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ISLAMIC UNIVERSITY

College of Medical Technology

Department of Pathological Analysis Techniques

Lecturer: Abbas Almulla

PRE-ANLYTICAL ERROR

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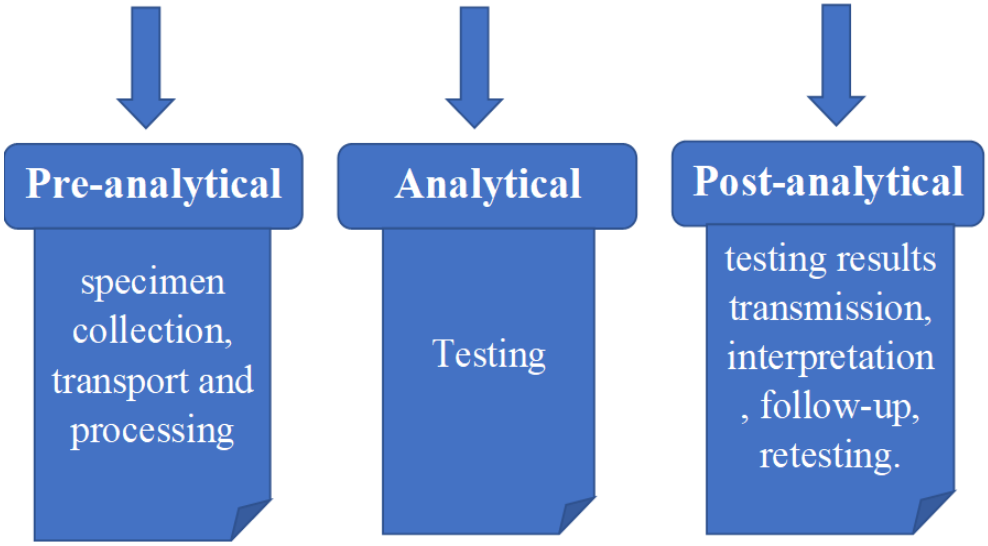
****Factors that influence test results:**

Laboratory tests involved three phases



*Preanalytical factors that influence test results

The preanalytical phase has long been recognized as a source of substantial variability in laboratory medicine. Laboratory errors, mostly due to some defect in the preanalytical phase, may lead to diagnostic errors. These factors may be divided into two major groups: influencing and interference factors.

1- Influencing factors:

Influencing factors lead to changes in the quantity of the analyte in a method-independent way. Influencing factors may be changeable (eg, diet, time of the day) or unchangeable (eg, gender, ethnicity, genetic background). The effect of influencing factors may be reduced through standardization of preanalytical conditions. Influencing factors characterized as controllable and not controllable including are:

a- Controllable Variables

1- Time of Sampling: Several key elements need to be considered when thinking about the best time for sampling: the best time of the day, time after the last meal, time after the last sample, time after the last dose of the drug, and so on.

2- Influence of Circadian Rhythm: Several analytes tend to fluctuate in terms of their plasma concentration over the course of a day Thus the concentration of potassium is lower in the afternoon than in the morning, whereas that of cortisol decreases during the day and increases at night.

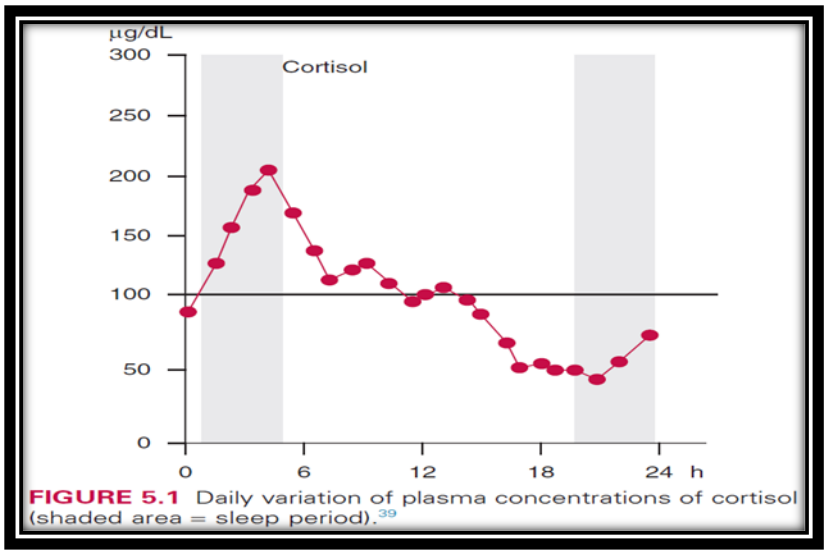


FIGURE 5.1 Daily variation of plasma concentrations of cortisol (shaded area = sleep period).[39]

The circadian rhythm can also be influenced by individual responses to meals, exercise, and sleep. These influences should not be confused with real circadian changes. Also, seasonal influences may take into consideration, whereas total triiodothyronine (T3) is 20% lower in the summer than in the winter,26 whereas 25 OH-cholecalciferol exhibits higher serum concentrations in the summer.

3- Menstrual Cycle: Changing of the hormones patterns during MC will direct effect on some analyte concentration. For example, aldosterone concentration in plasma is twice as high before ovulation than in the follicular phase. Likewise, renin can show a preovulatory increase. Even cholesterol exhibits a significant decrease during ovulation, and phosphate and iron decrease during menstruation. Pituitary gonadotropin and ovarian hormones are significantly affected by this cycle.

4- Influence of Diagnostic and Therapeutic Procedures: Samples should be taken before any diagnostic procedures with potential interfering effects. Likewise, interfering drugs should be administered after collecting a blood sample. The following diagnostic and therapeutic measures can result in both in vivo (frequent) and in vitro (less common) effects on laboratory tests.

a- Operations b- Infusions and transfusions c- Punctures, injections, biopsies, palpations, whole-body massage d- Endoscopy e- Dialysis f- Physical stress (eg, ergometry, exercise, ECG) g- Function tests (eg, oral glucose tolerance test) h- Immunoscintigraphy i- Contrast media, drugs j- Mental stress k- Ionizing radiation

"A sample taken at the wrong time is worse than taking no sample."

a-Long-Term Effects of Diet: Creatinine increase in plasma of up to 20% of ingesting cooked meats, A diet rich in fat leads to increased serum triglyceride concentration, reduced serum urate, and a depletion of the body's nitrogen pool.

b-Acute Effects of Diet: The activity of some enzymes (eg, ALP, AST, ALT) increases up to 20% following a meal. The turbidity of the plasma/serum sample caused by chylomicrons following absorption of lipids.

"12 hours of fasting and reduced activity (bed rest) required for right sample"

6- Effects of Fluid Intake Before Sampling

a- Caffeine. Caffeine stimulates the adrenal cortex and medulla, leading to the subsequent increase of the concentration of catecholamines and their metabolites as well as free cortisol. Increase in plasma glucose concentration and renin activity followed with caffeine ingestion.

b- Alcohol. Decrease of plasma glucose and increase of lactate, increases hepatic formation of uric acid, metabolic acidosis increases the activity of serum GGT and some other enzymes for example (isocitrate dehydrogenase).

7- Smoking Tobacco: Smoking increases the serum concentrations of fatty acids, epinephrine, free glycerol, aldosterone, and cortisol. These changes occur within 1 hour of smoking a cigarette. Nicotine causes the increase of the concentration of epinephrine

8- Body Position and Tourniquet: The higher pressure obtained in veins leads to the loss of water and low molecular weight substances, increasing the concentration of proteins, cells, and analytes bound to them. This

becomes clinically significant after 1 to 2 minutes. Therefore, the tourniquet should be released 1 minute after it has been applied.

9-Muscular Activity: Concentrations of creatine kinase (CK), creatine kinase MB (CK-MB), alanine aminotransferase (ALT), and lactate dehydrogenase (LD) are increased in individuals where physical activity is greater than 12 hours per week. Cardiac troponin (cTn) rises after maximal bicycle stress test.

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Notices to preparing for right blood sampling

These recommendations, the following general requirements should be applied to all blood tests:

1. Blood should be drawn preferably in the morning between 7 a.m. and 9 a.m.

2. Fasting should last for 12 hours, during which only water consumption is permitted.

3. Alcohol should be avoided for 24 hours before blood sampling.

4. In the morning before blood sampling, patients should refrain from cigarette smoking and caffeine-containing drinks (tea, coffee, etc.).

b- Noncontrollable Variables: which including Age, Race, Pregnancy, Altitude and Gender

Many analyte concentrations in the blood are depend on the age and gender and it's change across variation of the factors which we talk about. For example, Alkaline phosphatase, LDL-cholesterol, hormones, creatinine are different with age for that age-dependent reference intervals are provided.

2- Interferences factors

a- Hemolysis: Hemolysis is defined as a process of membrane disruption of erythrocytes and other blood cells, accompanied by the subsequent release of cell components into the plasma and red coloration of the serum (or plasma) to various degrees after centrifugation. Hemolysis is the most common preanalytical error and the most common cause of sample rejection. It occurs with a frequency of up to 30% and accounts for almost 60% of unsuitable specimens.

The mechanism of Hemolysis Interference including multiple types of interferences:

1- Spectrophotometric Interference: Spectrophotometric interference of hemolysis occurs due to the ability of hemoglobin to absorb light at 415-, 540-, and 570-nm wavelengths.

2- Release of the Cell Components into the Sample: Some components are present in blood cells in concentrations that are several times higher than those in the extracellular space (i.e, plasma or serum).

3- Sample Dilution: Some analytes are present in much higher concentrations in plasma than in blood cells like albumin, bilirubin, glucose, sodium, and a few others. For those parameters' hemolysis will cause a dilution effect, and their concentrations will be lower in hemolyzed samples.

4- Chemical Interference: Various blood cell components may affect the analyte measurement procedure by directly or indirectly competing for molecules in the reagents, inhibiting indicator reactions or modifying the analyte by complexation, proteolysis, or precipitation.

b- Lipemia: Lipemia is defined as a turbidity of the sample visible to the naked eye. Turbidity of the sample is caused by the light scattering due to the presence of large lipoprotein particles. To avoid postprandial lipemia, patients are therefore requested to fast for 12 hours before the blood sampling.

The mechanisms of interference caused by lipemia:

1- Spectrophotometric Interference: The ability of lipoprotein particles to absorb light is manifested in the range of wavelengths (300–700 nm).

2- Interference Caused by the Volume Depletion Effect: Plasma in healthy individuals in the fasting state consists of only minor portion of lipids (<10% of the total plasma volume).

c- Icterus: The normal concentration of bilirubin in human plasma (or serum) is up to 20 μmol/L. Change in the color of the serum (or plasma) becomes detectable when bilirubin concentration exceeds 34 μmol/L. Bilirubin concentrations above 100 μmol/L are clinically defined as icterus. Bilirubin interferes with numerous chemistry tests such as enzymes (ALT, alkaline phosphatase, creatine kinase, lipase), electrolytes, metabolites (urea, creatinine, glucose), lipids (cholesterol, triglycerides), proteins (albumin, total proteins, IgG), hormones (estradiol, beta-HCG, free T3), and even some drugs (gentamicin, phenobarbital, theophylline, tobramycin).

Mechanisms of Interference Caused by Icterus:

1- Spectrophotometric Interference of Bilirubin: Bilirubin causes spectrophotometric interference due to its ability to absorb light in the wide range of wavelengths between 400 and 540 nm.

2- Chemical Interference of Bilirubin: Bilirubin produces negative bias on assays that involve H2O2 as an intermediate reaction (eg, cholesterol, glucose, uric acid, triglycerides).

****Detection of Hemolytic, Icteric, and Lipemic Samples:** Hemolysis becomes visible at the concentration of 0.3 to 0.5 g/L of free hemoglobin, and the intensity of the red color of the serum or plasma further increases with the increase in concentration of free serum hemoglobin (Fig. 5.6A). Lipemia causes sample turbidity, which approximately corresponds to the concentration of serum triglycerides (Fig. 5.6B). Increased concentrations of serum bilirubin lead to yellow to orange coloration of the serum, and the change of the color correlates to the increasing concentration of the bilirubin in the serum.

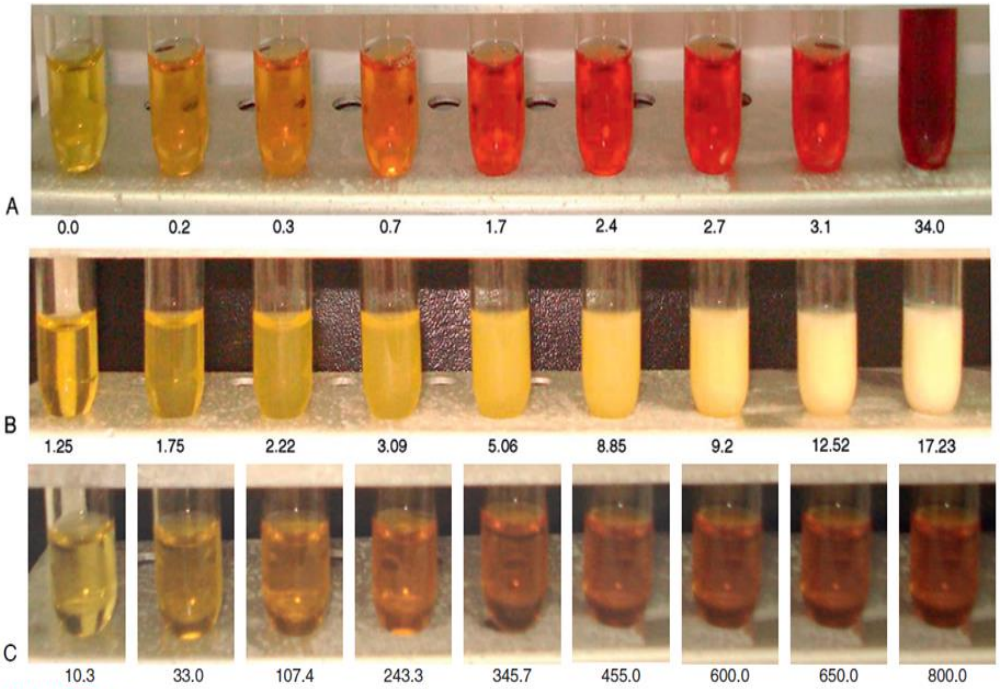

FIGURE 5.6 A, Hemolysis: the intensity of the red color of the serum and corresponding concentrations of free serum hemoglobin (in g/L). B, Lipemia: the degree of turbidity and corresponding concentrations (in mmol/L) of tryglicerides. C, Icterus: the intensity of the yellow color of the serum and corresponding concentrations of bilirubin (in µmol/L). (Color standard scales provided by Clinical Institute of Chemistry, University Hospital Center "Sestre milosrdnice," Zagreb, Croatia. Please see the online version of this figure for full color.)