BLOOD SAMPLE COLLECTION AND DIURNAL VARIATION : A REVIEW

Vishnu Kumar, Sharique Ahmad*, Zarina Farheen*

Department of Biochemistry, Department of Pathology*

Era's Lucknow Medical College & Hospital, Sarfarazganj Lucknow, U.P., India-226003

ABSTRACT

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Address for correspondence Dr. Sharique Ahmad

Department of Pathology

Era's Lucknow Medical College &

Hospital, Lucknow-226003

Email: diagnopath@gmail.com

Contact No: +91-9648351223

Pre-existing researches have shown that some biochemical machinery reveal diurnal variation and are perceptive to postural change. The latter is due to the consequence of gravitational force and hydrostatic pressure on the plasma level. Therefore laboratories are suggested to apply the rules of standardization based on preanalytical factors, which state that blood samples should be taken in the morning after at least 15 minutes of rest. The concentrations of many substances in blood vary considerably at different times of day e.g. Cortisol (8:am at peak and 4:00 pm dropped) Specimens for these analyses must be collected at the times

at different times of day e.g. Cortisol (8:am at peak and 4:00 pm dropped) Specimens for these analyses must be collected at the times specified by the laboratory, as there may be no reference ranges relating to their concentrations in blood at other times According to current recommendations, blood samples should be taken in the morning after 15 minutes' resting time. Multiple blood and saliva cortisol levels collected at different times, such as at 8 am and 4 pm, have been used successfully to evaluate both cortisol levels and diurnal variation. A 24-hour urine cortisol sample will not show diurnal variation; it will measure the total amount of unbound cortisol excreted in 24 hours. The heart service of any clinical laboratory is blood sampling and analysis. In connection with this, reference intervals or decision limits need to be established. Some components exhibit diurnal variation and impact of resting time prior to blood sampling on biochemical components, including albumin, thyrotropin (TSH), total calcium and sodium in plasma.

KEYWORDS: Diurnal variation, Blood sampling, Supine posture, Collection tubes, Check lis.

INTRODUCTION

Current day's diagnosis is heavily dependent upon reliable laboratory investigations Reports. It is therefore pertinent to ensure the credibility of the investigation reports emanating from the clinical laboratories. Entire process, from sample collection to report generation; now has been divided in to three phases. 1. Pre analytical phase, 2. Analytical phase and, 3. Post Analytical Phase, and errors arises during these phases are known as Pre Analytical errors, Analytical errors and Post Analytical errors respectively. In which pre analytical errors are most prevalent, and covers up to 73 % of total Laboratory errors, while analytical errors covers up to 7 % of total laboratory errors. And post analytical errors covers 20% of total laboratory errors. Laboratory services are absolutely essential for diagnosis of functional abnormalities of the diseases at cellular, extra cellular, tissue and organ levels. Biochemical investigations will help a vigilant clinician to understand the diseases in diagnosis, prognosis, and for follow-up of the treatment response. (1-10) Appropriate test is one in which clear question for which the result will provide an answer. Enabling the clinician to make a decision. Initiate some form of action leading to health benefit for the patient. Before ordering investigations, first nt times, such as at 8 am and 4 pm, have been used ation. A 24-hour urine cortisol sample will not show cortisol excreted in 24 hours. The heart service of any on with this, reference intervals or decision limits need and impact of resting time prior to blood sampling on I), total calcium and sodium in plasma. osture, Collection tubes, Check lis. you have to fill investigation request form very carefully Patient's details: Name, Age, Sex, IPD/OPD Number, name of the test and the source (IN CAPITAL LETTER). Brief clinical presentation of

CAPITAL LETTER). Brief clinical presentation of patient with provisional diagnosis or clinical history. Special notation e.g., medications taken or any other. Poor documentation gives impression of ignorance & carelessness. Generally 3 colors (white, pink and vellow) lab investigation request forms are used in Hospital, to send investigation request to clinical laboratories. White color investigation request form is for Biochemistry. Pink color investigation request form is for Hematology (Clinical Pathology). Yellow color investigation request form is for Microbiology. Biochemistry, Pathology and Microbiology tests have already written on relevant investigation request form. You have to tick only relevant investigation required, sign, stamp and send to HLS with well labeled sample vacuutainer. (11-15)

TYPES OF LABORATORY TESTS: Laboratory tests have been categorised in 4 Major Groups

1. Discretionary or on-off tests

Most common clinical biochemistry tests that are designed to answer specific questions e.g., Does the patient has increased blood urea/ glucose/ serum bilirubin concentration? Normally, these tests are useful to support the diagnosis.

2. Biochemical Profiles

These tests are based on the fact that more useful information on the patients disease status can be obtained by analysing more constituents rather than one e.g., plasma electrolytes (Na⁺, K⁺, CI, Ca⁺⁺, Li⁺), liver function tests (serum bilirubin, ALT, AST), Antenatal Profile, Lipid Profile, Thyroid Profile etc.

3. Dynamic Function Tests

These tests are designed to measure the body's response to external stimulus e.g., oral glucose tolerance test (to assess glucose homeostasis).

4. Urgent or Emergency Test

An urgent test is designated as one on which the clinician is likely to take immediate action. The main reason for asking for an analysis to be performed on an urgent basis is that immediate treatment depends on the result. i.e. Blood glucose, CBC, MP, NS1 Dengue, PT, PC, INR, CRP, PROCALCITONIN, Blood glucose, Na-K, CKMB, TROP T, TROP I, UREA, CREATININE etc. Generally clinical laboratories releases emergency reports within 2 hours, IPD reports 8 hours and OPD reports next day.

Collection of Specimens*

Lack of thought before collecting specimens or carelessness in collection may adversely affect the interpretation or impair the validity of the tests carried out on the specimens. (16-18)

Safety Considerations

A white laboratory coat or tunic is worn and fastened to the top. Gloves must be worn when handling blood samples. Any member of staff handling blood samples are advised to be vaccinated against Hepatitis B. (19 & 20)

Factors Affecting Collection Of Sample

Diet

Dietary constituents may alter the concentration of analytes in blood significantly (e.g. plasma [glucose] and [triglyceride] are affected by carbohydrate and fatcontaining meals, respectively).

Drugs

Many drugs influence the chemical composition of blood (e.g.) antiepileptic drugs. Details of relevant drug treatment must be given when requesting chemical analyses, especially when toxicological investigations are to be performed. (21-23)

Diurnal Variation

The concentrations of many substances in blood vary

considerably at different times of day and/or are sensitive to postural change e.g. Cortisol (on 8:am at peak and dropped at 4:00 pm). Specimens for these analyses must be collected at the times specified by the laboratory, as there may be no reference ranges relating to their concentrations in blood at other times. The patient should be seated or be in supine posture for 15 to 20 minutes before the specimen collection. The core service of any clinical chemical laboratory is blood sampling and analysis. In connection with this, reference intervals or decision limits need to be established. Therefore laboratories are recommended to apply the rules of standardization based on preanalytical factors, which state that blood samples should be taken in the morning after at least 15 minutes of rest. According to current recommendations, blood samples should be taken in the morning after 15 minutes' resting time. Some components exhibit diurnal variation and are sensitive to postural change. (24-40)

The phlebotomy equipments

 Disposable syringes, 2. Disposable lancets, 3. Gauze pads or adsorbent cotton, 4. Tourniquet 5. Alcohol swab, 6.Collection tube, 7. Waste container, 8. Needle destroyer. Tourniquet should not be applied for samples of electrolytes.

Types of Specimen Collection

Blood

Blood for analysis may be obtained from veins, arteries or capillaries. Venous Blood is usually the specimen of choice. Vein puncture is the method for obtaining the sample. Arterial blood is mainly used for blood gas analysis.

Preliminary Steps blood Collection

Confirm the identity of the patient. At least three items of identification should be used (eg. name, hospital ID no., room location of patient if hospitalized, address of OPD patient). Phelbotomist should be properly dressed in personal protective equipments eg. gloves. The patient should be seated or be in supine posture for 15 to 20 minutes before the specimen collection. Verify about fasting status. Minimum 8 hours fasting needed for fasting blood glucose. While for lipid profile 12 hours fasting needed. Arm with IV line , scarring or hematoma at intended collection site should be avoided. Estimate the volume of blood to be drawn. Select the appropriate number & the types of tubes for the blood. An appropriate needle should also be selected (21 to 24 Gauze). (41-45)

Location

The medial cubital vein in the antecubital fossa is the preferred site for collection of venous blood. Collect

blood through cannula that is being inserted for fluid infusion at the time of first insertion to avoid a second stick. Preparation of Site. Area around the puncture should be cleaned with alcohol swab or gauge saturated with 70% isopropanol. For ethanol determination the skin should be cleaned with benzalkonium chloride solution. Let the skin dry before you prick the skin.

Timing

Time at which the sample is obtained is important for those blood constituents that undergo marked diurnal variation eg. Corticosteroids.

Venous Occlusion

After cleaning tourniquet is applied 4-6 inches above the intended puncture site. Not to leave in place for more than one minute.Blood must be filled up to mark in vacuutainer.

Steps to Prevent A Haematoma

Puncture only the uppermost wall of the vein. Remove

the tourniquet before removing the needle. Use the major superficial veins. Make sure the needle fully penetrates the uppermost wall of the vein. Apply pressure to the vein-puncture site. (46-49)

Prolonged Tourniquet Application

The primary effect is hemoconcentration of nonfilterable elements such as proteins. Significant increases can be found in total protein, aspartate aminotransferase (AST), total lipids, cholesterol, and iron. Affects packed cell volume and other cellular elements.

Check List

Check list is a written sequence of phlebotomy steps to monitor and check blood sample collection steps to minimize pre analytical errors. Total No. of Steps in check list are 18. (50)

Collection tubes or vacuutainer

For blood sample analysis - following three types of tube required.

Step No.	Task	Pre Test		Post Test	
		YES	NO	YES	NO
1.	Do Check investigation ordered, fill investigation request form?				
2.	Do Greet, Identify & inform the patient?				
3.	Do Verify diet restriction?				
4.	Do Sanitize hand?				
5.	Do Prepare needle & put on gloves?				
6.	Do Position patient, Clean & air dry site?				
7.	Do apply tourniquet?				
8.	Do Patient clenching fist?				
9.	Do Insert needle (angle should be in range of 15 to 30 degree)?				
10.	Do remove tourniquet?				
11.	Do ask patient to open fist?				
12.	Do Establish blood flow?				
13.	Do Fill, remove needle & Apply gauze at puncture site and fold hand?				
14.	Do collect blood in different required Vacuutainers?				
15.	Do Destroy needle with needle destroyer?				
16.	Do Remove Gloves?				

Туре	Items	Additive	Cap color	Tube size	
	Plain tube	Non	Red		
Serum tube Plasma tube	Pro-coagulation tube	Clot activator	Red		
	Gel & clot activator tube	Separation gel with clot activator	Yellow	Δ 11 these tubes/	
	Glucose tube	Potassium oxalate/ sodium fluoride	Grey	Vacuutainer are available	
	PT tube	0.109 mol /L Sodium citrate (200 µl Sodium citrate 3.4%, must be in 1.8 ml blood & ratio must be 1:9).	Blue	in three sizes 13x75mm, 13x100mm and 16x100mm	
	Heparin tube	Lithium heparin Sodium heparin	Green		
	EDTA tube	EDTA-K2/ <mark>K</mark> 3	Purple or Lavender		
Whole blood tube	ESR tube	0.129 mol/L Sodium citrate & ratio must be 1:4.	Black		

Yellow top vacuutainer contains clot activator with gel separator uses for biochemistry and serology tests, red top vacuutainer only clot activator serology and biochemistry tests, lavender top vacuutainer contains Ethylenediaminetetraacetic acid (EDTA), is a molecule called a chelating agent. A chelating agent is a claw-like substance that can grab and stick to other molecules. Some types of EDTA stick to calcium. Lavender top vacuutainer uses for routine hematology tests, gray top vacuutainer contains Sodium Fluoride (NaF) which works as an anti-glycolytic agent & Potassium Oxalate which works as an anticoagulant uses for blood glucose and sodium lactate tests, light blue top vacuutainer contains Sodium citrate usese for prothrombin time (PT), green top vacuutainer contains heparin uses for toxicology test, black top vacuutainer used for Erythrocyte Sedimentation Rate (ESR) and contains Sodium citrate as an anticoagulant. (51)

Yellow Top tubes are designed for routine use in immuno-hematology and viral marker testing in screening and clinical laboratories. Gel forms stable barrier between serum and blood cells. Tubes are effective in therapeutic drug monitoring testing. The inner wall of each serum tube is coated with microscopic silica particles which activate the coagulation process when tubes are inverted. Tubes equipped with a gel separator contain a barrier gel in the base of the tube. During centrifugation, this gel forms a stable barrier between the serum and the blood cells, separating the serum from fibrin and cells. These tubes are particularly effective in therapeutic drug monitoring testing. Standard tubes are also available with no additive. Do not mix the contents of two different tubes as the additives will interfere in analysis. (52)

SKIN PUNCTURE: Skin puncture is an open sample collection technique. It is done in situations where - Sample volume is limited, severe vein damage due to repeated venipuncture when veins are not available eg. in a burn case.

Arterial Puncture

Preferred sites are Radial artery, Brachial artery and Femoral artery. In neonates indwelling catheter in umbilical artery is best to obtain sample for blood gas analysis. Arterialized capillary blood is obtained if arterial puncture not possible.

Specimen Processing Problems and Precautions

Haemolysis- Avoid keeping the tourniquet on the patient's arm for long periods of time. Use needles 21-gauge or larger (22- gauge thin-wall needles are acceptable). Do not expel blood into a tube through the needle. Do not shake blood vigorously in container.

Concentration changes

Never rinse syringes or needles with saline or anticoagulant solutions before use. Do not allow blood to stand in open containers for prolonged periods of time before centrifugation. Do not centrifuge blood in open containers.

Contamination of sample

Use sterile blood-handling methods wherever possible. Promptly process blood samples. Store

serum or plasma at refrigerator temperatures or freeze until analyzed.

Limitations & Pitfalls of the Examination

In hospitalised patients do not take blood from an existing peripheral venous access site because this may give false results. Haemolysis, contamination and presence of intravenous fluid and medication can all alter the results. Nursing staff and physicians may access central lines for specimens following protocols. However, specimens from central lines carry a risk of contamination or erroneous laboratory test results.

Urine

A clean, mid stream, early morning, fasting specimen is the most concentrated, used for microscopic examination & detection of abnormal amounts of constituents. If specimens are collected over a specific period of time the patient's adherence to instruction is important.

Urine Preservatives

Preservatives like Thymol, sodium benzoate, toluene are used to prevent bacterial action or chemical decomposition. Acidification of urine with HCl is widely used to preserve 24hrs specimen. Most acceptable forms of preservation of urine is refrigeration immediately after collection.

Cerebral Spinal Fluid

Obtained after lumber puncture. 3 ml of fluid is collected in plain sterile tubes. First tube should be used for Biochemistry/ Serology tests. Second tube goes for Microbiology tests. Third tube goes for Hematology examination. Uses EDTA Vacuutainer for hematology test, fluoride vacuutainer for glucose, plain vacuutainer for protein and LDH. (51-52)

Faeces

Analyzed for occult blood. Tests should be done on aliquots of excreted stools rather than on material obtained on the gloves while a rectal examination. No preservatives are added, sample is refrigerated. (53)

Errors in Medical laboratory

The error anywhere leads to chaos and especially laboratory errors may affect the patient care. The laboratory error has been defined by different bodies in different terms.

The error in laboratory may be an actual error which may have occurred at any phase of processing or it may be simply a perception of the clinician in whose views any laboratory result not corresponding to his clinical diagnosis is an error on the part of the laboratory.

Laboratory medicine, as a specialty that had prioritized quality control, has always been at the forefront of error reduction. In terms of quality control and error rates, laboratory medicine has a far better record than most other fields in health care. It is almost mandatory for laboratories to run Internal and External Quality Control Programs, identify and minimize the errors occurring.(54)

CONCLUSION

Proper identification of the specimens must be maintained. Every sample must be adequately labeled which should include patient's name, location, ID no., date & time of collection in legible handwriting. Label should be placed on the tube or cup and not on the cap. All samples should be treated as if they are potentially dangerous. Maximum benefits, from minimum tests. Prepare for sample collection according to patient's requirement. Different sample containers have specific uses. Ensure proper sample identification after collection. The concept of total quality management encompasses all the steps involved in sample processing, beginning from test ordering to the final interpretation of results by the clinicians to reduce or eliminate the errors that may arise during the various steps. The promotion of ideal phlebotomy practices and sample transport procedures is a prerequisite for the efficacy of laboratory functioning. The dependence on accurate laboratory results for diagnostics makes it mandatory for labs to ensure accountability and accuracy of results to negate incorrect diagnosis as a consequence of faulty reporting. A practice of keeping a record of the errors at all stages of analysis and then devising corrective strategies for their prevention can gradually makes free a laboratory from such errors. This review highlights the methodological challenges and developments that need to be considered in ensuring the use of valid information in developing health care through research findings.

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