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PRE – ANALYTICAL ERROR (CONTD.)

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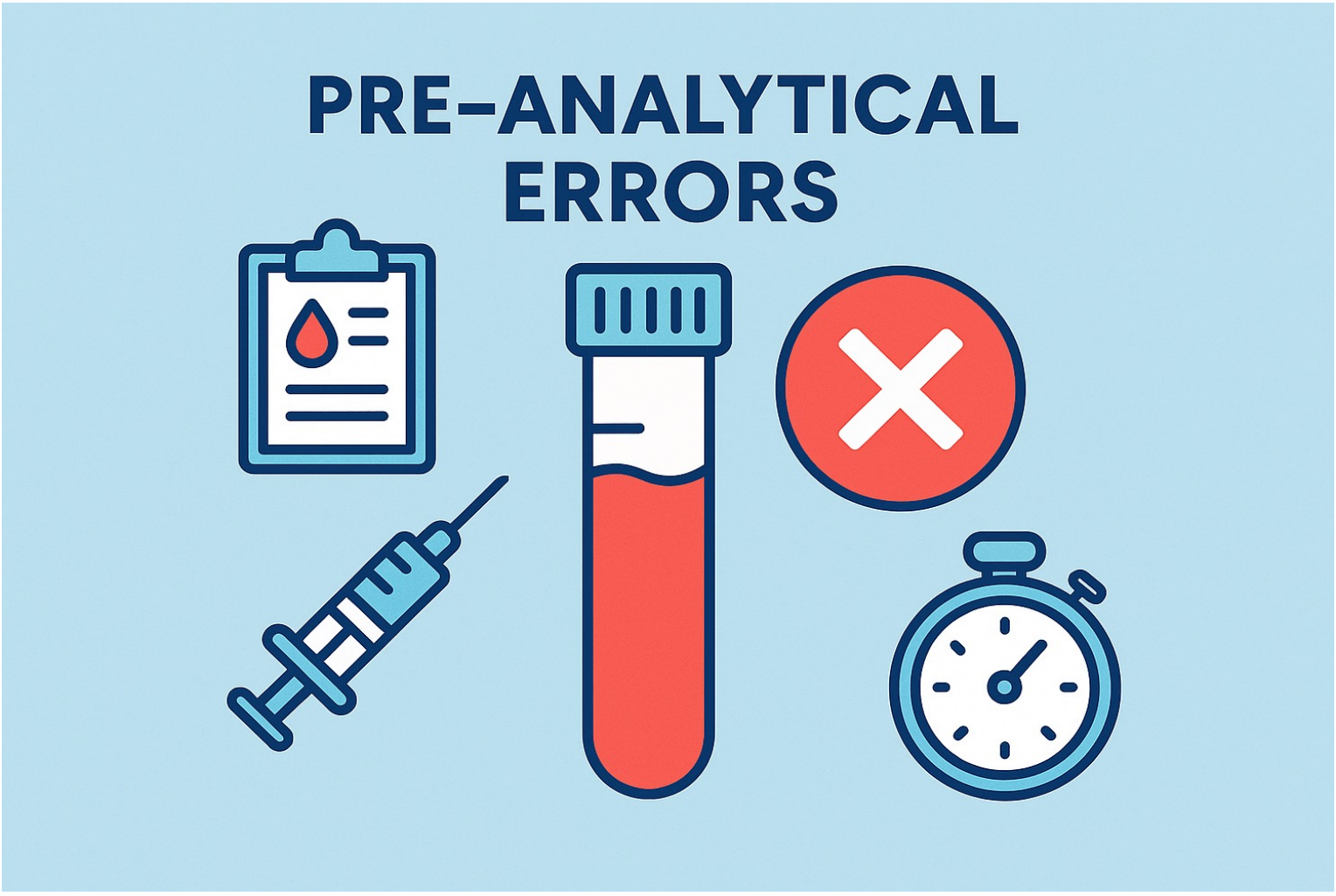

PRE ANALYTICAL ERROR (CONTD.)

Preanalytical errors can occur during the transport and storage of samples due to improper handling or exposure to foreign substances. These errors can alter the quality of the sample and results in changes of test outcome.

Preanalytical Error during Transport: Shipping specimens to **reference laboratories** is often necessary for specialized tests not performed in regular clinical labs. **Before shipping plasma or serum** should be separated from blood cells and **the separated portion must be transferred into a new, clean tube** instead of sending the original collection tube.

Preparing Specimens for Packaging: Following steps are important to ensure safe and stable transport:

1. **Secure the samples:** Place labeled tubes (with tightly closed caps) in a **tube rack** to keep them upright and stable.
2. **Seal the samples:** Put the rack inside a **large, zippered plastic bag** or another **leak-proof, unbreakable container**.
3. **Add secondary protection:** For extra safety, place the sealed bag inside another protective container.
4. Samples are generally shipped **frozen or refrigerated** in a **Styrofoam-insulated container**.

Packing in a Styrofoam-Insulated Shipping Container

1. Place **absorbent material** (enough to soak up all tube contents if breakage occurs) at the bottom of the container.
2. Add a layer of **frozen cold packs or dry ice**.
3. Place the **sealed sample package** (zippered plastic bag containing tubes) on top.
    - If multiple packages are included, insert **cold packs between them** to maintain temperature.
    - Add more absorbent material between packages if needed.
4. Add another layer of **cold packs or dry ice** on top of the packages, then cover with **absorbent material**.
5. Include a **shipping list** (with sample identification numbers) inside a sealed plastic bag on top of the absorbent layer.

- The list should also include the **name and contact number** of the sender or local contact person.

6. **Seal the Styrofoam box** securely with shipping tape.
7. Mark the box with **two "up" arrows** on opposite sides to indicate the correct orientation.
8. **Label the box** clearly according to the **country's shipping and safety regulations**, including all required hazard or bio-specimen labels.

Preanalytical Error due to Storage Conditions

The **CLSI guidelines** provide general recommendations for how blood specimens should be stored in the laboratory:

- **Serum or plasma** should be **separated from blood cells immediately after centrifugation**.
- Once separated, samples should be **tightly capped** to prevent **evaporation or concentration changes**.
- Storage recommendations:
  - Up to **8 hours at room temperature (20–25°C)**
  - Up to **48 hours at 4°C** (refrigerated)
  - After **48 hours**, samples should be **frozen at –20°C**

Samples should be **snap-frozen** using **dry ice or liquid nitrogen** to prevent uneven freezing (gradient formation).

Before testing, **allow samples to thaw at room temperature**, since **heating** can **damage analytes**. Gentle inversion after thawing helps redistribute any concentration gradients formed during freezing. If necessary, **centrifugation** can be performed again to remove any **cell debris or fibrin strands** formed during freezing. Repeated **freeze–thaw cycles** are **not recommended** because they can degrade analytes and affect test accuracy.

Important Storage-Related Precautions

- **Do not freeze whole blood**, as freezing causes **hemolysis (cell rupture)**.
  Hemolyzed blood gives inaccurate results, especially for **hematology** or **blood gas** analysis.
- **Prolonged storage of unseparated plasma at 4°C** can cause **cell leakage**, which **falsely increases potassium levels**.
- When red blood cells break down, **catecholamines (like adrenaline)**

are released, and their **reuptake slows**, leading to **falsely elevated values** if stored too long at 4°C.

- **Prothrombin time (PT)** increases with **long-term frozen storage**; therefore, **PT samples should be stored only at room temperature or 4°C** .
- **Exposure to fluorescent light** can degrade **bilirubin** and other **heme-related compounds**.
    - **Light also destroys vitamins** such as **carotene**, **vitamin A**, and **red blood cell folate**.
    - Some **drugs**, including **nifedipine** and **chloramphenicol**, are also **light-sensitive**.

To prevent these effects, such samples should be collected in **brown or amber-colored tubes**, or wrapped in aluminum foil during storage or transport.

Some chemical substances (analytes) in blood are unstable if serum or plasma is not separated from blood cells quickly. According to CLSI guidelines, separation should be done as soon as possible.

- **For serum samples:** Blood should be allowed enough time to clot before centrifugation.
    - Tubes containing clot activators need gentle mixing and at least **30 minutes** to clot.
    - Plain serum tubes may need up to **60 minutes**.
    - For patients on anticoagulants, clotting may take even longer.
- **For plasma samples:** Tubes should be mixed gently as per the manufacturer's instructions to allow the anticoagulant to work properly.
    - If mixing is insufficient, clotting may not occur properly, causing **platelet clumps or clots**, which interfere with proper separation of plasma or serum during centrifugation.
- **Do not open tubes** before or during centrifugation, as this can cause **evaporation** and change results.
- Some serum samples, like those used for **ionized calcium and pH**, should not be exposed to air. Air exposure causes **loss of CO_2**, leading to **higher pH** and **lower ionized calcium** levels.

Effect of Storage Conditions on Laboratory Results

CLSI gives general guidelines for storing specimens in the lab:

- After centrifugation, **serum or plasma should be separated from cells immediately**.
- Store separated specimens **tightly capped** to prevent evaporation.
    - Up to **8 hours** at room temperature (20–25°C)
    - Up to **48 hours** in a refrigerator (around 4°C)
    - After **48 hours**, freeze at **–20°C** or lower.
- Samples should be **snap-frozen** (using dry ice or liquid nitrogen) to avoid uneven freezing.
- Before testing, thaw samples **at room temperature** — do not heat them, as heating can damage the analytes.
- Gently invert samples after thawing to remove any separation layers that formed during freezing.
- A short centrifugation after thawing can remove any cell debris or fibrin strands.

Important points:

- Avoid **repeated freezing and thawing**, as this can damage the sample.
- **Never freeze whole blood**, because it causes **hemolysis** (breaking of red cells), which affects hematology and blood gas results.
- **Unseparated plasma** stored too long at 4°C may show **false high potassium** levels due to leakage from cells.
- **Catecholamines** (stress hormones) can also appear falsely high if stored too long.
- **Prothrombin time** increases if plasma is stored frozen for too long, so such samples should be kept at room temperature or in a refrigerator instead.
- **Light exposure** damages some substances:
    - **Bilirubin, Vitamin A, carotene, and folate** break down quickly under light.
    - Some drugs (like **nifedipine** and **chloramphenicol**) are also light sensitive.
      → Such samples should be collected in **brown tubes** or wrapped in **aluminum foil** to protect them from light.

Effect of Cross-Contamination on Laboratory Results

Cross-contamination happens when unwanted material (like another patient's sample, IV fluid, or chemicals) gets mixed into a specimen. This can occur during **collection, processing, or testing**.

- During **collection**, contamination may happen if blood is drawn in the wrong order or from an IV line containing fluids.
- During **aliquoting** (dividing samples), contamination can occur if the same pipette tip is used multiple times. To prevent this, use **fresh disposable pipette tips** for each sample and handle **one patient's specimen at a time**.

On **automated analyzers**, contamination may occur if probes are not cleaned properly or if disposable tips are not used. Manufacturers now use **wash steps** and **disposable tips** to reduce this risk

Specimen Misidentification

Accurate identification of patients and specimens is essential for **patient safety**. Organizations such as **The Joint Commission (TJC)**, **College of American Pathologists (CAP)**, and the **Institute of Medicine** emphasize this as a top priority.

- Identification begins when a patient arrives for testing.
- Collect at least **two unique identifiers** (for example, name and date of birth) and ensure they match the patient's medical records.
- **Do not collect** the specimen until all identification issues are resolved.
- The sample must be **labeled in front of the patient** and sent to the lab with the test request.
- In the lab, check that the identifiers on the specimen **match the test requisition or electronic order**.
- Identification should be **maintained carefully** through all steps — centrifugation, aliquoting, and analysis.
- Most laboratories now use **barcoded labels** to ensure continuous and accurate specimen tracking.

Interferences of hemolysis, lipemia and high bilirubin on laboratory tests

Preanalytical errors caused by endogenous interfering substances are among the most frequent sources of inaccuracies in laboratory testing.

Effect of Hemolysis on Laboratory Tests

Hemolysis refers to the rupture or breakdown of the erythrocyte membrane, leading to the release of hemoglobin and other intracellular constituents into the surrounding serum or plasma. The substances released from erythrocytes in significant amounts include enzymes such as **lactate dehydrogenase (LD)** and **aspartate aminotransferase (AST)**, as well as electrolytes like **potassium** and **magnesium**. In instances of severe hemolysis, the leakage of intracellular fluid from erythrocytes may cause dilution of certain serum or plasma analytes, such as **sodium**, which are normally present in low concentrations inside red blood cells.

Effect of Lipemia on Laboratory Tests

Lipemia-related test interference may arise from recent food intake, abnormalities in lipid metabolism, or the administration of lipid-containing infusions. The most frequent pre-analytical cause of lipemia is inadequate fasting before blood collection. Plasma triglyceride levels, mainly in the form of chylomicrons, increase significantly within one to four hours after a meal and may remain elevated for six to twelve hours thereafter. Therefore, patients should fast for at least 12 hours prior to sample collection. For individuals receiving parenteral lipid infusions, the treatment should be discontinued for a minimum of 8 hours before blood is drawn.

Common secondary causes of lipemia include diabetes mellitus, alcoholism, nonalcoholic fatty liver disease, and renal disorders. In whole blood, lipemia is difficult to detect visually unless triglyceride concentrations exceed 1000 mg/dL, while in serum or plasma, visible turbidity is usually apparent when triglycerides are above 300 mg/dL.

Lipemia interferes with laboratory tests primarily through light scattering and absorption caused by lipoprotein particles, leading to inaccuracies in assays utilizing spectrophotometric or nephelometric methods. It may also cause **volume displacement errors**, particularly in electrolyte measurements that employ **indirect ion-selective electrode techniques**. Additionally, lipids can affect analytical results through **physicochemical interactions** — analytes residing in the lipid layer may be less accessible to reagents or antibodies used in immunoassays, and lipid content can alter **electrophoretic or chromatographic separations** of analytes.

Several approaches are available to remove lipids from serum or plasma samples. **High-speed centrifugation** is a common and

effective method that produces a clear infranatant. The success of centrifugation depends on the type of lipids causing interference. When lipemia results from **chylomicrons**, centrifugation at **12,000 g** can effectively separate them. However, if the interference is due to **VLDL or LDL particles**, a much higher centrifugal force is necessary. Lipemia in **EDTA-anticoagulated samples** used for hematological analysis can also be minimized by appropriate centrifugation.

Effect of Icterus on Laboratory Tests

Increased levels of **bilirubin** are another common cause of **endogenous interference** in laboratory tests. Bilirubin strongly absorbs light at wavelengths between **340 nm and 500 nm**, so any test that measures absorbance in this range may be affected. Besides its light-absorbing properties, bilirubin can also **chemically react with reagents** used in certain tests. For example, in **oxidase/peroxidase-based assays** (such as those used to measure glucose, cholesterol, triglycerides, creatinine, and uric acid), bilirubin reacts with the **hydrogen peroxide** formed during the reaction. This causes **lower-than-expected results**.

Bilirubin can also **interfere with dyes that bind to albumin**. In some versions of the **Jaffe method for creatinine**, bilirubin interference occurs because it is **oxidized in an alkaline environment**, reducing its absorbance. In contrast, in a **strongly acidic environment**, the absorbance of **conjugated bilirubin** shifts toward **ultraviolet (UV) wavelengths**, which can cause interference in tests such as the **phospho-molybdate method for phosphate**.

Detecting high bilirubin levels (hyperbilirubinemia) by simply **looking at the sample** is not very reliable, especially if the sample is also **hemolyzed**, which makes detection more difficult. Therefore, the **recommended approach** is to use **automated analyzers** that can detect bilirubin and other interfering substances.

While **spectral interference** from bilirubin can be corrected using **blanking procedures** (which account for its absorbance), **chemical interference** cannot be removed this way. Adding reagents like **potassium ferrocyanide** or **bilirubin oxidase** can help eliminate bilirubin interference in tests that depend on **hydrogen peroxide formation**.

Conclusions

As in other areas of medicine, errors are unavoidable in the whole diagnostic process involving laboratory testing. A good

understanding of the sources of error, frequently involving pre-analytical factors, together with a quantitative evaluation of the clinical significance of the magnitude of analytical errors, aided by the establishment of limits of acceptability based on statistical principles of analytical and intraindividual biological variation, are critical to design a quality program to minimize the clinical impact of errors in the clinical laboratory.

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