Factors influencing laboratory results

Chapter Overview

Laboratory testing is an integral part of modern medicine. To assess a patient's health status, as much information as possible is needed, which can be obtained from various sources: medical history, physical examination, laboratory testing, imaging, and functional diagnostics. Approximately **60–70% of clinical decisions** are based on laboratory data.

For these decisions to be accurate, it is essential that the laboratory process is precise, standardized, and minimally prone to error. The entire laboratory process can be divided into three main phases: **pre-analytical, analytical, and post-analytical**. Each has its own specific features and potential sources of error, which can significantly affect test results and their interpretation.

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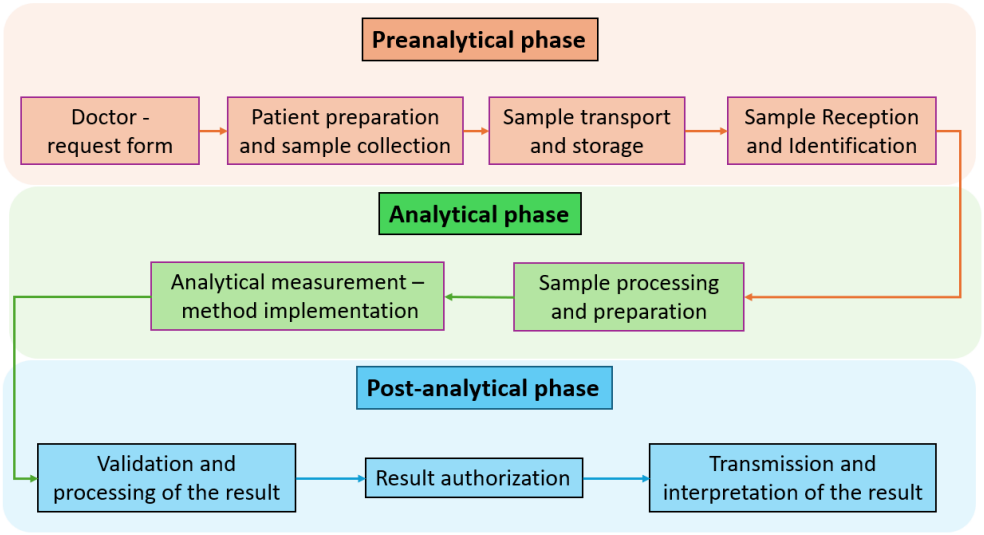


1 Pre-analytical Phase of Laboratory Testing

The pre-analytical phase is the most important and at the same time the most vulnerable part of the laboratory process. It begins at the moment a laboratory test is ordered and ends just before the actual analysis of the sample in a specialized laboratory. According to studies, up to **70% of all laboratory errors** occur in this phase.

1.1 Factors Influencing Laboratory Test Results

The quality of a laboratory result depends not only on the accuracy of the analytical method itself but also on the patient's condition, sample collection, transport, and storage. Errors at any of these steps may distort or completely invalidate the results.

1.1.1 Biological Factors of the Patient

Even before sampling takes place, the patient's condition plays a role. Laboratory values are influenced by a number of non-modifiable factors—for example, sex (men physiologically have higher hemoglobin levels), age (newborns, children, and elderly people differ in many parameters), ethnicity, biological rhythms (e.g., cortisol varies during the day), pregnancy, or the presence of another disease. The biological half-life of individual analytes also matters, i.e., the rate at which substances are metabolized or eliminated from the body.

In addition, there are modifiable factors—patient behavior prior to sampling. These include physical activity (shortly after exercise, lactate, CK (creatine kinase), or AST (aspartate aminotransferase) levels increase), psychological stress (raises stress hormone levels and alters glycemia or white blood cell count), food intake (affects glucose and lipids), alcohol, smoking, and medications.

Therefore, it is essential to instruct the patient before testing (e.g., to come fasting, avoid alcohol, strenuous exercise, or smoking). Only in this way can results be obtained that truly reflect the patient's health status.

? ***Question:*** *How would you proceed if you came for a blood test and realized that you had exercised the day before and eaten a large dinner?*

1.1.2 Key Steps in Sample Collection

Once the patient is prepared, the next step is the actual collection. It is important to choose the correct type of biological material (whole blood, plasma, serum, urine, cerebrospinal fluid, etc.) and to time the collection properly (e.g., in the morning, fasting). The patient's position also affects the results—fluid distribution differs between lying down and standing.

The next step is the choice of **collection tube** (the color differentiation of the test tubes may vary between manufacturers, here from the manufacturer Sarstedt):

- **Red (EDTA):** hematology (whole blood, anticoagulated)
- **Brown (separation gel):** biochemistry (serum)
- **Green (sodium citrate):** coagulation
- **Yellow (sodium fluoride):** glucose
- **Purple (sodium citrate):** sedimentation

Popis odběrové zkumavky Chemická aditiva	Uzávěr	Barva uzávěru	Použití
Srážlivá krev S aktivátorem srážení a gelem		Hnědá	Příprava séra
Nesrážlivá krev EDTA K3EDTA		Červená	Vyšetření krevního obrazu, krevní skupiny, glykovaného hemoglobinu, sedimentace erytrocytů
Nesrážlivá krev citrát sodný (1:9) Citrát sodný 3,2 %		Zelená	Koagulační vyšetření
Nesrážlivá krev citrát sodný (1:4) Citrát sodný 3,2 %		Fialová	Sedimentace erytrocytů (manuálně)
Nesrážlivá krev EDTA a fluorid sodný		Žlutá	Vyšetření glykémie, laktátu a homocysteinu
Nesrážlivá krev heparin Heparinát lithný		Oranžová	Příprava plazmy s heparinem

(Source: http://ukbd.fnhk.cz/odberovy-system.html#01)

The technique of collection also matters: prolonged application of a tourniquet (> 1 minute) leads to hemoconcentration and false results, while improper disinfection may contaminate the sample.

1.1.3 Transport and Storage of Samples

After collection, the next steps are transport and storage. The goal here is to minimize the time until analysis and preserve sample stability.

- Whole blood: composition changes during standing (potassium leaks from erythrocytes, glucose and oxygen are consumed).
- Serum: more stable; can be stored short-term in a refrigerator, or frozen for longer storage.
- Urine: without preservatives, it quickly undergoes bacterial changes → requires preservation or cooling.
- Sensitive substances (e.g., bilirubin): must be protected from light.

***Note**: **Whole blood** contains both plasma and blood cells (erythrocytes, leukocytes, platelets). It is mainly used in hematology and coagulation tests and must be collected in a tube with an anticoagulant to prevent clotting.*
***Serum** is the liquid portion of blood obtained after clotting and centrifugation. It does not contain fibrinogen and is primarily used in biochemistry and serology. Compared with whole blood, it is more stable and easier to store.*

***Question:** Which laboratory parameters must be collected exclusively in the morning and why? What changes occur if whole blood is left standing too long without stabilization?*

2 Analytical Phase and Analytical Properties of Laboratory Methods

The analytical phase represents the central part of the laboratory process. At this stage, the patient's sample has already been collected and prepared, and the actual analysis of the biological material is performed using instruments, reagents, and methods.

Modern laboratories employ automated analyzers and sophisticated control systems, which makes the analytical phase the most closely monitored and reliable part of the process. Nevertheless, errors cannot be entirely excluded.

2.1 What Can Influence Results in the Analytical Phase?

- **Technical condition of instruments**: analyzers require regular calibration and maintenance. Contaminated optical paths, malfunctioning pipetting units, or clogged probes can produce incorrect results.
- **Quality of reagents**: reagents have a limited shelf life and are sensitive to temperature. Improperly stored or expired reagents can alter the intensity of color reactions, leading to false values.
- **Automation vs. manual analysis:** automated systems reduce the risk of human error, but software errors can affect dozens of samples at once. In contrast, manual procedures depend heavily on the experience and accuracy of the laboratory worker.
- **Standard operating procedures (SOPs):** failure to follow protocols precisely (e.g., incubation times, pipetted volumes) undermines reproducibility and reliability of results.

***Note**: Error rates in the analytical phase are generally lower than in the pre-analytical phase, thanks to a high degree of automation and control mechanisms. However, mistakes in this phase can still have serious consequences—leading to **false-negative or false-positive findings**, which may significantly affect patient diagnosis and treatment.*

2.2 What Errors Can Occur?

In real measurements, both **random** and **systematic errors** may occur. The measured value never exactly equals the true value—it always comes with some degree of error or deviation.

2.2.1 Random Errors

Random errors arise from small, hard-to-control factors (temperature fluctuations, instrument vibrations, microscopic pipetting differences). As a result, measured values "scatter" around the true value.

These errors follow the laws of probability. They cannot be precisely predicted or fully eliminated. If we measure the same quantity repeatedly under the same conditions, the results will differ slightly from one another.

2.2.2 Systematic Errors

Systematic errors cause all results to shift in one direction—either consistently higher or consistently lower than the true value.

- **Example:** poor instrument calibration. If an analyzer consistently shows values 10% lower than reality, all patient results will be distorted.
- Such errors may remain constant or vary according to certain conditions (e.g., temperature).

The danger is that repeated measurement of the same sample will not reveal the error—results will appear reproducible but still incorrect. To detect systematic errors, conditions must be varied and **control samples with known values** must be used.

***Note**: If calibration is off, a healthy patient might be labeled as hypoglycemic, or a diabetic may appear "well controlled."*

2.3 How to Describe and Evaluate Errors?

To objectively evaluate the quality of methods, laboratories use several key parameters.

2.3.1 Precision

Precision describes how close results are to one another when repeatedly measuring the same sample.

- Example: measuring glucose concentration five times in the same sample, with only minimal differences between results → the method is precise.
- Deviations usually arise from random errors (pipetting, temperature fluctuations, small instrument drifts).

Types of precision:

- **Repeatability:** consistent results under identical conditions (same instrument, same operator, same day).
- **Reproducibility:** consistent results under varying conditions (different days, operators, or instruments).

Statistical measures:

- **Standard deviation (s):** describes how spread out results are around the mean.
- **Coefficient of variation (CV = s/ $\bar{x}$ × 100%):** expresses relative imprecision as a percentage, allowing comparison between methods or labs.

***Note**:* If two laboratories measure nearly the same cholesterol value for the same patient, the method shows good reproducibility.

2.3.2 Trueness

Trueness expresses how close the measured result is to the true (reference) value. If a method repeatedly gives results that match reality, we can say it has good trueness.

It is evaluated using reference or control samples with known analyte concentrations. If the measured values deviate from these known values, this indicates a systematic error. Such an error shifts all results in the same direction—for example, faulty calibration of an instrument may cause all glucose results to be 0.5 mmol/L lower than reality.

Mathematical expressions:

- **Absolute error (bias):** the difference between the measured value and the true value.

$$\text{Bias} = x_{\text{measured}} - x_{\text{true}}$$

- **Relative error:** the deviation expressed as a percentage.

$$\text{Relative error} = \frac{\text{Bias}}{x_{\text{true}}} \times 100\%$$

? ***Question**: If the true cholesterol value is 5.0 mmol/L and the method consistently shows 4.5 mmol/L, what is the bias and the relative error?*

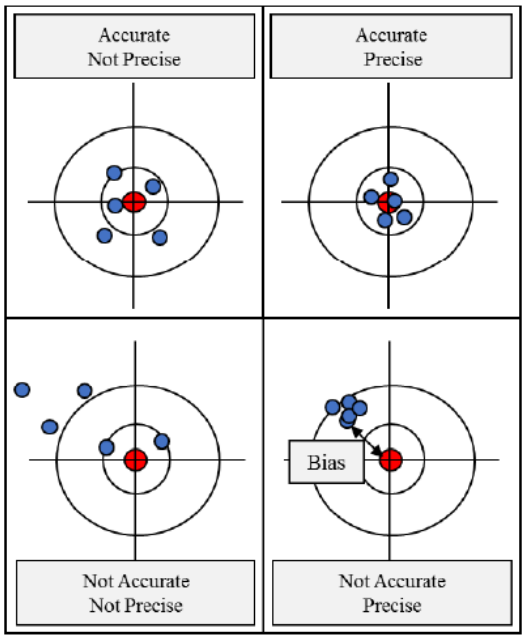
2.3.3 Accuracy

Accuracy is a comprehensive parameter that expresses the overall quality of a method. It takes into account both precision (agreement between repeated results) and trueness (closeness to the true value).

- If a method is precise but not true, the results are consistent but systematically incorrect. For example, a glucometer may always show values 10% higher—all results will be close to each other but not to reality.
- If a method is true but not precise, the average value matches reality, but individual results are scattered. This means the mean is correct, but single measurements are unreliable.
- The optimal method is one that is both precise and true—results are stable and correspond to reality.

Visual analogy (target with shots):

- **Shots close together but off-center →** precise but not true.
- **Shots scattered around the center →** true but not precise.
- **Shots close together at the center →** both precise and true → accurate.



? ***Question:*** *How would you characterize the following results?*
1) A laboratory measures sodium levels, and the results are nearly identical each time, but all are 5 mmol/L lower → the method is …? 2) Another method shows values scattered between 130–150 mmol/L, but the average matches the true value of 140 mmol/L → the method is …?

2.3.4 Other Analytical Characteristics

- **Analytical sensitivity:** the ability of a method to detect very low concentrations of an analyte.
- **Analytical specificity:** the ability of a method to measure only the analyte of interest, without interference from other substances.
- **Limit of detection (LOD) and limit of quantification (LOQ):** the lowest concentration that can be reliably distinguished from zero (LOD) and the lowest concentration that can be accurately quantified (LOQ).
- **Robustness:** the ability of a method to withstand minor changes in conditions (temperature, pH, operator).

2.4 How Can Errors Be Prevented?

In the analytical phase, one cannot simply rely on the idea that "the instrument works." A whole system of measures exists to ensure correct results:

Calibration and maintenance of instruments

Instruments must be regularly checked and adjusted—this is called calibration. It is performed both internally (by laboratory staff according to standard procedures) and externally (by a specialized company or accredited service). Without calibration, an analyzer may systematically report incorrect values.

Quality and stability of reagents

Chemical reagents have a limited shelf life and must be stored under proper conditions of temperature, humidity, and light protection. For example, bilirubin is very light-sensitive—if reagents are not properly stored, results will appear falsely low. Using expired or contaminated reagents is a frequent cause of incorrect results.

Method validation

Every new method must be validated before being introduced into routine practice. The laboratory must be certain that the method measures exactly what it is intended to measure, correctly distinguishes the target analyte from other substances, and that minor changes in conditions (e.g., room temperature) do not significantly affect the results. Without validation, there is a risk of obtaining clinically unusable results.

Standard Operating Procedures (SOPs)

SOPs are detailed written instructions specifying exactly how staff should proceed at each step of analysis—from sample preparation to result evaluation. This ensures that even if a sample is processed by a different worker or laboratory, the procedure remains the same. This is essential for reproducibility and comparability of results.

Internal and external quality control

Each laboratory uses control samples with known analyte concentrations on a daily basis. If the control results fall outside the defined range, it is clear that a problem has occurred (e.g., faulty reagent or instrument malfunction). In addition, laboratories participate in external quality control—interlaboratory comparisons, where identical samples are analyzed and results compared across laboratories. Only this ensures that results from different sites are mutually comparable.

3 Post-analytical Phase of the Laboratory Process

The post-analytical phase follows the analysis itself and represents the final step of the laboratory process. It includes processing, checking, validation, and delivery of results. Although often considered less "technical," it is absolutely crucial—a poorly validated or incorrectly transmitted result can have the same consequences for the patient as an error in collection or analysis.

3.1 Key Steps of the Post-analytical Phase

Validation of results

A biochemist or other specialist evaluates whether the result makes sense. They check if it is consistent with the patient's previous results, with other laboratory parameters, and especially with the clinical condition. At this stage, improbable values caused by sampling or analytical errors can be identified.

Authorization of results

Final confirmation by a specialist (clinical biochemist, hematologist, etc.) that the results are correct and can be officially released. Authorization is both a legal and professional responsibility—it certifies that the laboratory guarantees the quality of the test.

Transfer of results to the Hospital Information System (HIS)

Results are electronically entered into the hospital database. Here, secure and error-free transfer is crucial—an incorrectly matched result (e.g., assigned to the wrong patient) could have fatal consequences.

Interpretation of results by the clinician

It is the physician who places the result in the context of the specific patient. The laboratory provides numbers and expert commentary, but only the clinician decides whether a value represents an acute threat or a clinically insignificant deviation.

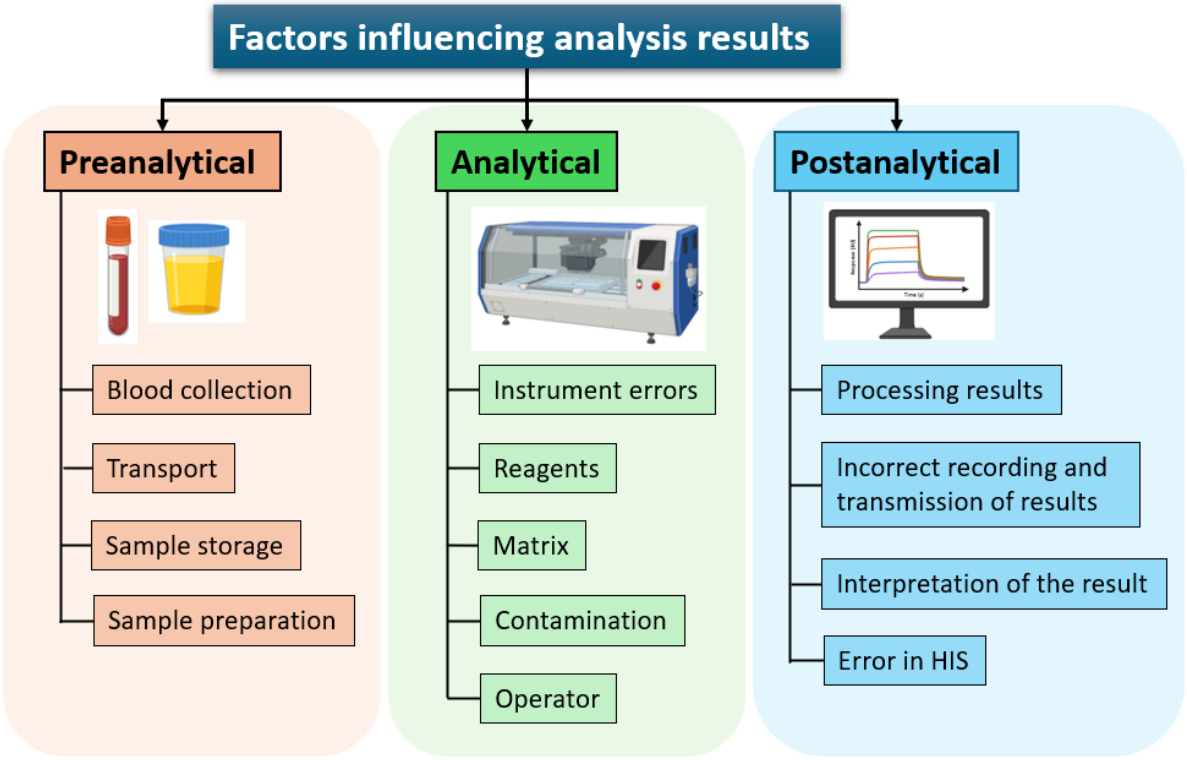
3.2 Examples of Errors in the Post-analytical Phase

- **Validation without context:** A biochemist validates a troponin result without considering that the sample was collected into the wrong tube → result is falsely negative → delayed treatment of acute myocardial infarction.
- **Error during authorization:** Overlooking an extreme value that is biologically impossible (e.g., blood glucose 50 mmol/L in a patient without symptoms).
- **Error in data transfer to HIS:** Patient mix-up where the result appears under another patient.
- **Incorrect interpretation:** The physician ignores the possibility of hemolysis → falsely high potassium → incorrect treatment for hyperkalemia is given.

? ***Question:*** *Which phase do you think requires the greatest cooperation between the laboratory and the clinician? How would you classify a patient sample mix-up versus assigning a result to the wrong patient? Are these serious errors? What consequences might they have?*

Summary

The laboratory process consists of three main phases—pre-analytical, analytical, and post-analytical. Most errors occur in the pre-analytical phase (up to 70%), making proper test indication, patient preparation, sample collection, and transport crucial. The analytical phase is stable today thanks to automation but still requires careful calibration and high-quality reagents. The post-analytical phase includes validation and authorization of results, their correct transfer, and interpretation in the clinical context.



Control Questions

1. In which phase of the laboratory process do most errors occur, and why?
2. What is the difference between validation and authorization of a result?
3. How does hemolysis affect the potassium result?
4. Which laboratory parameters must be collected in the morning and on an empty stomach?
5. What is the importance of SOPs (standard operating procedures) in the laboratory?
6. Which laboratory parameter changes if the sample is taken immediately after physical activity?