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SEED HAEMATOLOGY

Pre-analytics: create the correct preconditions to achieve high-quality analysis results – part I

Pathology laboratories are an essential backbone of patient care and diagnosis. Although there is a lot of automation in haematology and clinical pathology laboratories, there are still many variables influencing the laboratory results. Correct reporting requires that all the phases – pre-analytical, analytical and post-analytical – should be as free from errors as possible. Pre-analytical errors are reported to account for up to 70 % [1]. Since quality depends on a multitude of interconnected factors, precision and accuracy are not the only guarantors of quality. From the very beginning, all three phases need to be monitored and quality controlled. The objective behind quality control is to minimise laboratory errors. By minimising the errors of every step in the pre-analytical phase, a laboratory can improve the quality of the analytical results, reduce the number of re-collected specimens, and so improve turnaround time and patient management.

What is pre-analytics?

Pre-analytics comprises all processes prior to the actual laboratory analysis. Pre-analytical factors, such as specimen collection, specimen handling, interfering substances and patient factors, are common causes of inaccurate test results.

Patient-related variables can be subdivided as shown in Table 1.

Table 1 *Patient-related variables*

Permanent	Long-term	Short-term
Race	Age	Body position
Gender	Pregnancy	Physical stress
	Altitude	Mental stress
	Nicotine	Circadian variation
	Alcohol	Nutrition
	Drugs	
	Nutrition	

Some of the pre-analytical variables, such as specimen variables, can be controlled, while knowledge of uncontrollable variables need to be well understood in order to be able to separate their effects from disease-related changes affecting laboratory results. Some of these mentioned variables might influence haematological results [2]:

Race

Significant differences can be found in both genders of the African population in contrast to the Caucasian population. African people have, for instance, markedly lower numbers of white blood cells (WBC) or WBC subpopulations.

Gender

Besides gender-specific variables like hormones, the number of red blood cells (RBC) and the haemoglobin (HGB) concentration are lower in females than in males.

Age

Many measurable parameters do not reach adult values before adolescence. The WBC subpopulation counts, such as lymphocytes and neutrophils, are significantly different in children up to the age of 6 years, compared to adult reference ranges [3].

Pregnancy

During pregnancy, although unproblematic in most cases, some haematological particularities can occur. During a healthy pregnancy, the mean plasma volume rises from about 2.6 L to 3.9 L, with probably little change occurring in the first 10 weeks of gestation, and a subsequent progressive rise up to the 35th week, at which time the values level off [2].

Altitude

Some blood constituents exhibit significant changes at high altitude compared to findings at sea level. Significant increases with altitude are observed, for example, for haematocrit (HCT) and HGB (up to 8 % at 1,400 m) [2].

Nicotine

It is important to ask the patient whether he or she is a smoker. The chronic consumption of nicotine increases the number of WBC, HGB and HCT [4].

Nutrition

Diet might influence the results which are obtained from the analysers. Anaemia has long been recognised as a major medical problem affecting a considerable proportion of the world's population at all ages. It has been calculated that about 24.8 % [5] of the world's population is suffering from anaemia. Approximately 50 % of all anaemia are caused by iron deficiency. Iron deficiency anaemia attributable to nutritional deficiency and/or blood loss still remains the most common, treatable anaemia in the world. The HGB concentrations and the reticulocyte haemoglobin equivalent (RET-H_e) in these cases [6] are observed to be lower than gender and age-dependent reference values.

Body position

There can be considerably different results between the sitting and the lying body position. If changing from a lying to an upright position, a patient's plasma volume can be reduced by 12 %. A change in plasma volume leads to an apparent concentration change in cells, macromolecules and small molecules [2].

Physical and mental stress

During mental and physical stress conditions, the WBC counts might be higher compared to the normal patient's counts [7, 8].

Drugs

Addictive drugs, such as amphetamine, morphine, heroin, cannabis and cocaine, can influence the results of laboratory tests [9]. Additionally, case reports and few formal epidemiological studies claim that drugs can be the aetiology of agranulocytosis and aplastic anaemia [10]. Also cytotoxic drugs, which are used during chemotherapy, may cause low WBC counts as a side effect [11].

Which is the proper time for taking a blood sample?

In order to receive comparable results, the conditions for blood sampling have to be as constant as possible. Cell counts largely depend on the blood circulation, which means that blood samples should always be taken at the same time of the day. As the day progresses, an organism has to adapt to both environmental and individual conditions.

Recommendations [2] concerning the timing of sampling:

- If possible, samples should be taken between 7 am and 9 am.
- Sampling should be carried out 8 – 16 hours after the last meal (ideally 12 hours).
- Samples should be taken before interfering diagnostic and therapeutic procedures are performed.
- In drug monitoring, consider the peak after drug administration and the steady state phase before the next dose.
- Always document the exact time of sampling in the charts and requests.

How does the sample-taking procedure influence haematological results?

It is the purpose of each examination to obtain a sample that is representative for the patient's state, appropriate for the requested examination, and only minimally affected by the sample-taking procedure, additives, transport and storage conditions.

If a blood sample is poorly collected, the results may be inaccurate and misleading to the clinician, and the patient may have to undergo the inconvenience of repeat testing. The major issues resulting from errors in collection are haemolysis, contamination, inaccurate labelling and platelet (PLT) clumps.

Factors that increase the risk of haemolysis include:

- Use of a needle of a too small diameter (23 G or under)
- Pressing the syringe plunger too much in order to force the blood into a tube, thus increasing the shear force on the RBC
- Drawing blood specimens from an intravenous or central line
- Underfilling a tube so that the ratio of anticoagulant to blood is distorted
- Reusing tubes that have been refilled by hand with inappropriate amounts of anticoagulants
- Mixing a tube too vigorously
- Failing to let alcohol or disinfectant dry
- Using a too great vacuum, e. g. using a large tube for a paediatric patient, or using a too large syringe (10 – 20 mL) [12]

According to the tests that have to be performed, the sample type should be identified [12]:

- Venous blood for haematological routine check-ups and various therapeutic purposes
- Capillary blood (finger or heel pricks) for the blood analysis of neonates, infants and under certain circumstances for adults. Patients with: poor vein conditions, burns or scars at the body locations for venous blood sampling, high concentration of adipose, frequent blood tests, IV catheters in both arms or hands, point of care testing and those who are scheduled for intravenous medication or chemotherapy, are suitable for capillary blood testing.
- Arterial blood gases for patients on mechanical ventilation and to monitor blood oxygenation

What needs to be considered for blood sampling?

- The blood collection tube should not be used after its expiration date, in order to ensure that the specimen is suitable for clinical testing. In blood collection tubes that contain additive used after its expiration date, the vacuum may not draw the required volume to fill the tube and this will result in short-draw specimens [13].
- Draw blood collection tubes in the correct order to avoid cross-contamination of additives between tubes. For illustration purposes, Table 2 shows the revised, simplified recommended order of draw for vacuum tubes or syringe and needle [12].

Table 2 *Recommended order of draw for selected sampling tubes*

Order of use	Type of tube	Additive
1	Blood culture	Broth mixture
2	Non-additive	
3	Coagulation	Sodium citrate
4	Clot activator	Clot activator
5	Serum separator	None
6	Sodium heparin / Lithium heparin	Sodium heparin or lithium heparin
7	Plasma separating tube (PST)	Lithium heparin anticoagulant and a gel separator
8	EDTA	EDTA
9	Blood	Acid-citrate-dextrose (ACD, ACDA or ACDB)
10	Oxalate-fluoride	Sodium fluoride and potassium oxalate

Impact of blood collection tubes and additives

Blood collection tubes are not inert containers for blood but have several constituents, including anticoagulants that can potentially interfere with assays. The components used to manufacture the venous and capillary blood collection tubes and the collection technique used to fill these tubes are assumed not to contribute to the total error or otherwise degrade the performance of the assays for which the tubes are intended. To ensure there is no contribution to measurand interference, laboratories should review clinical literature and evaluation information of blood collection tube manufacturers.

In order to prevent blood from clotting, anticoagulants are used to obtain plasma and whole blood specimens. The most commonly used anticoagulants for whole blood analysis are ethylenediaminetetraacetic acid (EDTA), heparin and citrate. Anticoagulants added to specimens in appropriate concentrations to preserve certain measurands may cause problems with the assay of other measurands, frequently by interfering with binding or precipitation of the antigen – antibody complex.

EDTA

EDTA is a chelating agent that binds calcium and prevents clot generation. It is the anticoagulant of choice for haematology testing. Three different forms are available, of which K2-EDTA is the anticoagulant recommended by the International Council for Standardization in Haematology (ICSH). Owing to its chelating properties, EDTA can interfere with some assays. For instance, it can bind metallic ions, such as europium, or zinc and magnesium, which are cofactors for enzymes. Because of this, the blood to EDTA ratio is critical for optimal test results. EDTA in high concentrations can hypertonically shrink RBC and affect red blood cell size causing morphological changes.

Heparin

Heparin as anticoagulant is commonly used in chemistry and special chemistry testing. Heparin acts primarily through a complex that it forms with antithrombin III. Heparin may interfere with some antibody – antigen reaction. The use of heparin decreases the rate of reaction of some antibodies, particularly at the precipitation step in second-antibody systems [14]. Lithium heparin is the recommended form of heparin to be used because it is least likely to interfere when performing tests for other ions. Heparin is the only anticoagulant that should be used in a blood collection device for the determination of pH, blood gases, electrolytes, ionised calcium and cytogenetics. Heparin should not be used for coagulation or haematology testing.

Trisodium citrate

Trisodium citrate, buffered or unbuffered, is the standard anticoagulant for coagulation testing. Only liquid additives are currently available; this is to preserve the 9:1 ratio (blood:citrate) recommended for coagulation testing.

In some individuals, EDTA may cause inaccurate platelet results. These anomalies, platelet clumping and platelet satellitism, may be the result of changes in the membrane structure occurring when the calcium ion is removed by the chelating agent, allowing the binding of pre-formed antibodies. In this instance, sodium citrate tubes are sometimes collected to obtain more accurate platelet counts [2].

Does the filling of an EDTA tube affect the results?

Good laboratory practice includes that a correctly filled test tube is one of the requirements for precise analysis. Generally the tubes should be filled to the recommended volume. Insufficiently filled EDTA tubes may lead to incorrect mean corpuscular volume or mean cell volume (MCV), mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution width (RDW) and RBC results. They can also cause morphological changes in WBC and RBC. EDTA is hypertonic, resulting in the occurrence of echinocytes. An overfilled tube results in an excessively low EDTA concentration, which leads to clotting.

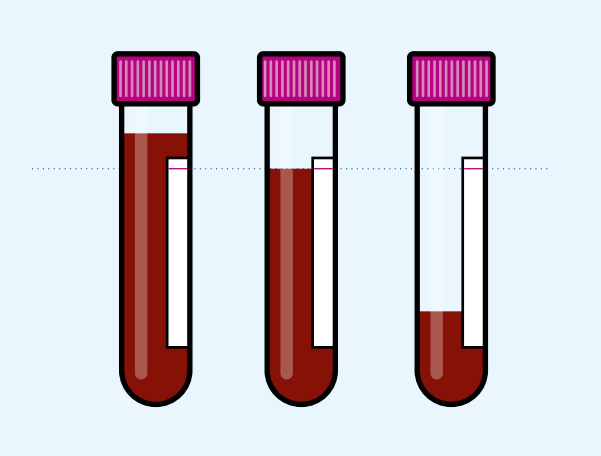

Fig. 1 *EDTA tubes from left to right: overfilled, correctly filled and underfilled.*

Transportation and storage

Obtaining a blood sample correctly plays an important role in the pre-analytical process.

Transport and storage of the test material sometimes tend to play a rather subordinate role in the pre-analytical process. The ICSH suggests [15] that the material for haematological tests should be refrigerated at 4 °C. In Table 3 the stability of the haematology parameters is shown:

Table 3 *Stability of haematology parameters in EDTA blood samples*

Parameter	Storage at 4 °C	Effect of delayed processing
HGB	Stable up to 72 h	
RBC count	Stable up to 72 h	
MCV	Stable up to 6 – 12 h	Tends to increase
HCT	Stable up to 6 – 12 h	Tends to increase
PLT	Stable up to 24 h	
Reticulocytes (RET)	Stable up to 72 h	
WBC	Stable up to 72 h	

The stability of the haematology parameters can vary depending on the test method, the technology or the reagents used by the analytical system. It is highly recommended to follow the manufacturer's instructions.

Specimens must be transported in the appropriate biohazard bags or containers, to the laboratory in as short a time as possible, at room temperature. Prompt removal of specimens from the collection area is especially important if the area temperature is above 22 °C, which may cause some constituents to deteriorate [16].

Summary

A summary of the factors that contribute to making analysis in a haematology laboratory more difficult:

- Errors in patient preparation
- Missing or incorrect patient identification
- Missing time and date of sample
- Too little test material
- Incorrect additives
- Incorrect proportion of the additives to the test material
- Unsuitable storage
- Material too old
- Freezing of whole blood
- Incorrect transport or storage temperature
- Insufficient mixing
- Unsuitable sample type
- Interference factors, such as haemolysis, icterus, lipaemia
- Inadequate communication between laboratory/ physicians/medical staff

Conclusion

The awareness of the pre-analytical variables and their effects on haematology testing is the first step in ensuring that results can become consistently accurate. By minimising the incidence of pre-analytical errors, patient care could be optimised, laboratory costs reduced and the relationship between physician and laboratory enhanced.

In part II of 'Pre-analytics: create the correct preconditions to achieve high-quality analysis results' we provide guidance on blood sampling and explain the accepted principles for drawing and collecting blood.

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Sysmex Europe GmbH
Bornbarch 1, 22848 Norderstedt, Germany · Phone +49 40 52726-0 · Fax +49 40 52726-100 · info@sysmex-europe.com · **www.sysmex-europe.com**
You will find your local Sysmex representative's address under www.sysmex-europe.com/contacts

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