

Dr Lal PathLabs **REFERENCE GUIDE**

LPL/G/DAT/005

***Dedicated to the memory of my father
and our founder Late Dr. (Major) S K Lal***

13th Edition

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SERVICES

Logistics

To ensure specimen integrity and quick turnaround times, LPL runs an efficient courier service with vast experience in providing logistics over broad geographical boundaries. Trained Logistic personnel, provide support in specimen pickup, distribution of supplies and test reports. LPL utilizes the services of various modes like air, road and trains to ensure sample delivery to the nearest laboratory depending on the test menu. The logistic team uses specially designed transport boxes to maintain sample stability at the recommended transport temperature. Data loggers are placed within the boxes to record temperature variations if any, during the transport.

Customer care

Qualified Customer support representatives are available 7 days a week (Mon through Sat 7.30 am to 10 pm and Sunday 7.30 am to 9 pm) to handle all customer queries. In addition they also deal with activities pertaining to customer complaints & customer satisfaction assessment. Customer care channelizes calls to the technical personnel to provide interpretive information whenever required.

Home Collection

To facilitate patient convenience trained home collection team is available on appointment by calling STD code + 49885050 only for Pathology tests. In addition clients can also book home collection of samples through the website and mobile App.

Clinical Trials

Dr.Lal Pathlabs Clinical Trials provides global central laboratory services, to meet the needs of our pharmaceutical and Clinical Research Organisations. For this purpose, dedicated staff is designated to provide customized, efficient and timely services to its clients.

Testing Policies

Repeat determination: Performed routinely as part of the laboratory's ongoing Quality Assurance Program. In addition, tests can be repeated when requested by the attending physician. Samples are routinely retained as per the Lab Retention Policy at appropriate temperatures to maintain stability during storage. All Pre analytical / Analytical / Post analytical repeats requested require fresh sampling within 7 days of original sample collection. Contact Customer care services for repeat determinations.

Test Additions / Cancellations: Tests may be cancelled without charge while specimens are in transit. Once the specimen has been registered, cancellation requires authorization from concerned department through the Customer care. Cancellations shall not be entertained once the specimen has been assayed. Test Additions can be requested provided the specimen is adequate and viable. Addition of tests requires authorization from concerned department through the Customer care.

Veterinary Testing

LPL has a Veterinary Pathology Diagnostic centre "PathVets" (Block B, 40/50 GF, Chittaranjan Park, New Delhi-110 019, Tel. 011-49058406/407) where Pan India samples are tested. This diagnostic centre provides facilities for Pathology tests for all Domestic pets, Poultry farms, Dairy animals, Animal breeding farms & National Zoological parks.

Critical Alert notifications

A critical alert is defined as a value that presents a pathophysiological state at such variance with normal or expected values that it is considered life threatening unless a corrective action is undertaken. Critical values do not necessarily correspond with normal reference ranges, toxic range or therapeutic ranges but are based on a level at which medical action is considered necessary. All critical value limits are informed the concerned client through SMS & emails. The SMS facility is available till 8 pm.

Notification of Delay / Repeat results

LPL maintains a specific procedure for addressing delays / repeats of patient tests. The clients are informed through SMS / email and on their registration portal app (one reg).

Turnaround Time (TAT)

The most meaningful and quantifiable measures in healthcare are accuracy and speed of diagnosis. LPL is committed to providing the fastest turnaround time possible to improve patient management. Lab monitors the TAT stringently and evaluates areas for improvement on a daily basis.

Primary Sample Storage

The collection centres / patient service centres store the primary sample at required temperatures till their dispatch to the testing laboratory where all samples requiring scheduled tests are stored at the designated temperatures till they are tested. All tested samples are stored as per their documented retention time.

HIV Counselling

As per NACO guidelines, LPL offers Pre & Post test counselling to all its clients registered for an HIV test. Special forms have been designed for Pre-Test counselling and patient consent. Post Test counselling is available at all our laboratories.

Genetic Counselling

The Genomics division- Genevolve has Genetic counsellors to guide & advise the individuals and families affected or at risk of genetic disorders to help them understand and adapt to the medical, psychological & familial implications of genetic contribution to disease.

Business Continuity Plan & Disaster Management

In accordance with Good Lab Practices, LPL has a well-defined Business Continuity Plan with redundancy built into the IT systems to provide alternate source of servers and networking which can take over the functions of the laboratory within 30 minutes. The main server is located in Mumbai while back up server is in Delhi which carries complete patient and lab information.

In the event of a crises affecting patient care services, LPL has a well-documented Disaster management plan for patient support services. Notification of clients, alternative transport facilities, timely pickup of specimens and proper backup procedures form a part of the contingency plan. Reference & Satellite laboratories provide facilities for alternative laboratory testing during crises.

Holiday Coverage

Dr.Lal Pathlabs National Reference Laboratory, is open 24 x7, 365 days a year except on Sunday evenings (Sunday timing – 6 am to 7pm). The Satellite Laboratories usually work 12 hours during the day as per laboratory specified timings. Collection centres & Patient Service Centres are open till 7:30 am to 8:00 pm daily except Sunday & Holidays (Sunday/Holiday timing – 7:30 am to 1:00 pm).

LPL's listed holidays are-New Year's Day, Republic Day, Holi, Independence Day, Gandhi Jayanthi, Dussehra, Diwali & Christmas. Labs remain open till 1.00 pm to provide sample collection services. Reports are provided the next working day.

GENERAL INSTRUCTIONS

PRE-ANALYTICAL VARIABLES

Human body is composed of different compounds and elements whose concentration and activity may reflect an individual's health or pathophysiological state. Many factors other than disease affect the concentration or activity of these analytes. Preanalytical variables can be controllable or non-controllable.

CONTROLLABLE VARIABLES

Posture

In an adult, change from lying to upright position reduces blood volume by about 10% thereby reducing plasma volume of the blood and increasing plasma protein concentration. Normally decrease with change from lying to standing position is complete in 10 minutes. However an interval of 30 minutes is required for reverse change to occur from standing to lying position.

Application of tourniquet at the time of blood collection mimics the effect of change from lying to standing position thereby increasing the plasma concentrations of proteins, enzymes, protein bound constituents, blood cell counts, hematocrit and hemoglobin.

VARIATION IN ANALYTE CONCENTRATION WITH CHANGE IN POSTURE FROM LYING TO STANDING

ANALYTE	AVERAGE INCREASE IN %
Alanine aminotransferase	7
Albumin	9
Alkaline phosphatase	7
Amylase	6
Aspartate aminotransferase	5
Calcium	3
Cholesterol	7
IgA	7
IgG	7
IgM	5
Thyroxine	11
Triglycerides	6

Prolonged bed rest

Within a few days of the start of bed rest, plasma volume decreases thereby increasing hematocrit by 10% within 4 days. Prolonged bed rest is associated with increased urinary nitrogen excretion. Calcium excretion increases to a maximum of 60% after 7 weeks of bed rest. Excretion of sodium, potassium, phosphate and sulphate increases to a smaller extent. VMA excretion is reduced by one fourth after 2-3 weeks of bed rest. When an individual becomes active after a period of bed rest, > 3 weeks is required before calcium excretion reverts to normal and another 3 weeks before positive calcium balance is achieved.

Exercise

Nature and extent of exercise plays an important role in affecting serum analytes. Beta endorphins and catecholamines almost double within a minute of strenuous exercise. Moderate exercise increases blood glucose which stimulates insulin secretion. Strenuous exercise for 10 minutes increases plasma renin activity by 400%. Cortisol secretion is stimulated and normal diurnal variation is abolished.

EFFECT OF STRENUOUS EXERCISE ON SERUM CONSTITUENTS

CONSTITUENT	% INCREASE
Acid phosphatase	11
Alanine aminotransferase	41
Alkaline phosphatase	3
Aspartate aminotransferase	31
Calcium	1
Chloride	1
Cholesterol	3
Creatinine	17
Phosphate	12
Total Protein	3
Urea Nitrogen	3
Uric acid	4
CONSTITUENT	% DECREASE
Albumin	4
Bilirubin	4
Iron	11
LDH	1
Potassium	8
Sodium	1
Total Lipids	12

Physical training

Athletes generally have a high serum activity of enzymes of skeletal muscle origin as compared to non-athletes. Serum concentrations of urea, uric acid, creatinine and thyroxine are higher in athletes due to increased muscle mass. Physical training also increases HDL cholesterol and decreases LDL cholesterol and triglycerides.

Circadian variation

Many body constituents exhibit cyclical variation throughout the day. Factors contributing to this variation include posture, activity, food ingestion, stress, daylight or darkness and sleep or wakefulness. Concentration of serum iron increases as much as 50% from 8 am to 2 pm. Hormones are specially affected by cyclical variations –

- Corticotropin secretion increases 3-5 fold from minimum value between afternoon and midnight to its maximum around waking.
- Cortisol is maximum between 6-8 am
- Aldosterone and Renin activity is maximum in the early hours of morning during sleep reducing to a minimum by afternoon.
- GFR is 20% greater in the afternoon.
- Urinary 17 Ketosteroids and 17 Hydroxycorticosteroids are lowest at night reaching maximum at mid afternoon.
- Prolactin concentration is greatest during sleep
- Serum TSH is maximum between 2-4 am and minimum between 6-10 pm
- Higher glucose values occur when glucose tolerance test is done in the afternoon

Travel

Travel across several time zones affects normal circadian rhythm due to altered pituitary and adrenal function. During a 20 hour flight serum glucose and triglyceride levels increase and fluid and sodium retention occurs.

Diet

High protein diet: Increases serum cholesterol, phosphate, urea, uric acid, ammonia & urinary urea & uric acid

High fat diet: Increases serum triglycerides

High carbohydrate diet: Decreases serum LDL cholesterol, triglyceride, total cholesterol and protein

Food Ingestion

Plasma analytes are affected by the time between ingestion of meal and collection of blood. Many analytes require overnight fasting for 10-14 hours before blood collection making it an optimum time. A protein rich meal in the evening may increase serum urea nitrogen, phosphorus and uric acid upto 12 hours. The effect of carbohydrate meals is less than protein meals.

Bran : Habitual ingestion of bran affects absorption of certain compounds like calcium, cholesterol and triglycerides from the gastrointestinal tract.

Food constituents : Many fruits like bananas and vegetables contain serotonin which increases excretion of 5-HIAA. Avocados impair glucose tolerance by affecting insulin secretion. Onions reduce plasma glucose and insulin response to glucose. Garlic ingestion may reduce cholesterol level by about 9%.

Caffeine: It stimulates adrenal medulla resulting in 2-3 fold increase in plasma epinephrine concentration. It also affects adrenal cortex, increasing plasma cortisol with increased excretion of free cortisol, 11-hydroxycorticoids and 5-HIAA. Ingestion of 2 cups of coffee may increase plasma free fatty acid level by 30%. Serum gastrin level may be increased by 5 times on drinking 3 cups of coffee.

Vegetarianism

Long standing vegetarians show reduced levels of VLDL cholesterol by 12% as compared with non-vegetarians. Serum creatinine levels may also be slightly low due to reduced ingestion of proteins. An individual on a vegetarian diet for 3 months shows 20% reduction in serum copper, 10% reduction in selenium and zinc. Urine pH is usually higher in vegetarians due to reduced intake of precursors of acid metabolites.

Malnutrition

Plasma concentrations of most proteins like total proteins, albumin, pre-albumin and beta globulin are reduced in malnutrition. Serum cholesterol and triglycerides may be only 50% of the concentration in healthy individuals. Activity of most of the enzymes is reduced.

Long Term fasting and starvation

Within 3 days of start of a fast, blood glucose decreases by 18 mg/dL. Insulin secretion is also greatly reduced. Amino acids are increased but urea decreases. Organic acids accumulate leading to metabolic acidosis. A fasting > 60 hours shows reduced insulin by 50% and C-peptide by 30%. Glucagon, Epinephrine and Norepinephrine are doubled and Growth hormone is increased 5 fold.

Smoking

Nicotine increases concentration of epinephrine in plasma and urinary excretion of catecholamines. Glucose levels increase by 10 mg/dL within 10 minutes of smoking a cigarette and this increase persists for 1 hour. The lactate pyruvate ratio increases significantly within 10 minutes. Plasma growth hormone levels are very sensitive to smoking and increase 10 fold within 30 minutes after smoking. Cholesterol, Triglycerides and LDL cholesterol levels are high in smokers. Smoking also affects the immune response of the body lowering IgG, IgA & IgM levels. CEA is reported to be 70% higher in habitual smokers. Sperm count of male smokers is reduced and number of abnormal forms is greater.

Alcohol

Prolonged moderate ingestion of alcohol increases the blood glucose by 20-50%. Serum triglyceride levels increase by >20 mg/dL, plasma aldosterone by 150% and prolactin by 40-50%. HDL cholesterol also rises while LDL cholesterol reduces. Chronic alcoholism increases GGT by 1000 fold, AST by 200% and ALT by 60%.

Drug Administration

Large doses of a drug administered over a long time affect the concentration of body fluids by their therapeutic intent, side effects and patient idiosyncratic response to their administration. Co-administration of certain drugs may affect their metabolism and pharmacological effects. CK, Aldolase and LDH - muscle component increase in the serum on intramuscular administration.

Herbal Preparations

Herbal preparations lack regulatory standardization and may affect metabolism of therapeutic drugs. Ginseng ingestion reduces Prothrombin time and INR in patients taking Warfarin. Grapefruit juice increases bioavailability of drugs like Methadone, Amiodarone and Simvastatin. Aloe vera and Senna have laxative effects and prolonged use may lead to hypokalemia. Liver damage may be caused by impure constituents of herbal mixtures.

NON-CONTROLLABLE VARIABLES

Biological influences

Genetics plays an important role in determining the concentration of blood constituents like cholesterol, glucose, urea, urate and bilirubin. Females with blood group 'O' have a lower hemoglobin concentration than other blood groups.

Age: Has a notable effect on reference intervals of each analyte and in general population is divided into 4 groups namely Newborn, Older child to puberty, Sexually mature adult and Elderly adult.

Gender: After puberty, characteristic changes in the concentrations of sex hormones affect test results. Serum levels of Alkaline phosphatase, ALT, AST, CK & Aldolase are greater in men than in women. Hemoglobin and bilirubin levels are slightly lower in women but reticulocyte counts are higher.

Race: Total serum protein concentration is higher in blacks than in whites though serum albumin is less. Carbohydrate and Lipid metabolism differ in black and white races. Glucose tolerance is less in Blacks, Polynesians and Native Americans. Lipoprotein (a) level in blacks is twice higher than whites.

Environmental factors

Altitude: Individuals living at high altitude have higher hemoglobin and PCV levels due to reduced atmospheric oxygen. Basal Growth hormone levels are high in individuals living in high altitudes but concentrations of Renin and Aldosterone are low.

Temperature: Acute exposure to heat expands the plasma volume and reduces glomerular filtration. Extensive sweating can lead to hemoconcentration rather than hemodilution.

Place of residence: Geographic location affects concentration of body fluids with significant increase in serum levels of cholesterol, triglycerides and magnesium in areas rich in hard water. Carboxyhemoglobin concentration is higher in places with heavier automobile traffic as compared to rural areas.

Seasonal influences

During summer in northern hemisphere, blood volume increases and in winter plasma proteins increase by 10%. Cholesterol is higher in winter than in summer whereas Triglycerides are higher by 10% in summer.

Menstrual cycle

Plasma corticosterone level is 50% higher in luteal phase than in follicular phase. Plasma androstenedione and aldosterone levels increase from follicular to luteal phase. Cholesterol and triglyceride levels are higher in mid cycle corresponding to maximum estrogen secretion. This cyclical variation of cholesterol is not observed in women with anovulatory cycles.

Obesity

Serum levels of cholesterol, triglycerides and beta lipoproteins are positively correlated with obesity. Lactate dehydrogenase and glucose are increased in both sexes with increase in body weight. In males AST, creatinine, Total protein & hemoglobin rise with increasing body weight whereas in females calcium rises with weight gain. Acute phase reactants are higher in obese as compared to lean individuals.

Pregnancy

Since blood volume increases during pregnancy from 2600 mL in early pregnancy to 3500 mL at 35 weeks gestation, plasma proteins are reduced specially albumin levels. Urine volume is 35% greater and glomerular filtration is 50% greater during 3rd trimester of pregnancy. All acute phase reactant proteins increase during pregnancy and ESR rises by 5 fold.

Stress

Anxiety stimulates increased secretion of aldosterone, angiotensin, catecholamines, cortisol, prolactin, growth hormone, TSH & renin. Stress decreases albumin by 5% but cholesterol levels increase.

Fever

Fever provokes many hormonal responses and appears to reduce the secretion of Thyroxine. It accelerates lipid metabolism and is often associated with respiratory alkalosis.

Shock and Trauma

Regardless of the cause of shock and trauma, certain characteristic biochemical changes occur like 3-5 fold increase in serum cortisol concentration, increased excretion of 17-hydroxycorticosteroid. Stress of surgery reduces serum T3 levels by 50% in patients without thyroid disease.

Transfusion and Infusion

Transfusions to replace blood loss because of injury reduces sodium & chloride. Extensive blood transfusions can lead to siderosis and increased serum iron concentration. Infusions of glucose solution usually reduce plasma phosphate and potassium concentrations. After an infusion it is not advised to collect blood for 8 hours as it affects accuracy of test results.

ANALYTICAL VARIABLES

Biologically common phenomena which can lead to analytic variation are:

- Cold agglutinins
- Rouleaux formation
- Osmotic matrix effects
- Platelet agglutination
- Giant platelets
- Nucleated erythrocytes
- Megakaryocytes
- Red cell inclusions
- Circulating mucin
- Leukocytosis
- In vitro hemolysis
- Bilirubinemia
- Lipemia

Immunoassays are affected by:

- Interference by Paraproteins
- Heterophile antibodies
- Very high hormone levels – Hook effect
- Macro forms of Prolactin, Amylase, Creatinine kinase & LDH elevate test results

POST-ANALYTICAL VARIABLES

Manual & telephone reporting must be discouraged as it is subject to transcription errors at the receiver end. Report delivery process & design should be such that correct & timely reporting with correct reference range & test interpretation is achieved.

SPECIMEN COLLECTION

Patient Identification

- Phlebotomist must confirm the identity of the patient by requesting patient to call out his / her full name. Same is matched with the test request form. Positive identification of the patient is made before Primary sample collection. In cases of pediatric patients, the attendant must be asked the same.
- For certain tests like HLA, additional patient identification is required by checking the photographs stapled to the lab form and matched visually.

Personal Protective Equipment (PPE)

- Phlebotomists must wear gloves, face mask and lab coat before approaching the patient and take standard precautions against potentially infectious material and limit the spread of infectious disease from one patient to another
- In certain cases like collection of Throat /Nasal swabs in Swine flu patients, special collection kit has to be worn prior to sample collection.

Pre-test requirement checks

If a fasting specimen or dietary restriction is required, confirm patient has fasted or eliminated foods / drugs from diet ordered by the Physician.

Patient position

Patient should be comfortable, seated or supine and should be in this position as long as possible before sample is drawn. Venipuncture should never be performed on a standing patient.

Selection of Venipuncture site

- Median Cubital vein in the antecubital fossa or crook of the elbow is the preferred site for collecting venous blood in adults because the vein is large and close to the surface of skin.
- Veins on the back of the hand or at the ankle may be used but these are less desirable and should be avoided in patients with Diabetes and other individuals with poor circulation.
- An arm with an inserted intravenous line, cannula, arteriovenous fistula, extensive scarring or hematoma should be avoided.
- Arm on the same side as Mastectomy should not be used as surgery causes lymphostasis there by affecting blood composition specially if the surgery is within 6 months.
- For severely ill patients and those requiring intravenous injections, alternative blood drawing site should be selected.
- For patients on prolonged intravenous fluid therapy with difficulty in finding proper veins, the IV drip should be shut off for at least 3 minutes before specimen is collected. Specimens below the infusion site in the same arm are satisfactory for most tests except for those analytes which are contained in the infusion solution like glucose and electrolytes.

Prior to Venipuncture

Phlebotomist should estimate the volume of blood to be drawn and select appropriate number and types of tubes required for blood samples as per tests requested. In case of multiple collections, order of draw should be decided.

Proper Labeling Of Tubes

Each submitted specimen tube must be labeled with the patient's name, (written exactly as it appears on the Lab Form / Test Request Form) and the tests to be conducted. The tubes should be bar coded . The time of specimen collection and the identity of the phlebotomist collecting the Primary sample is recorded.

Barcoding Instructions

1. All tubes to be barcoded prior to sample collection.
2. Affix barcode longitudinally on the tube keeping barcode number on the left side as shown in figure.
3. Bar code 'F' is used for Blood Sugar Fasting and barcode ' PP' for Blood Sugar Post Prandial. Do not use these barcodes for random sugar samples
4. Precautions:
 - (a) Do not change the numbers on the barcode by hand.
 - (b) Do not use ink or write on the barcode label by hand.
 - (c) Do not soil the barcode.
 - (d) Affix barcodes without any folds.
 - (e) Barcode should not cover the entire tube as this blocks physical viewing of the sample for gross hemolysis, lipemia and sample volume.



Preparing Venipuncture site

The area around the intended puncture site should be cleaned with the spirit swab containing 70% alcohol in a circular motion from inside to outside.

Skin should be air-dried and no alcohol should remain on the skin as traces may lead to hemolysis. Do not touch the site after it has been cleaned.

Venous Puncture Technique using Evacuated tubes / Vacutainer

- Sanitize hands & wear gloves with consideration of latex allergy.
- Apply a tourniquet preferably of Velcro or soft rubber strips and ask the patient to make a fist without vigorous hand pumping. Tourniquet should not be tied for more than 3 minutes as it affects biochemistry parameters. Ideally tourniquet should not be tied for more than 1 minute.
- Holding the green colored section of the needle shield in one hand, twist and remove the white section of the needle with the other hand.
- Screw needle onto the holder. Leave the green colored shield (21 inch gauge) or if necessary black colored shield (22 inch gauge) on the needle.
- Puncture the skin with the needle at approximately 15 degree angle with the bevel of the needle facing upwards.
- Insert the needle smoothly and fairly rapidly to minimize patient discomfort. If using an evacuated system, as soon as the needle is in the vein, insert the vacutainer tubes forward in the holder, puncturing the diaphragm of the stopper. Remember to insert vacutainer tubes maintaining the correct order of draw. Release the tourniquet when blood begins to flow into the vacutainer. When the tubes have filled upto the indicator mark, apply soft pressure with the thumb against the flange of the holder to disengage stopper from the needle and remove tube from the holder. If more samples are needed, repeat the same procedure.
- Remove the last tube from the holder before withdrawing needle from the vein. Never withdraw the needle without removing the tourniquet.
- Apply pressure to the venipuncture site with a dry swab & place adhesive band aid when blood stops flowing.
- Mix and invert all tubes as per chart below. Do not shake the tubes vigorously.
- Dispose of contaminated material in designated containers using universal precautions as per local state guidelines.
- In case of contamination of the holder / glove, discard and replace with a new one.

Blood collection with Syringe

- Syringes are used for patients with difficult veins.
- Place the needle firmly over the nozzle of the syringe and remove cover of the needle.
- Keep bevel of the needle upwards.
- Align syringe and needle with the vein to be punctured and push the needle into the vein at an angle of 15 degrees.
- As soon as the vein is pierced, stop the forward pressure on the syringe and draw blood by gently pulling back the plunger of the syringe.
- After adequate blood volume has been drawn, remove the syringe with the needle from the vein and immediately place a dry swab over the puncture site.
- Puncture the color coded top of the evacuated tubes with the same needle and allow the tube to fill passively. Do not uncapped and forcefully push the blood into the evacuated tube.

Venipuncture in children

Technique for venipuncture in adults and children is similar. However children are likely to make unexpected movements, hence assistance in holding them is required. Use Microtainer evacuated tubes and preferably 23 gauge butterfly needle with attached tubing to collect specimens.

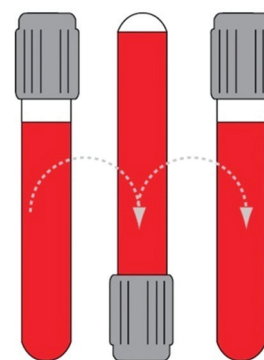
Completion of sample collection

- When blood collection is complete and needle is withdrawn, patient should be instructed to press the puncture site with a dry swab and arm raised without bending at the elbow, to lessen the leakage of blood.
- Apply band aid once the bleeding has stopped.
- Discard needle / syringe / swabs as per the waste disposal guidelines.

Recommended Order of Draw for multiple specimen collection

Color of Stopper	Additive	Inversions
Blood Culture bottle	Broth	8
White Top	No Additive	0
Light Blue Top	Sodium citrate	3-4
Gold Top - SST	Gel Separator	5
Red Top	No Additive	5
Green Top	Sodium heparin	8
Royal Blue Top	K2 EDTA	8
Lavender Top	EDTA	8
Grey Top	Sodium fluoride	8
Yellow Top	ACD	8

Figure of Inversion



Note: It is critical that complete mixing of blood collected with additive is accomplished as quickly as possible as shown in figure above.

COMMON ERRORS IN SPECIMEN COLLECTION

1. Misidentification of patient
2. Mislabeling of specimen
3. Short draws / wrong anticoagulant to blood ratio

4. Mixing problems / clots
5. Wrong tubes / wrong anticoagulant
6. Hemolysis / lipemia
7. Hemoconcentration from prolonged tourniquet tying
8. Exposure to light / extreme temperatures
9. Improperly timed specimens / Delayed delivery to laboratory
10. Processing errors- Incomplete centrifugation, incorrect log-in, improper storage

FACTORS AFFECTING TEST RESULTS

1. Hemolysis – means lysis of RBC which affects certain test results like Potassium, Magnesium, Iron, LDH, Phosphorus, Ammonia & Total protein.

Causes of hemolysis :

- Needle gauge too thin
 - Syringe plunger pulled back too fast
 - Expelling blood vigorously in the tubes
 - Mixing tubes vigorously
 - Collecting blood before alcohol has dried at venipuncture site
2. Lipemia
 3. Clots in anticoagulated specimen
 4. Non fasting specimen when test requires fasting
 5. Improper blood collection tube
 6. Short draws / wrong volume
 7. Improper transport conditions
 8. Discrepancies between requisition & specimen label
 9. Unlabeled or mislabeled specimen
 10. Contaminated specimen / leaking container

BLOOD COLLECTION PROCEDURE IN NEWBORNS ON FILTER PAPER

Materials required:

- Sterile, disposable lancet or automated lancet device (maximum tip length 2.4 mm)
- 70% alcohol swab
- 3 sterile cotton wool or gauze swabs
- Disposable gloves
- Blood collection card / Filter paper

Sample Collection Procedure

- Wear gloves.
- Ask the mother to cuddle the baby on her knee to assist you and comfort the baby.

- Place a paper towel on the lap of the person holding the baby to protect from any blood drips.

- Ensure that the heel is warm. Hold the heel in warm hands for 3 minutes or dip heel in warm (not hot) water. Warming the heel will enhance the blood flow and positioning the foot in a downward position from the heart will help to collect specimen adequately.

- Clean the heel with sterile spirit swab and air dry.

- Do not leave any alcohol on the skin as this may dilute the sample and adversely affect the test results. Encircle the heel with fingers and thumb and squeeze gently until skin is taut and suffused with blood.

- Puncture the heel with a firm deliberate stab with lancet.

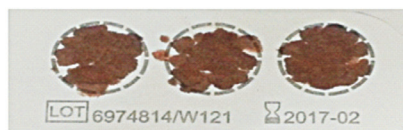
- The length of the puncture should not exceed 2.4mm.

- If a second puncture is necessary, make this a few millimeters away from the first puncture or use the other foot.
- After the puncture, wait for five seconds as vasoconstriction occurs initially. Then gently apply intermittent pressure with the thumb to the area surrounding the puncture site. Avoid excessive pressure as this may bruise the site.
- Always wipe away the first drop of blood as it is often diluted with tissue fluid and may lead to false negative results.
- Touch the circle marked on the card gently to the hanging drop so that blood soaks through to the other side. Do not ever try to put blood drop on both sides of the card. It is very important that blood should soak through the card.
- Do not press the card on to the skin.
- Do not apply multiple drops to fill each circle or one drop on top of another.
- Do not touch blood spot with finger.
- Press clean cotton wool firmly on the puncture site until bleeding stops. It is not advisable to place adhesive bandages over skin puncture sites in newborns.
- Dry the sample at room temperature on a clean non-adsorbent surface for 4 hours.
- Alternatively, venous blood can also be taken by syringe and drops of blood are poured on filter paper.
- Skin punctures must never be performed on the fingers of newborns.
- Never prick bruised heel.

ACCEPTABLE SAMPLES



UNACCEPTABLE SAMPLES



Filling Out IEM Cards

It is extremely important that all requested information on screening card is filled completely and legibly. The information requested is vitally important for screening and follow up.

Accurate and complete patient and physician information is critical for rapid follow up in the event of abnormal test result. Name, date of birth and specimen date are particularly important. Ensure physician information is accurate and complete. The timing of collection and medical information on transfusion, medication, pre-maturity and other requested data are needed to interpret

results and determine appropriate follow up procedures. **Use ball point pen only to enter information on the card.** Do not use a typewriter to fill the form because it may contaminate the filter paper.

Mailing Instructions for filter paper specimens

- Preferably send the cards on the same day of collection. If it is not possible to send the sample on the day of collection, then store at 4°C after the sample has dried.
- If stacking several cards, do not directly place one blood spot on top of another as this can cause contamination; rotate each card in the stack to alternate the collection areas
- Place the blood specimen card in paper envelope. If paper envelope is not available, wrap a sheet of clean white paper around the cards ensuring that the blood spots are completely covered.
- There is minimal risk of infection from dried blood samples.
- Dried blood specimens must NOT be packaged in airtight, leak proof plastic bags. The lack of air exchange in a sealed plastic bag causes heat buildup and moisture accumulation which can damage the dried blood spot.
- High temperatures can degrade enzymes. Sample card must be transported refrigerated.

Precautions for filter paper collections

- Circles have to be completely filled with blood drops and avoid over filling.
- Milking or squeezing the puncture site can cause hemolysis and mixing of tissue fluids with blood.
- Layering or applying successive drops of blood in the same printed circle causes caking and /or non-uniform concentrations of blood.
- Ensure that blood soaks through the card. Do not apply blood on both sides of the card.
- Contamination of sample during collection, drying, or mailing with urine samples will render the results unreliable. Such samples will be rejected.
- Inadequate or inappropriate drying.
- Humidity and moisture adversely affect the quality of sample and analyte recovery.
- Excess heat or sunlight bakes the sample.
- Excess cold leads to separation of red blood cells and serum.
- Placing the sample in a plastic bag generates moisture and promotes bacterial growth.

Urine Specimen Collection

- A clean early morning fasting specimen is usually the most concentrated and is preferred for microscopic examination and detection of abnormal amounts of constituents like proteins and HCG.
- First 10 mL of urine voided is most appropriate for bacterial examination.
- Mid stream specimen is best for investigating bladder disorders.
- Catheter specimens can be used in critically ill patients or in those with urinary tract obstruction.
- It is advisable to clean patient's genitalia before voiding urine for routine and culture examinations in order to obtain true concentration of white cells.

24 HOUR URINE COLLECTION

Collection technique

- Patient must be properly instructed on the collection technique.
- Discard first morning urine sample.
- Collect all the urine samples in the required container including first morning sample of next day.

- If container has preservatives like HCl, each void should be in a separate container which is emptied into the larger container. This two step procedure prevents danger of patients splashing themselves with the acid. Add appropriate volume of 50% or 6N HCl as per table 1 to stabilize the pH between 1 & 3.
- Keep the container at 40C during the entire collection period or alternatively keep the container in a bucket of ice.
- For 24 hour collection a 5 litre urine container suffices.
- Urine should not be collected at the same time for 2 or more tests requiring different preservatives.
- Removal of an aliquot is not permissible during 24 hour collection, because excretion of most compounds varies throughout the day and test results will be affected.
- Prior to testing, smaller aliquots must be made after thoroughly mixing the urine in the 24 hour container to ensure homogeneity. Measure and note the total volume of the well-mixed 24 hour urine collection and send 50mL aliquot in a screw-cap plastic container refrigerated (2-80C), for analysis. Record 24-hour urine volume on test request form (TRF) and urine container.

For specialized urine tests

- **Trace metal analysis:** It is mandatory to use trace metal containers. Do not measure 24 hour volume. For arsenic test, do not eat shellfish 48 hours prior to specimen collection.
- **Calcium:** Do not take laxatives during the collection period.
- **5-HIAA:** For 48 hours prior to specimen collection, limit the following to one serving per day – fruits/vegetables/nuts/caffeinated beverages or food/medicines like aspirin, antihistamines, cough syrups.
- **VMA/Catecholamines/Metanephrines:** food & beverage restrictions 72 hours prior to specimen collection.

Table 1

Urine Output in mL	Volume of HCl to be added
50–100	0.5 mL
101–200	1 mL
201–400	2 mL
401–600	3 mL
601–800	4 mL
801–1000	5 mL
1001–1200	6 mL
1201–1400	7 mL
1401–1600	8 mL
1601–1800	9 mL
1801–2000	10 mL

Table 2

24 hr Urine Collection Preservatives	Tests
None	Amino acids, Amylase, Citrate, Chloride, Creatinine, ALA, Immunoelectrophoresis, Phosphorus, Potassium, Protein, Protein electrophoresis, Sodium, Urea, Uric acid
10 g Boric acid	Aldosterone, Cortisol
10 mL 6 N HCl	Catecholamines, Cystine, HVA, Hydroxyproline, Metanephrine, Oxalate, VMA, 5HIAA, Calcium
0.5 g Sodium fluoride	Glucose
50% Alcohol	Cytological examination

Random Urine Collection

- Random urine specimen should be acidified with 50% HCl (prepared by mixing equal volumes of concentrated HCl and deionized water) to a pH between 1.0 and 3.0 immediately after collection. Acid should be added drop-by-drop, checking the pH regularly till it falls between the desired range.
- Avoid excess addition of acid; do not use concentrated acid.
- Well-mixed 50mL aliquot of this collection should be sent/stored refrigerated (2–8°C) for analysis.

HISTOPATHOLOGY SPECIMEN COLLECTION

SPECIMEN HANDLING FOR ROUTINE SUBMISSIONS:

- Immediately place each specimen in a tightly secured container with **10% Neutral buffered formalin**. Specimen must be totally immersed in formalin. **Do not allow specimen to dry.**
- **Use a separate container for each separately identified specimen but register all specimens under single Lab number.**
- Do not crush the specimen with forceps, hemostats, or other instruments. Cautery will cause heat artifact. **DO NOT FREEZE** formalin fixed specimens.
- **Do not force a large specimen into a small container. Formalin must surround the specimen for proper fixation. Formalin volume to Specimen ratio should be 10:1.**
- **Label each container (not the lid) with patient's name, source of specimen & bar code.**
- **Complete Histopathology test requisition** form and send with specimen(s). Only one Histopathology test requisition form is needed per patient. Each container and specimen must be separately identified on the test requisition form. The Requisition Form must be filled by the referring Doctor/Clinician/Centre from where specimen is received.
- **The test requisition form should contain pertinent clinical information including Patient's date of birth, gender, clinical information and anatomic location of tissue removed.**

SPECIMEN HANDLING FOR SPECIAL TESTS

IMMUNOHISTOCHEMISTRY (IHC)

- Formalin fixed paraffin embedded blocks to be submitted with appropriate clinical history and copy of previous Histopathology report if available.
- Alternatively submit specimens as for Routine Histopathology.
- Immunohistochemistry is a specialized test which requires accurate clinical history and findings for processing of the specimen.

DIRECT IMMUNOFLUORESCENCE

Submit minimum 3 mm punch biopsy of **Skin / Renal / Conjunctival** specimen in buffered normal saline. Ship refrigerated. **Immunofluorescence cannot be performed on formalin fixed tissues.**

SPECIAL STAINS

Submit specimens as for Routine Histopathology or submit formalin fixed paraffin blocks. Attach Histopathology report and relevant clinical history.

ONCOLOGY RESECTION SPECIMENS

- **Collect specimens as for routine histopathology.**

- **Complete surgical pathology requisition form for Oncology Resections and send with the specimen.** Each container and specimen must be separately identified on the test requisition form. The Requisition Form must be filled by the referring Doctor/Clinician/Centre from where specimen is received.
- **The test requisition form should contain pertinent clinical information including Patient's date of birth, gender, clinical information and anatomic location of tissue removed.**

ELECTRON MICROSCOPY (EM)

Transmission electron microscopy (TEM) is a technique in which a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through and creating its high resolution image. This analysis is performed by special microscopes called Transmission Electron Microscopes (TEMs).

TEMs are capable of imaging at a significantly higher resolution than light microscopes, owing to the small wavelength of electrons. This enables the instrument's user to examine fine detail which is thousands of times smaller than the smallest resolvable object in a light microscope.

TEMs find application in diagnostic pathology, cancer research, virology, materials science as well as pollution, nanotechnology, and semiconductor research. The high magnification of the electron microscope enables observations not possible by light microscopy, and electron microscopy is considered to be an essential component of human diagnostic renal pathology, neuromuscular pathology, and is a useful tool in difficult cases in oncosurgical pathology.

In renal pathology, ultrastructural features may enable a diagnosis to be made where the light microscopy is apparently normal, eg. Minimal change, Thin membrane disease, Hereditary nephropathy, Fibrillary and Immunotactoid glomerulonephritis. In addition, it can provide information to confirm the diagnosis, as in Immune complex glomerulonephritis, Renal amyloidosis, Dense deposit disease, Diabetes etc.

In muscle biopsy, the characteristic diagnostic features of several Myopathies, Glycogen storage vacuoles, Nemaline myopathy, Actinopathies, and Hyaline body myopathy etc. are seen only with the use of TEM. Additionally, when samples for electron microscopy are inadequate, valuable diagnostic information can be obtained from ultrastructural investigations on reprocessed paraffin- embedded material.

Electron Microscope facility at LPL:

LPL has a dedicated Transmission Electron Microscopy laboratory, backed by experienced personnel and housing state of the art 120 kV JEOL Transmission Electron Microscope.

Sample collection for Electron microscopy (TEM):

Samples should be collected in special vials containing 3% buffered glutaraldehyde solution, available from LPL. The specimen should be shipped refrigerated.

Detailed clinical history should accompany the specimen along with contact number of the referring doctor.

WATER COLLECTION

Collection of Water Samples For Presumptive Coliform Count:

Hold the sterile bottle by the base in one hand. Use the other hand to remove the cap keeping the bottle vertical. The cap should be held in the hand face up, while the bottle is filled. Screw back the cap immediately. To prevent contamination, do not touch the surface or inside the cap. If the bottle becomes contaminated it should not be used. Minimum 180 mL of water sample is mandatory.

Collection of a sample from Tap:

1. Clean the nozzle of tap with spirit.
2. Turn the tap on full and allow the water to flow for 1minute.
3. Collect the water in a sterile bottle.

Collection of water from rivers / lakes:

The stopper should be removed carefully with one hand and with the other hand bottle held at its base should be inserted, mouth downwards, a foot below the surface of the water. The bottle is then turned so that mouth is directed to the current and water flows into the bottle without coming into contact with hand. If there is no current the bottle is moved horizontally, so that water flows into it. The bottle is then brought to the surface and capped immediately.

CENTRIFUGATION

SST/Red top tubes must be placed vertically for 30 minutes to allow blood to clot prior to centrifugation. For all blood samples collected, plasma/serum should be separated from cells within 2 hours. Uncentrifuged samples should be kept at room temperature to decrease hemolysis. These samples should not be refrigerated at 4°C unless centrifuged. Plasma samples for Molecular diagnostics should be centrifuged immediately and frozen. Blood samples must be centrifuged at 3500 rpm for minimum 10 minutes for proper separation of serum/plasma.

Urine and other body fluids may be centrifuged at 1000 rpm for 5 minutes to concentrate particulate matter as sediment.

Precautions:

- Tubes should be well stoppered and balanced. Weight of buckets, tubes & their contents on opposite side of the rotor should not differ by more than 1%. Tubes filled with water may be used to equalize the weight if required.
- The speed control switch should be at zero before starting the centrifuge & adjusted to the required speed.
- Do not open the lid while the centrifuge is in operation.
- Do not stop the rotating tubes with your hands. Let them stop on their own.
- In case a tube breaks, the bucket, cushion & chamber should be carefully cleaned with 1% Sodium hypochlorite solution.
- Always wear gloves to protect yourselves as any spillage can be hazardous.
- Keep serum/plasma/urine refrigerated /frozen till packed and transported to the laboratory at the required temperature as per the Alphabetical List of Tests.

Note :

- Premature separation of serum should be avoided as continued formation of fibrin can clog sampling devices in testing equipment.
- Incomplete centrifugation can affect test results. Centrifugation time should be strictly followed.
- Tubes must be stoppered and then centrifuged to reduce evaporation and prevent aerosolization of infectious particles and volatile substances like Ethanol. Stoppers also maintain anaerobic conditions which are important for measurement of Carbon dioxide and Ionized calcium. It also helps to preserve blood pH which is important for enzymatic measurement of Acid phosphatase.
- Samples for Molecular diagnostics should not be exposed to repetitive cycles of freezing and thawing as this can lead to shearing of DNA.

INSTRUCTIONS FOR PACKAGING SPECIMENS AND TEST REQUISITIONS

Specimens must be packed and shipped properly for accurate testing, which helps ensure that patients receive optimal treatment. A specimen may not be viable for testing if it becomes too cold or too hot. It may be necessary to collect another specimen from the patient, which may delay treatment.

LPL's goal is to ensure that all medical specimens arrive at the testing facility:

- At the correct temperature for testing
- Intact in the container, without breakage or leakage

- In the shortest possible time
- In compliance with all applicable regulations

This guide has been prepared to help understand the role in accomplishing these goals. By following the guidelines for proper specimen preparation, packing, shipping, and documentation, one can comply with regulations and safely transport specimens.

- Enter the Patient Information, Tests requested and other details in the Test Request form. Fold the test requisition form with the patient's name and bar code facing out.
- The specimen bag has two pouches. Place the specimen(s) wrapped in absorbant paper, in the rear pouch (printed side) and the requisition form in the front pouch (un-printed side) with the bar code facing out.
- Seal the Zip Lock bag. This will prevent the sample from contaminating the Test Request Form in cases of accidental leakage.

Transportation to Laboratory

Transport requirements are of four types: Frozen, Refrigerated, Room Temperature and at 18–22°C. Use of transport boxes supplied by LPL are recommended. **Please refer to the Stability and Minimum Volumes in the Alphabetical List of Tests before using appropriate mode of transport to laboratory.**

Frozen (0-2° C)

1. Remove all contents of transport box
2. Place a layer of perforated sponge at bottom of the box
3. Place a prefrozen gel pack over the perforated sponge (gel packs must be prefrozen at 0°C for 24 hours prior to use)
4. Place specimens sealed in Zip lock bag over the gel pack
5. Place another prefrozen gel pack over the samples
6. Cover with second layer of perforated sponge
7. Place unperforated sponge and close the lid of the box
8. Seal the cardboard box and transport to laboratory immediately
9. Indicate '**Frozen Samples**' on cardboard box

Note: In case a transport bag is being used, place prefrozen gel packs in the pockets of the bag and ziplocked samples to be placed in these pockets.

Alternatively samples can be transported Frozen in **Dry Ice**.

1. Remove all contents of transport box
2. Break dry ice into small pieces & place at bottom of the box
3. Place samples sealed in zip lock bag over the dry ice
4. Place perforated sponge over the samples
5. Close the lid of the box
6. Seal the cardboard box and transport to laboratory immediately
7. Indicate "**Frozen Samples in Dry Ice**" on cardboard box

Refrigerated: (2-8° C)

1. Remove all contents of transport box
2. Place a prefrozen gel pack at bottom of the box (gel packs must be prefrozen at 0°C for 24 hours prior to use)

3. Place a layer of perforated sponge over the prefrozen gel pack
4. Place specimens sealed in a zip lock bag over the perforated sponge
5. Cover specimens with second layer of perforated sponge
6. Place another prefrozen gel pack over the samples
7. Place unperforated sponge and close thermocol lid
8. Seal the cardboard box and transport to laboratory immediately
9. Indicate 'Refrigerated Samples' on cardboard box

Note: In case a transport bag is being used, place prefrozen gel packs in the pockets of the bag and ziplocked samples to be placed in the centre of the bag.

Ambient (18–22°C):

1. Remove all contents of transport box
2. Place a prefrozen gel pack at bottom of the box (gel packs must be prefrozen at 0° C for 24 hours prior to use)
3. Place a layer of perforated sponge over the prefrozen gel pack
4. Place a second layer of perforated sponge
5. Place another prefrozen gel pack over the perforated sponge
6. Place unperforated sponge over the second gel pack
7. Place specimens sealed in zip lock bag over the unperforated sponge
8. Seal the cardboard box and transport to laboratory immediately
9. Indicate '**Ambient**' Samples on cardboard box

Note: In case a transport bag is being used, place the samples in the outer pockets of the bag (without gel packs).

BILLING

Payments can be made by cash / credit card / PayTM / Demand Draft in favour of “Dr. Lal PathLabs Ltd” payable at New Delhi **OR** Electronic transfer directly to LPL bank accounts. An additional courier charge of Rs.1500 / Rs.5500 as applicable per test per patient should be included for samples to be sent to USA.

TEST REPORTS

The turnaround time for each test is indicated in the Alphabetical List of Tests. For samples sent to USA, the reports are available in 2 to 3 weeks time from the date of dispatch to USA. **Due to unforeseen circumstances, reports may not be available on the due date.** Clients are telephonically informed of all delays by Customercare department.

MODE OF COLLECTION OF TEST REPORTS

- **Self:** Collection of Test reports by the patients or their representatives on production of the receipt issued.
- **Courier/Registered Post/e-mail** on request at the time of Sample Collection.
- **Website:** Patients may access their reports online on our website (www.lalpathlabs.com) via the link “**View Test Report**” on the home page. Under this link there are two columns “**Lab/Visit ID**” and “**Password**”. ‘Lab/Visit ID’ is patient’s unique

identification number and 'password' to be used is patient's surname. In case patient does not have a surname, the password would be his/her first name. Verification code must be typed in capitals. Online reports can be viewed forever on the website with effect from August, 2016.

Mobile App:

Lal PathLabs Mobile App on Android and iOS helps in finding the nearest centre / Lab based on patient's location, gives information about various tests and helps in booking a Home collection. Patient will also be able to see the current and all the past reports on the App itself.

Procedure for Accessing reports

- Open menu draw in the App
- Click on 'My reports'
- Select 'Check your report using lab ID and password' and press enter after filling in the same
- Click on the download icon to download the report or send the report to your email ID

Cumulative Reports

- Current + last 3 reports available simultaneously
- Valid for test results with quantitative values only