Case study

1- A 74-year-old retired man presented to his general practitioner with haemoptysis and weight loss. He had smoked 20 cigarettes a day for 55 years. Chest radiograph revealed a left upper lobe shadow, and bronchoscopy confirmed a primary lung carcinoma. His blood pressure was 132/80 mmHg and clinically he was euvolaemic. There was no evidence of adrenal insufficiency or thyroid disease and he was not taking any medications. Some results were as follows:

Plasma

Sodium 112 mmol/L (135–145)

Potassium 3.6 mmol/L (3.5–5.0)

Urea 3.6 mmol/L (2.5–7.0)

Creatinine 98 μmol/L (70–110)

Urine

Spot sodium 58 mmol/L

DISCUSSION

The most likely explanation for the severe hyponatraemia is syndrome of inappropriate antidiuretic hormone secretion (SIADH). Some lung carcinomas ectopically release ADH. Note that the patient was clinically euvolaemic and there was no other evidence of other causes of euvolaemic hyponatraemia, such as adrenal insufficiency or hypothyroidism. The urinary sodium concentration is inappropriate for the hyponatraemia (> 20 mmol/L).

2- A 74-year-old woman underwent a left total hip replacement and had received 5 L of 5 per cent dextrose intravenously over one day. She had the following post-operative results:

Plasma

Sodium 117 mmol/L (135–145)

Potassium 3.7 mmol/L (3.5–5.0)

Urea 3.4 mmol/L (2.5–7.0)

Creatinine 76 μmol/L (70–110)

The pre-operative results were as follows:

Plasma

Sodium 138 mmol/L (135–145)

Potassium 4.2 mmol/L (3.5–5.0)

Urea 5.6 mmol/L (2.5–7.0)

Creatinine 90 μmol/L (70–110)

DISCUSSION

The patient was being infused with large amounts of 5 per cent dextrose intravenously. This was the most likely cause of her post-operative hyponatraemia. The administration of hypotonic and sodium-free fluid was causing a dilutional hyponatraemia. This is a particularly dangerous practice post-operatively, when antidiuretic hormone (ADH) secretion is increased due to the stress of the operation and of nausea and pain, thus increasing the likelihood of water retention.

3- A 5-year-old girl was admitted to hospital because of a 4-day history of diarrhoea and vomiting. On examination she was found to be clinically ‘dehydrated’ with loss of skin turgor; her pulse was

120 beats/min and her blood pressure 74/50 mmHg. Her admission results were as follows:

Plasma

Sodium 167 mmol/L (135–145)

Potassium 3.0 mmol/L (3.5–5.0)

Urea 19 mmol/L (2.5–7.0)

Creatinine 110 μmol/L (70–110)

Urine Spot sodium < 10 mmol/L

DISCUSSION

The severe hypernatraemia is in keeping with hypotonic fluid loss due to the diarrhoea and vomiting. The loss of skin turgor, tachycardia and hypotension suggest hypovolaemia. The low urinary sodium concentration indicates renal sodium conservation. Children are particularly susceptible to hypernatraemia induced by hypotonic fluid loss.

4- Below are the pre-operative blood results of a 44-year-old man. The sample was reported by the laboratory to be grossly ‘lipaemic’.

Plasma

Sodium 118 mmol/L (135–145)

Potassium 4.0 mmol/L (3.5–5.0)

Urea 6.0 mmol/L (2.5–7.0)

Creatinine 95 μmol/L (70–110)

Glucose 4.0 mmol/L (3.5–5.5)

Cholesterol 17.3 mmol/L (3–5)

Triglycerides 68 mmol/L (<1.5)

Osmolality 290 mmol/kg (275–295)

Further tests excluded other common causes of hyponatraemia.

DISCUSSION

The plasma osmolality was not low despite hyponatraemia, suggesting pseudohyponatraemia. This is probably due to the gross lipaemia when measured by indirect sodium ion-detecting electrodes which some laboratories use to assay sodium (direct electrodes do not usually detect pseudohyponatraemia).

Note that calculated plasma osmolality is 2[118 + 4] + 6.0 + 4.0 mmol/L = 254 mmol/kg, which differs markedly from the measured value of 290 mmol/kg.

5- A 17-year-old man was involved in a road traffic accident. Both femurs were fractured and his spleen was ruptured. Two days after surgery and transfusion of 16 units of blood, the following results were found:

Plasma

Sodium 136 mmol/L (135–145)

Potassium 6.1 mmol/L (3.5–5.0)

Urea 20.9 mmol/L (2.5–7.0)

Creatinine 190 μmol/L (70–110)

Albumin-adjusted calcium 2.40 mmol/L (2.15–2.55)

Phosphate 2.8 mmol/L (0.80–1.35)

Bicarbonate 17 mmol/L (24–32)

The patient was producing only 10 mL of urine per hour and a spot urinary sodium was 8 mmol/L.

DISCUSSION

The results are compatible with pre-renal acute kidney injury (AKI), secondary to massive blood loss. Note the oliguria, low urinary sodium concentration, hyperkalaemia, hyperphosphataemia and also low plasma bicarbonate concentration, suggestive of a metabolic acidosis.

6- A 56-year-old man attended the renal out-patient clinic because of polycystic kidneys, which had been diagnosed 20 years previously. He was hypertensive and the following blood results were returned:

Plasma

Sodium 136 mmol/L (135–145)

Potassium 6.2 mmol/L (3.5–5.0)

Urea 23.7 mmol/L (2.5–7.0)

Creatinine 360 μmol/L (70–110)

Estimated glomerular filtration rate (eGFR) 14 mL/ min per 1.73 m2

Albumin-adjusted calcium 1.80 mmol/L (2.15–2.55)

Phosphate 2.6 mmol/L (0.80–1.35)

Bicarbonate 13 mmol/L (24–32)

DISCUSSION

These results are typical of a patient with chronic kidney disease (CKD) with raised plasma urea and creatinine concentrations. The patient has hyperkalaemia and a low plasma bicarbonate concentration, suggestive of a metabolic acidosis. The hypocalcaemia and hyperphosphataemia are also in keeping with CKD stage 5.

7- A 21-year-old man presented to the urology outpatient clinic because of renal calculi. There was also a family history of renal calculi.

Plasma

Sodium 137 mmol/L (135–145)

Potassium 4.2 mmol/L (3.5–5.0)

Urea 5.9 mmol/L (2.5–7.0)

Creatinine 108 μmol/L (70–110)

Estimated glomerular filtration rate (eGFR) > 90 mL/min per 1.73m2

Albumin-adjusted calcium 2.43 mmol/L (2.15–2.55)

Phosphate 1.1 mmol/L (0.80–1.35)

Bicarbonate 27 mmol/L (24–32)

Urate 0.33 mmol/L (0.20–0.43)

Urinary excretion of both calcium and oxalate fell within the laboratory reference ranges. However, cystine was detected in the urine.

DISCUSSION

In conjunction with the family history and relatively young age of presentation, the results are suggestive of cystinuria manifesting cystine stones. This is one of the most common amino acidurias, although a rare cause of renal calculi, and is treated by increasing fluid intake and alkalinizing the urine.

8- A 7-year-old boy was admitted unconscious to a casualty department. On examination he was found to be hyperventilating. He had inadvertently consumed ethylene glycol antifreeze, which he had found in his parents' garage stored in a lemonade bottle. Blood results were as follows:

Plasma

Sodium 134 mmol/L (135–145)

Potassium 6.0 mmol/L (3.5–5.0)

Bicarbonate 10 mmol/L (24–32)

Chloride 93 mmol/L (95–105)

Glucose 5.3 mmol/L (3.5–6.0)

Arterial blood gases

pH 7.2 (7.35–7.45)

PaCO2 3.18 kPa (4.6–6.0)

PaO2 13.1 kPa (9.3–13.3)

DISCUSSION

The results show a high anion gap, normally about 15–20 mmol/L, but in this case 37 mmol/L, with metabolic acidosis, i.e. (134 + 6) – (10 + 93). Hyperkalaemia is present due to the movement of intracellular K+ out of cells because of the acidosis. The compensatory mechanism of hyperventilation ‘blows off ’ volatile acid in the form of CO2, hence the low PCO2.

9- A 52-year-old woman with Sjögren’s syndrome and distal or type I renal tubular acidosis attended a renal out-patient clinic. Her blood results were as follows:

Plasma

Sodium 144 mmol/L (135–145)

Potassium 3.0 mmol/L (3.5–5.0)

Bicarbonate 13 mmol/L (24–32)

Chloride 118 mmol/L (95–105)

DISCUSSION

The results are suggestive of a normal anion gap metabolic acidosis or hyperchloraemic acidosis. Hypokalaemia is unusual in the face of an acidosis, one of the exceptions being renal tubular acidosis type I or II. Note that the anion gap here is (144 + 3) – (13 + 118) = 16 mmol/L, which is a normal gap although with high plasma [Cl–] and low [HCO3 –].

10- A 67-year-old retired printer presented to casualty because of increasing breathlessness. He had smoked 20 cigarettes a day for 50 years. On examination he was found to be centrally cyanosed and coughing copious green phlegm. His arterial blood results were as follows:

pH 7.31 (7.35–7.45)

*P*aCO2 9.3 kPa (4.6–6.0)

*P*aO2 6.9 kPa (9.3–13.3)

Bicarbonate 37 mmol/L (24–32)

DISCUSSION

The patient had chronic obstructive pulmonary disease, and the blood gases show a respiratory acidosis with hypercapnia and hypoxia. The latter has resulted in central cyanosis. Compensation is via the kidneys, with increased acid excretion and HCO3 – reclamation. Chronic cases of respiratory acidosis are usually almost totally compensated as there is time for the kidneys and buffer systems to adapt. This is unlike an acute respiratory acidosis due to bilateral pneumothorax, in which the rapid acute changes do not give sufficient time for the compensatory mechanisms to take place. This patient had an acute exacerbation of his lung disease and the CO2 retention exceeded the compensatory mechanisms.

Introduction to Common Laboratory Assays and Technology

Point-of-care testing may be thought of as any specimen testing that exists outside the walls of the large, central hospital laboratory. Community clinics, physician offices, emergency rooms, or patients' homes are common examples. Currently, hand-held analyzers exist that are capable of accurately measuring many common analytes such as electrolytes, blood gases, pH, blood urea nitrogen (BUN), creatinine, glucose, lipids, coagulation factors, hemoglobin, and hematocrit. Wireless devices, embedded computers are becoming commonplace at the patient's bedside.

The principle technology that underlies the function of these smaller instruments usually involves biosensor systems. *Biosensor systems* consist of two components: a bioreceptor and a transducer. The bioreceptor is a molecule such as an antibody, enzyme, receptor protein, or nucleic acid that recognizes a target analyte. The interaction between the bioreceptor and the target analyte generates either a specific molecular species or results in a physiochemical change that can be measured by electrochemical methods. The transducer detects this change and converts it into a measurable signal that is proportional to the concentration of the analyte. Common transducers used in these systems are based on amperometry, such as measurement of hydrogen peroxide and oxygen; potentiometry, measurement of pH and ions; and photometry, which uses optical fibers. In contrast to conventional assay methods that involve multiple steps and liquid reagents, both components are integrated into one sensor in these portable instruments. In the future,

the centralized laboratory will provide highly specialized testing and will be a source of information storage and retrieval, while point-of-care testing will likely continue to expand, providing clinicians with immediate results.

PHOTOMETRY

Photometry is used to identify and/or quantify a given substance by measuring either the light absorbed or emitted on excitation by a specific narrow wavelength of light. In clinical laboratory instruments, the range of wavelengths measured is between 150 (the low ultraviolet) and 2500 nm (the near infrared region). These instruments are classified by the source of light as well as whether the light is absorbed or emitted. Four types of photometric instruments are currently in use in laboratories:

1- **molecular absorption**
2- **molecular emission (fluorometers)**
3- **atomic emission (flame photometers)**
4- **atomic absorption spectrophotometers**

1- Molecular Absorption Spectrophotometers

Molecular absorption spectrophotometers, usually referred to as *spectrophotometers*, are commonly employed in conjunction with other methodologies, such as nephelometry and enzyme immunoassay (EIA). Spectrophotometers are easy to use, have relatively high specificity, and the results are highly accurate. The high specificity and accuracy are obtained by isolated analytes reacting with various substances that produce colorimetric reactions.

The basic components of two types of spectrophotometers (single and double beam).

Single-beam instruments have a

1- light source (e.g., a tungsten bulb or laser), which passes through an entrance slit that minimizes stray light

2- monochromator, Specific wavelengths of light are selected by the use of a monochromator Light of a specific wavelength
3- Cuvette passes through the exit slit and illuminates the contents of the analytical cell cuvette.
4- Detector After passing through the test solution the light strikes a detector, usually a photomultiplier tube,
5- Recorder This tube amplifies the electronic signal, which is then sent to a recording device .

The result is then compared with a standard curve to yield a specific concentration of analyte.

The double-beam instrument, similar in design to single-beam instruments, is designed to compensate for changes in absorbance of the reagent blank and light source intensity.

It utilizes a mirror (VI) to split the light from a single source into two beams, one passing through the test solution and one through the reagent blank. By doing so, it automatically corrects optical errors as the wavelength changes.

Most measurements are made in the visible range of the spectrum. The greatest sensitivity is achieved by selecting the wavelength of light in the range of maximum absorption. If substances are known to interfere at this wavelength, measurements may be made at a different wavelength in the absorption spectrum. This modified procedure allows detection or measurement of the analyte with minimal interference from other substances.

2- Molecular Emission Spectrophotometers

Molecular emission spectrophotometry is usually referred to as *fluorometry*. The technology found in these instruments is based on the

principle of luminescence, that is, an energy exchange process that occurs when electrons absorb electromagnetic radiation and then emit this excited energy level at a lower level. Three types of fluorescence phenomena

1- **fluorescence**
2- **phosphorescence**
3- **chemiluminescence**

1- ***Fluorescence*** results from a three-stage process that occurs in certain molecules known as *fluorophores*. The first stage involves the absorption of radiant energy by an electron in the ground state creating an excited singlet state. During the very short lifetime of this state (order of nanoseconds), energy from the electronic-vibrational excited state is partially dissipated through a radiationless transfer of energy that results from interactions with the molecular environment and leads to the formation of a relaxed excited singlet state. This is followed by relaxation to the electronic ground state by the emission of radiation (fluorescence). Because energy is dissipated, the energy of the emitted photon is lower and the wavelength is longer than the absorption photon. The difference between these two energies is known as *Stokes shift*. This principle is the basis for the sensitivity of the different fluorescence techniques since the emission photons can be detected at a different wavelength band than the excitation photons. Consequently, the background is lower than with absorption spectrophotometry where the transmitted light is detected against a background of incident light at the same wavelength.

The phenomenon of *phosphorescence* is similar to fluorescence since it also results from the absorption of radiant energy by a molecule.

The phenomenon of *chemiluminescence* is also similar to that of fluorescence in that it results from light emitted from an excited singlet state. However, unlike both fluorescence and phosphorescence, the excitation energy is caused by a chemical or electrochemical reaction. The energy is typically derived from the oxidation of an organic compound, such as luminol, luciferin, and acridinium ester. Light is derived from the excited products that are formed in the reaction.

Application of Molecular Emission Spectrophotometers

One of the most common applications of fluorescence polarization is competitive immunoassays, used to measure a wide range of analytes including therapeutic and illicit drugs, hormones, and enzymes. This important methodology involves the addition of a known quantity of fluorescent-labeled analyte molecules to a serum antibody (specific to the analyte) mixture. The labeled analyte will emit depolarized light because its motion is not constrained. However, when it binds to an antibody, its motion will decrease and the emitted light will be more polarized. When an unknown quantity of an unlabeled analyte is added to the mixture, competitive binding for the antibody will occur and reduce the polarization of the labeled analyte. By using standard curves of known drug concentrations versus polarization, the concentration of the unlabeled analyte can be determined.[10]

.Another important application of fluorometry is flow cytometry

3-Atomic Emission and Atomic Absorption Spectrophotometers

Atomic emission (flame photometry) and *atomic absorption spectrophotometry* have limited use in modern laboratories. In the past, concentrations of metallic elements such as sodium, potassium, and

lithium were commonly determined by flame photometry. The technique is based on the elementary quantum principle that electrons in an atom are excited to a higher energy level by heat. The electrons, being unstable in this state, return to a lower energy state. In doing so, the excess energy is liberated as photons in the visible light range. Usually, multiple energy levels are involved and the resulting spectral patterns are characteristic of each element. When conditions are held constant, the concentration of each ion is proportional to the light intensity at its characteristic wavelength.

Atomic absorption spectrophotometry procedures are currently associated mainly with toxicology laboratories where poisonous substances, such as lead and arsenic, need to be identified. Unlike flame photometry, the element that is analyzed by this technique is not appreciably excited. Rather, the element is dissociated from its chemical bonds. In this state, the element is in its lowest energy state and capable of absorbing energy in a narrow range that corresponds to its line spectrum. Atomic absorption spectrophotometers are much more sensitive than flame photometers and more specific.

4-TURBIDIMETRY AND NEPHELOMETRY

When light passes through a solution, it can be either absorbed or scattered. *Turbidimetry* is the technique for measuring the percent light absorbed. A major advantage of turbidimetry is that measurements can be made with laboratory instruments, such as a spectrophotometer, used for other procedures in laboratory testing. Errors associated with this method usually involve sample and reagent preparation. For example, since the amount of light blocked depends on both the concentration and size of each particle, differences in particle size between the sample and the standard is one cause of error. The length of time between sample

preparation and measurement, another cause of error, should be consistent since particles settle to varying degrees, allowing more or less light to pass. Large concentrations are necessary because this test measures small differences in large numbers.

Nephelometry, similar to turbidimetry, is the technique used for measuring the scatter of light by particles. The main differences are that (1) the light source is usually a laser, and (2) the detector, used to measure scattered light, is at a right angle to the incident light. Beam light scattered by particles is a function of the size and number of the particles. Nephelometric measurements are more precise than turbidimetric ones since the smaller signal generated for low analyte concentrations is more easily detected against a very low background.[12] Because antigen–antibody complexes are easily detected by this method, it is commonly employed in combination with EIAs.

5-REFRACTOMETRY

Refractometry measurements are based on the principle that light bends as it passes through different media. The ability of a liquid to bend light depends on several factors: wavelength of the incident light, temperature, physical characteristics of the medium, and the solute concentration in the medium. By keeping the first three parameters constant, refractometers can measure the total solute concentration of a liquid. This procedure is particularly useful, especially as a rapid screening test, since no chemical reagents and reactions are involved.

Refractometers are commonly used to measure total dissolved plasma solids (mostly proteins) and urine specific gravity. In the refractometer, light is passed through the sample and then through a series of prisms. The refracted light is projected on an eyepiece scale. The scale is

calibrated in grams per deciliter for serum protein, and in the case of urine, for specific gravity.

7-OSMOMETRY

In the clinical laboratory, osmometer readings are interpreted as a measure of total concentration of solute particles and are used to measure the osmolality of biological fluids such as serum, plasma, and urine. When osmotically active particles are dissolved in a solvent (water, in the case of biological fluids), four physical properties of the water are affected: the osmotic pressure and the boiling point are increased, and the vapor pressure and the freezing point are decreased. Since each property is related, they can be expressed mathematically in terms of the others (colligative properties) and to osmolality. Consequently, several methods can be used to measure osmolality including freezing-point depression, colloid osmotic pressure (COP), and vapor pressure osmometry.

In certain situations, it is important to measure the COP, a direct measure of the contribution of plasma proteins to the osmolality. Because of the large molecular weight of plasma proteins, their contribution to the total osmolality is very small as measured by freezing-point depression and vapor pressure osmometers. Since a low COP favors a shift of fluid from the intravascular compartment to the interstitial compartment, measurement of the COP is particularly important in monitoring intravascular volume and useful in guiding fluid therapy in different circumstances to prevent peripheral and pulmonary edema.

The *COP osmometer*, also known as a *membrane osmometer*, consists of two fluid-filled chambers separated by a semipermeable membrane. One chamber is filled with a colloid-free physiologic saline solution that is in contact with a pressure transducer. When the plasma or serum is placed in the sample chamber, fluid moves by osmosis from the saline chamber to the sample chamber, thus causing a negative pressure to develop in the saline chamber. The resultant pressure is the colloidal osmotic pressure.

ELECTROCHEMISTRY

In the clinical laboratory, analytic electrochemical techniques involve the measurement of current or voltage produced by the activity of different types of ions. These analytic techniques are based on the fundamental electrochemical phenomena of

1- **potentiometry**
2- **coulometry**
3- **voltammetry**
4- **conductometry.**

ELECTROPHORESIS

Routine diagnostic applications of *electrophoresis* technology exist for infectious diseases, malignancies, genetic diseases, paternity testing, forensic analysis, and tissue typing for transplantation. Electrophoresis tests, an important clinical laboratory method for molecular separations, involve the movement of charged molecules in a solution or on a support medium, associated with a direct current electrical field. The movement of molecules in this electrical field is dependent on molecular charge, shape, and size. Since most molecules of biologic importance are both water-soluble and charged, this analytical tool is one of the most important techniques for molecular separation in the clinical laboratory.
The main types of electrophoresis techniques used in both clinical and research laboratories include

1- cellulose acetate,
2- agarose gel,

3- polyacrylamide gel,
4- isoelectric focusing,
5- two-dimensional,
6- capillary electrophoresis (CE)

The primary application of electrophoresis is the analysis and purification of very large molecules such as proteins and nucleic acids. Electrophoresis also can be applied to the separation of smaller molecules, including charged sugars, amino acids, peptides, nucleotides, and simple ions. Through the proper selection of the medium for electrophoretic separations, extremely high resolution and sensitivity of separation can be achieved. Electrophoretic systems are usually combined with highly sensitive detection methods to monitor and analyze the separations that suit the specific application.

The basic electrophoresis apparatus consists of a high voltage direct current supply, electrodes, a buffer, and a support for the buffer or a capillary tube. Supports for the buffer include filter paper, cellulose acetate membranes, agarose, and polyacrylamide gels. When an electrostatic force (EOF) is applied across the electrophoresis apparatus, the charged molecules will migrate to the anode or the cathode of the system depending on their charge.

Gel Electrophoresis

Cellulose Acetate and Agarose Gel Electrophoresis

Cellulose acetate and *agarose gel electrophoresis* are commonly used for both serum protein and hemoglobin separations. Serum protein electrophoresis is often used as a screening procedure for the detection of disease states, such as inflammation, protein loss, monoclonal gammopathies, and other dysproteinemias. When the molecules have been separated into bands, specific stains are used to visualize them.

Densitometry is typically used to quantify each band. When a monoclonal immunoglobulin pattern is identified, another technique, immunofixation electrophoresis, is used to quantify the immunoglobulins IgG, IgA, IgM, IgD, and IgE. Once these proteins are separated on an agarose gel, specific antibodies directed at the immunoglobulins are added. The sample is then fixed and stained to visualize and quantify the bands.

ELISA

What Is ELISA?

ELISA is the basic assay technique, known as enzyme-linked immunosorbent assay (also referred to as EIA: Enzyme Immunoassay) that is carried out to detect and measure antibodies, hormones, peptides and proteins in the blood.

Antibodies are blood proteins produced in response to a specific antigen. It helps to examine the presence of antibodies in the body, in case of certain infectious diseases.

ELISA is a distinguished analysis compared to other antibody-assays as it yields quantitative results and separation of non-specific and specific interactions that take place through serial binding to solid surfaces, which is normally a polystyrene multiwell plate.

Types Of ELISA

ELISA tests can be classified into three types depending upon the different methods used for binding between antigen and antibodies, namely:

1- **Indirect ELISA** – Antigen is coated to the microtiter well
2- **Sandwich ELISA** – Antibody is coated on the microtiter well
3- **Competitive ELISA** – Microtiter well which is antigen-coated is filled with the antigen-antibody mixture.

1- Indirect ELISA

- Indirect ELISA detects the presence of an antibody in a sample.
- The antigen is attached to the wells of the microtitre plate.

- A sample containing the antibodies is added to the antigen-coated wells for binding with the antigen.
- The free primary antibodies are washed away and the antigen-antibody complex is detected by adding a secondary antibody conjugated with an enzyme that can bind with the primary antibody.
- All the free secondary antibodies are washed away. A specific substrate is added which gives a coloured product.
- The absorbance of the coloured product is measured by spectrophotometry.

2- Sandwich ELISA

- Sandwich ELISA helps to detect the presence of antigen in a sample.
- The microtitre well is coated by the antibody.
- The sample containing the antigen is added to the well and washed to remove free antigens.
- Then an enzyme-linked secondary antibody, which binds to another epitope on the antigen is added. The well is washed to remove any free secondary antibodies.
- The enzyme-specific substrate is added to the plate to form a coloured product, which can be measured.

3- Competitive ELISA

- Competitive ELISA helps to detect antigen concentration in a sample.
- The microtitre wells are coated with the antigen.
- Antibodies are incubated in a solution having the antigen.
- The solution of the antigen-antibody complex is added to the microtitre wells. The well is then washed to remove any unbound antibodies.
- More the concentration of antigen in the sample, lesser the free antibodies available to interact with the antigen, which is coated in the well.
- The enzyme-linked secondary antibody is added to detect the number of primary antibodies present in the well.

- The concentration is then determined by spectrophotometry.

Principle of ELISA

ELISA works on the principle that specific antibodies bind the target antigen and detect the presence and quantity of antigens binding. In order to increase the sensitivity and precision of the assay, the plate must be coated with antibodies with high affinity. ELISA can provide a useful measurement of antigen-antibody concentration.

Advantages Of ELISA

Following are some of the advantages of the ELISA technique:

1- Results fetched from ELISA gives an accurate diagnosis of a particular disease since two antibodies are used.

2- Can be carried out for complex samples as the antigen is not required to get purified to detect.

3- It is highly responsive since direct and indirect analysis methods can be carried out.

4- It is a rapid test, yields results quickly.

5- Possible detection for ELISA ranges from the quantitative, semi-quantitative, standard curve, qualitative, calibration curve models etc.

6- Easier to perform and uncomplicated process as compared to other assays which require the presence of radioactive materials.

Applications of ELISA

The applications of ELISA are discussed below:

1. The presence of antibodies and antigens in a sample can be determined.
2. It is used in the food industry to detect any food allergens present.
3. To determine the concentration of serum antibody in a virus test.
4. During a disease outbreak, to evaluate the spread of the disease.

SPECIMEN COLLECTION, HANDLING, and TRANSPORT

Purpose

Laboratory testing provides information about a patient's health to assist Physicians in diagnostic and therapeutic decisions. Specimen Integrity is dependent on accurate pre-analytical processes to include patient preparation, specimen collection, handling, and transportation. Improper collection and handling of samples can give erroneous results and compromise the care of the patient. These guidelines cover some of the key steps in handling blood samples to provide optimal specimens for testing.

Principle

There are multiple Pre-Analytical factors associated with the handling and processing of laboratory specimens that can lead to test result inaccuracy. Strict adherence to all phases of proper collection and processing is essential for accurate test results.

Pre-Analytical Factors:

1. Specimen Collection:

a. Improper Patient Identification

b. Incorrect Order of Draw

c. Incorrect Tube Selection

d. Traumatic draws leading to hemolysis

e. Inadequate mixing or insufficient sample

2. Specimen Handling/Processing:

a. Serum tubes not thoroughly clotted before centrifugation

b. Delay in Centrifugation

c. Storing specimens in incorrect temperatures

3. Specimen Transportation

a. Frozen specimens thawing during transport

b. Unspun specimens transported >2 hours from collection

Specimen Collection

Patient results are only as good as the specimen collected. The integrity of the sample must be preserved and requirements for collection and handling must be followed. It is critical that adequate volumes are collected on each patient and the patient preparation is adhered to follow test requirements such as fasting.

1. Collection Process: Following proper phlebotomy techniques will assist in preventing inaccurate test results:

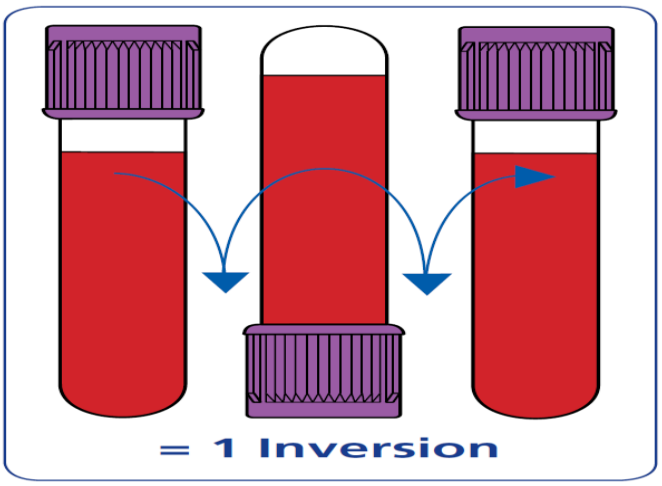
a. Tourniquet left on <1 minute to prevent Hemolysis

b. **All** tubes collected must be collected in the correct Order of Draw and inverted gently to ensure proper mixing of additive or anticoagulant:

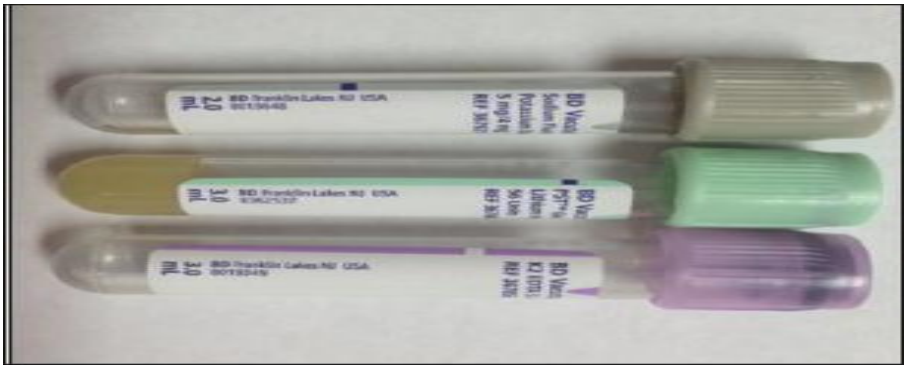
Order of Draw and Inversion Chart

Closure Color	Collection Tube	Mix by Inverting
BD Vacutainer® Blood Collection Tubes *(glass or plastic)*		
	• Blood Cultures - SPS	8 to 10 times
	• Citrate Tube*	3 to 4 times
or	• BD Vacutainer® SST™ Gel Separator Tube	5 times
	• Serum Tube *(glass or plastic)*	5 times (plastic) none (glass)
	• BD Vacutainer® Rapid Serum Tube (RST)	5 to 6 times
or	• BD Vacutainer® PST™ Gel Separator Tube With Heparin	8 to 10 times
	• Heparin Tube	8 to 10 times
or	• EDTA Tube	8 to 10 times
	• BD Vacutainer® PPT™ Separator Tube K_2EDTA with Gel	8 to 10 times
	• Fluoride (glucose) Tube	8 to 10 times

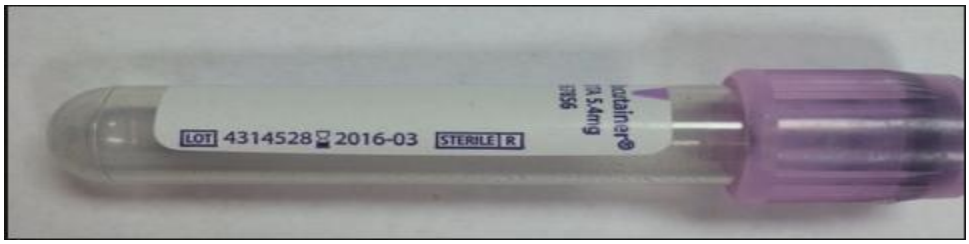
Note: Gold top tubes (SST) contains an additive (Clot activator) and must be inverted.



c. All collection tubes must be filled with the required volume (no short samples). Fill lines are indicated by the black and white notches on the side of the label:



d. Do not use expired tubes. Expiration dates can be found on each paper label on the tube



e. Incorrect Order of Draw will introduce contamination with anticoagulants and often produce inaccurate results. An example would be increased Potassium if the Lavender tube is drawn prior to collection of Gold.

2. Specimen Labeling: All specimens must contain two specimen identifiers:

a. Patient's first and last name

b. Patient's Date of Birth

c. Unique Identifying number to assist with identification such as Social Security number

Note: Hospital policy requires Patient's first and last name and Date of birth.

Specimen Handling and Processing (Centrifugation)

1. Serum tubes must be placed in an upright vertical position and allowed to clot for a minimum of 30 minutes before centrifuging. After the

specimen has been allowed to fully clot, the tube is to be centrifuged within 1 hour of collection and no longer than 2 hours after collection.

**** Failure to separate red cells from serum or plasma within 2 hours of collection, may lead to inaccurate results****
Note: Patients on anticoagulant therapy may need longer time to clot.
2. Centrifugation: All serum tubes must be properly balanced and tubes spun within the appropriate speed and time based on the centrifuge in use. ***The centrifuges listed below are the only models approved for separation of blood samples.***

Centrifuge Name	Type	RPM	Time	Directions
Horizon Mini 642B (Drucker)	Horizontal Rotor	Manufacturer Set (3380 RPM – 1590g)	Manually Set Time to 10 minutes.	Close lid and turn dial to 10 minutes.
Horizon Mini 642E (Drucker)	Horizontal Rotor	Manufacturer Set (3380 RPM – 1590g)	Manufacture set to 10 minutes.	Close lid and Press Start
Horizon 653V (Drucker)	Horizontal Rotor	3200 RPM - 1490g	Manufacture set to 10 minutes.	Turn dial to 3.2 RPM, Close Lid and Press Start
Horizon 653ES (Drucker)	Horizontal Rotor	Manufacturer Set 3150 RPM – 1550g	Manually set time to 10 minutes	Select 10 minutes, Close lid and Press Start
Horizon 642VFD-PLUS (Drucker)	Horizontal Rotor	Manufacturer Set 3800 RPM – 2000g	Manufacture set to 10 minutes.	Close lid and Press Start
Horizon 755VES (Drucker)	Horizontal Rotor	Manually set to 3500 RPM	Manually set time to 10 minutes	Select RPM to 3500 and Select time to 10 Minutes. Press Start

3. Observe each tube after centrifugation. Verify that the gel is completely separating cells from
serum. If complete separation is not visible, DO NOT RECENTRIFUGE.

1. Transfer serum or plasma to an aliquot tube using a pipette leaving a small amount on top of the gel or packed cells.
2. Centrifuge the aliquot tube following the recommendations of your centrifuge.

3. Observe the aliquot tube for red cells at the bottom of the tube (red button). Transfer the serum/plasma into another aliquot tube leaving a layer of serum/plasma above the red button.

Limitations:
Do not disturb the red button which are the red cells that have been separated.
●Label each aliquot tube to properly identify your patient through each step of processing.
●Do not place unspun tubes in the refrigerator. The refrigeration of the unspun tube will
increase Potassium levels by 135%
●Light Sensitive requirement: Pour plasma/serum into a dark aliquot tube to protect the
specimen from any light source to ensure specimen integrity. If a dark aliquot tube is not
available, wrap aluminum foil or paper towel around the tube (not the stopper)tightly. Please ensure the tube will not be exposed to light during storage and transport.

Specimen Transport
To minimize exposure to bloodborne pathogens in transport of specimens, Standard Precautions must be used. ALL blood and other potentially infectious material are treated as if they are known to be infectious with HIV or hepatitis and other bloodborne pathogens. All specimens must be transported in a sealed biohazard bag.

1. **Room Temp Requirements**: If your specimen does not have a specific storage requirement and will be stored at room temp before Courier pickup, please place in a sealed orange/red biohazard labeled specimen bag.

2. **Refrigeration**: If your specimen requires refrigerated temperatures during transport, place in a "blue" biohazard labeled specimen bag. Please use a permanent marker and place an "X" in the box designating "Refrigerator" temperature. Place the specimen in your refrigerator until Courier pickup. The blue bag will alert the Courier that this specimen will need to be transported on ice to retain the refrigerator temperature.

3. Frozen: If your test requires the specimen to be frozen after processing, the specimen must be centrifuged and serum/plasma must be transferred to an aliquot tube by pipette without disturbing gel or packed

cells. Following labeling requirements for all aliquots. The aliquot is to be placed in a blue biohazard labeled specimen bag.
use “X” in the box designating “Frozen” temperature. Place specimen bag in your freezer (or on dry ice) until Courier pickup. The blue bag will alert the Courier that this specimen will need to be transported on dry ice to retain the frozen temperature.

4. STAT: If your specimen has a “STAT” priority, please call your Courier for pickup. Place the sample in a “Red” biohazard labeled specimen bag. The red bag will easily be seen as a STAT specimen upon delivery to the laboratory.

5- other requirement

1.Remove all needles and sharps from all specimens before transporting

2.All specimens transported via courier must be transported in sealed biohazard, leak-proof, puncture resistant container tightly closed before transportation.

3.Please place specimens in the Ziploc portion of the specimen bag. Completed requisition is to be placed in the outside pocket