay in the availability of appropriate reports. It may also warrant repeat sampling and thus unnecessarily add to the cost for treatment.

### **Patient preparation**

Before collecting specimens for clinical chemistry, consideration must be given to certain patient variables. For analytes, like glucose and cholesterol, overnight fasting (at least 12 hours) is required to avoid erroneous results. Diurnal variation is known with analytes, such as cortisol and adrenocorticotropin. In such cases, an average of the samples drawn at two different times of the day may be considered.

### **Selecting the site**

The site of venipuncture can make a difference towards quality of the sample. The most commonly selected site is the median cubital vein. The next choice is the cephalic vein followed by basilic vein. Caution must be exercised so that only the basilic vein is punctured and the neighbouring brachial artery and median nerve remains unharmed.

### **Site preparation**

First, the site of venipuncture should be cleaned with alcohol. Cleaning should begin with the center of the site of venipuncture, and should continue outwards in concentric circles. Before starting the venipuncture, the alcohol should be allowed to air dry. Contamination with alcohol can lead to haemolysis. Haemolysis can result in the spurious elevation of the levels of analytes such as potassium, lactate dehydrogenase (LD), iron and magnesium in the chemistry laboratory.

### **Tourniquet application and time**

The tourniquet should be applied 3"-4" above the venipuncture site. The tourniquet should be applied on the arm for no longer than one minute.<sup>13</sup> It is essential to obtain accurate results for critical analytes like calcium (prolonged application results in false high levels).

**Table 1: Common sources and consequences of sample collection related errors**

<b>Phase</b>	<b>Error</b>	<b>Consequence</b>	<b>Practices to minimize errors</b>
Patient identification	Wrong or incomplete information	Misdiagnosis or delayed diagnosis due to repeat sampling	Patient identification bands
Patient preparation	Collection after meal for tests requiring fasting sample	False high values for parameters which increase after a meal eg. glucose, lipemia resulting from collection after a heavy meal can interfere with optical measurements and electrolyte analysis	Proper instruction and patient preparation i.e overnight fasting
	Analytes exhibiting diurnal variation	False high or low values	An average of the samples drawn at two different times of the day
Site of blood collection	Wrong site selected	False high or low values e.g arterial blood preferred for ABG analysis, venous blood can give false low pO <sub>2</sub> and false high pCO <sub>2</sub> values	Choosing the correct site
Site preparation	Contamination with alcohol	Hemolysis which in turn can lead to false high values for potassium, lactate dehydrogenase (LD), iron and magnesium etc	Allowing the site of puncture to completely dry before puncture
Tourniquet Application and Time	Prolonged application	False high calcium and potassium values	Should be applied on the arm for no longer than one minute
Proper Venipuncture Technique	Excessive probing and or fishing	Poor quality sample and hemolysis	Proper training for phlebotomy
Order of Draw	Incorrect order can lead to potential cross contamination of K <sub>2</sub> or K <sub>3</sub> EDTA on the needle from the lavender top tube to the chemistry tube	Can lead to an elevated result for potassium leading to misdiagnosis or wrong treatment	Order of draw for evacuated blood collection tubes: tube for blood culture, citrate tube, serum separator / serum tube, Heparin, EDTA and fluoride tube
Specimen volume and Tube Mixing	Faulty phlebotomy and improper tube mixing	Erroneous results due to improper sample to additive ratio, analyzer probe blocks due to clots in sample	All tubes with additives need to be inverted to mix the additive evenly with the correct volume of blood as indicated
Tube Handling and Specimen Processing	Improper handling	Erroneous results due to faulty handling	To follow guidelines for each type of specimen collection tube
Handling of Blood Specimens in special conditions	Faulty handling	Erroneous results due to faulty handling as a result of sample instability eg. adrenocorticotrophic hormone (ACTH), angiotensin converting enzyme (ACE) to be transported in ice slurry	To train phlebotomists regarding guidelines for specimens requiring special handling

### **Proper venipuncture technique**

During phlebotomy, probing to find the vein and achieve blood flow should be avoided. Excessive probing and/or “fishing” to find a vein can result in a poor quality sample and increase the chances of haemolysis.

### **Order of draw**

Selecting the correct order of draw during venipuncture will ensure accurate test results. The Becton, Dickinson and Company (BD) and Clinical and Laboratory Standards Institute (CLSI), formerly National Committee for Clinical Laboratory Standards (NCCLS) has recommended the following order of draw for evacuated blood collection tubes: tube for blood culture, citrate tube, serum separator / serum tube, Heparin, ethylene diamine tetra acetic acid (EDTA) and fluoride tube.<sup>13</sup> If the proper order for draw is not followed, potential cross contamination of K2 or K3 EDTA on the needle from the lavender top tube to the chemistry tube can lead to high serum potassium levels.

### **Proper tube mixing**

Tubes with additives should be mixed by inverting to ensure mixing of the additive evenly with the blood. Serum separator tubes contain clot activator and should be inverted 5 times to mix the activator to facilitate clotting of blood. Other tubes containing additives, such as heparin, EDTA and fluoride have to be inverted 8-10 times for proper mixing of the anticoagulant with the blood and prevent clotting and should not be shaken vigorously (to prevent haemolysis).

### **Correct specimen volume**

All collection tubes have to be filled to the correct volume of blood to ensure appropriate blood to additive ratio.<sup>14</sup> Expiration dates should also be checked on the evacuated tubes.<sup>15</sup>

### **Proper tube handling and specimen processing**

Processing of the tubes is the next procedure that can affect the test results. Serum and plasma tubes should be processed separately, as both specimen types have their own special handling requirements.

It should be ensured in case of Red top tubes (serum tubes) and gel tubes that the blood in the tubes clots completely prior to centrifugation and processing. Red top tubes should be allowed for 45 to 60 minutes, serum separator tubes should be allowed to clot for 30 minutes to ensure complete formation of the clot.<sup>15</sup> Blood samples from patients receiving anticoagulants may take longer time to clot. Tubes should be allowed to clot at room temperature, upright in a test tube rack, with the tubes closed. Centrifuging too soon may result in a gelatinous and/or fibrinous serum sample that will require respinning.

Plasma specimens do not require any waiting period. This allows the tube of blood to be drawn, mixed and centrifuged immediately, reducing the turn-around-time for test results.

### **Centrifugation**

Both serum and plasma separator tubes are to be centrifuged at the same speed and for the same period. Serum and plasma tubes without gel can be spun at a speed of 1000 relative centrifugal force (RCF) for ten minutes.<sup>15</sup> The timing is important as the gel needs to form solid barrier between cells and plasma/serum. Otherwise, cells and platelets may remain in the plasma and could cause interference with some analytes.

### **Special handling of blood specimens**

Some analytes may require the sample to be chilled after collection in order to maintain the stability of the analyte. Examples include adrenocorticotrophic hormone (ACTH), angiotensin converting enzyme (ACE), acetone, ammonia, catecholamines, free fatty

acids, lactic acid, pyruvate and renin. Slurry of ice and water is recommended for chilling tubes of blood.

### **Stability for whole blood, serum and plasma**

If a specimen of whole blood requires centrifugation (removal of serum or plasma from the red blood cells), this process should be completed within two hours after the venipuncture.<sup>16</sup> Once the serum has been removed or separated, the sample will be stable at room temperature for eight hours, and up to 48 hours at 2-4 °C. After 48 hours, the serum specimen should be frozen at -20 °C in an aliquot tube.<sup>16</sup> For analytes which are photo-sensitive, and need to be protected from light, stability can be ensured by wrapping the tube in aluminum foil. The most common example of a light-sensitive analyte is bilirubin.

Therefore, detailed attention to the sample collection related preanalytical variables will help to ensure accurate test results and improve the efficiency in all areas of the clinical laboratory.

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## **URINE SPECIMENS**

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After blood, urine is the most commonly used specimen for diagnostic testing, monitoring of disease status and for detection of drugs. Like blood, urine samples need to be collected and preserved under ideal conditions. The CLSI Guidelines make the following recommendations for urine collection.<sup>17</sup> Primary (routine) specimen containers should have a wide base and a capacity of at least 50 mL. 24 hour specimen containers should have a capacity of at least 3 litres. Specimen containers should have secure closures so as to protect from contaminants and prevent loss of specimen.

Amber colored containers should be used for specimens required for assay of light sensitive analytes such as urobilinogen and porphyrins.

In addition to routine checks performed for all specimens, some additional check items are

required for urine specimens. These include: labeling, collection date and time, volume, specimen preservation and collection method.

### **Labels**

If a collection container is used for transport, the label should be placed on the container and not on the lid to avoid wrong identification of the specimen.

### **Collection time and date**

The time and date of collection should be included in the specimen label. The start and stop times of the collection for timed specimens and the time of receipt by the laboratory must also be documented for verification of proper handling and transport after collection.

### **Volume**

It is important for specimen collection personnel to ensure that there is sufficient volume to perform the required tests.

### **Specimen preservation**

Non-pH buffered boric acid may be harmful to certain organisms while buffered boric acid preservatives can reduce the harmful effects of the preservative on the organisms.<sup>18</sup> Preserved urine specimens can be stored at room temperature until the time of testing. When a preservative is required, it should be added to the collection container before the urine collection begins.

### **Method of collection**

The method of collection should be confirmed when the specimen is received in the laboratory to ensure that the type of specimen submitted meets the needs of the required test(s). First morning specimens are the specimen of choice for urinalysis and microscopic analysis since the urine is generally more concentrated due to the length of time the urine is allowed to remain in the bladder. Therefore, the sample contains relatively higher levels of cellular elements and analytes. Abnormal constituents are also likely

to be present in higher concentration and, thus, are more likely to be detected.

Timed collection of specimen is required for quantitative measurement of certain analytes, including those subject to diurnal variation. These include creatinine, urea, potassium, sodium, uric acid, cortisol, calcium, citrate, amino acids, catecholamines, metanephrines, vanillylmandelic acid (VMA), 5-hydroxyindoleacetic acid, protein, oxalate, copper, 17-ketosteroids, and 17-hydroxysteroids. Timed specimens should be refrigerated during the collection period, unless otherwise directed by the laboratory. Accurate timing is very important as this information forms a critical part of the calculations performed to determine urine clearance values (e.g. creatinine clearance). Interpretations based on faulty calculations can result in improper diagnoses or medical treatment.

### **Collection of urine specimens from catheters**

Collection of urine specimens from catheters (e.g., Foley's catheter) is done using a syringe, followed by transfer to a specimen tube or cup. Supra-pubic aspiration may be necessary in non-ambulatory patients. Thus, a urine sample should be collected and preserved under optimum conditions, in order to obtain reliable and accurate test results.

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### **CEREBROSPINAL FLUID SPECIMENS**

CSF analysis forms an important area of analysis in the clinical chemistry laboratory. For the collection of CSF, sterile, screw capped containers should be used. It should be collected only by the physician. The physician should wear sterile gloves and use aseptic precautions during the procedure. Instructions should be given to despatch the specimen immediately to the laboratory, as any delay can cause death of organisms like meningococci and can disintegrate leukocytes. If a delay in transport is anticipated, the sample should be kept at room temperature, since refrigeration tends to

kill *H. influenza*.<sup>19</sup> In addition to microbial culture, cytology, and immunological studies, physicians depend upon the biochemical analysis of patients' CSF for various diseases. Apart from routine glucose and protein determinations,  $\beta$ 2-microglobulin, neuron-specific enolase (NSE), S-100 protein, and myelin basic protein can be measured. A traumatic lumbar puncture (faulty technique) increases the risk of blood contamination of the CSF sample; therefore, it is always recommended to discard the first 1-2 mL to avoid any effect due to haemolysis and immediately centrifuge the sample before freezing.<sup>19</sup> It must be borne in mind that CSF samples must always be centrifuged prior to analysis in order to precipitate any cells. Otherwise falsely high values for CSF protein will be obtained. The utmost caution must be exercised while pipetting and handling CSF samples. It is also recommended to aliquot the samples in small volumes (0.25 or 0.5 mL tube) and fill the tube up to 75%, to minimize the risk of adsorption and evaporation.<sup>20</sup>

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### **LESSONS FOR PRACTICE**

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In descending order, the most common sources of error in the preanalytical phase arise from mistakes in the filling of tubes, inadequate anticoagulant-blood ratio, followed by patient misidentification.<sup>21</sup> Negative impact of preventable laboratory errors on patient outcomes was observed in 24.4% of cases, leading to repetition of tests and increasing the length of hospital stay and costs.<sup>8</sup>

Thus, a quality manual for preanalytical variables is needed for implementing measures to recognize and control preanalytical variables. This forms a critical component of laboratory quality, which cannot be compensated by analytical and quality control procedures. The pre-analytical quality manual should address both patient and specimen variables. Standard instructions to be given to patients. Sample

processing guidelines, transportation and specimen storage conditions should also be included in the manual. The quality manual should have a comprehensive listing of interference factors encountered. Finally, an updated bibliography of preanalytical standards should be included. The laboratory personnel should also be educated for awareness and preventing preanalytical errors<sup>22</sup> and about guidelines laid down by international standardization bodies such as the ISO 6710 and the NCCLS/ CSLI in the U.S.A.

Apart from this, training the phlebotomists who are usually medical interns, residents, nurses and technicians in the basics of blood sample collection and the potential sources of errors and the practices to be implemented to minimize them. In a study<sup>23</sup> that adopted the role play model for training interns on the importance of pre-analytical phase, it was found that all medical interns were taking the correct precautions while collecting and sending the blood samples for analysis to the laboratory, thus ensuring better quality of results. Role playing is an upcoming teaching approach in medical education and it provides better understanding of the subject and in the acquisition and retention of knowledge about various medical subjects.<sup>24</sup> In order to document the effectiveness of the method, five interns were interviewed in an informal manner at their work place. The medical interns trained were of the opinion that this method is stimulating, thought provoking and is livelier than the usual method of teaching. They would be able to correlate better with actual situations they will encounter in the future.

The pre-analytical errors are defined as human dependent and preventable sources of mistakes occurring from the time a laboratory test is ordered by the physician until the sample is ready for analysis. Most of these can be easily prevented with continuous education of the personnel involved (clinicians and laboratory

staff). Awareness among medical practitioners about the preanalytical errors and how to prevent them will thereby help in reducing errors in the test result, and ultimately improving the quality of healthcare.

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### ACKNOWLEDGEMENTS

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We are grateful to Dr Ruchika Mehndiratta for her editorial guidance and assistance.

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