Verification of the suitability of biological samples

The education, training and empowerment of all professionals involved in the collection, transport, analysis and storage of biological samples is essential to reduce errors in the pre-analytical phase, since the performance of unsuitable procedures can generate serious clinical, organizational and economic consequences.
Most errors in the laboratory arise from the extra-analytical phases of the diagnostic process (especially in the pre-analytical phase).

The verification of the suitability of biological samples is the activity and responsibility of the Laboratory Operators.

Laboratory Operators must verify the suitability of biological samples (SIBioC 2020 Guidelines Recommendations for the detection and management of unsuitable samples in laboratories):

- suitable material;
- suitable container;
- correctly identified/labeled sample;
- verification of correct storage of "frozen" samples on ice;
- verification of correct storage at controlled temperature for targeted requests such as "ammonium";
- verification of the correct filling level of the tube for haematology and coagulation tests;
- verification of suitability and correct filling level of the tubes related to the request for "quantiferon";
- verifies the presence of hemolyzed, coagulated, scarce samples.

Main causes of possible unsuitability of the samples in relation to the required examinations.

Biological sample management and reporting methods

From SIBioC guidelines "Recommendations for the detection and management of unsuitable specimens in clinical laboratories" clinical biochemistry, 2020, vol. 44, n. 1

Detectable mainly during the Acceptance activity

Identification errors
Absence of information that allows the indubitable and certain identification of the patient:
• Samples received with incomplete requests or no request;
• Unlabeled, illegible, incorrectly labeled samples.

Handling of unsuitable biological samples due to sample identification issues
If legitimate doubts persist about the correct identification, either on the basis of objective criteria (obvious identification errors, detected by laboratory personnel at the time of receipt of the sample) or probabilistic criteria (finding of obvious inconsistencies in the results of the sample with respect to the patient's clinic or unjustifiable deviations from previous values), the laboratory should request another sample.

Based on the indications of CAP and JCAHO by virtue of the possible clinical and medico-legal implications, procedures for relabeling samples by laboratory personnel, personnel of the operating unit of origin of the sample or third parties are strongly discouraged.
If it is impossible to obtain further samples, the entire report must be cancelled by replacing the results with a coded comment "Sample cannot be analyzed due to identification errors"

Exam request errors
• Examinations not congruent with the clinical question;
• Samples received without an accompanying form/consent duly completed when necessary (e.g. toxicology, virology (HIV) applications).

Transcription errors
• Request for incorrect exams (compilation error);
• Request with missing exams;
• Requests with redundant or unnecessary examinations.

Incomprehensible or incomplete requests
• Incomprehensible requests

Unsuitable sample due to inappropriate collection time and/or incorrect patient preparation
• Incorrect time of sampling for tests influenced by the circadian rhythm;
• Unsuitable preparation of the patient before sampling (e.g. not fasting, taking medication)

Mainly detectable during laboratory activity in the pre-analytical and analytical phase

Sample collected in the wrong container
• Incorrect matrix (e.g. serum/plasma);
• Incorrect test tube or container with respect to the required examination.

In the presence of non-compliant samples, the laboratory must request a second suitable sample; if this is not possible, it is recommended to replace the analyte value with a comment coded in the report "Determination not executable due to inadequate sample"

Transport and storage issues
Conditions of transport and storage of the sample do not comply with the defined criteria.

• Samples not received;
• Samples not properly stored prior to analysis;
• Samples damaged during transport;
• Samples transported at unsuitable temperatures;
• Samples with excessive shipping time.

Incorrect filling
• Insufficient sample volume;
• Incorrect sample/anticoagulant ratio

This circumstance/anomaly is of absolute importance for examinations in which a correct relationship between the concentration of anticoagulant and blood is essential, such as blood coagulation and blood count tests.
Inadequate filling of the tube alters the reliability of the results.
The recommended practice is not to perform the analysis when filling ±10% of the nominal volume of the primary tube.
If insufficient samples are present, the laboratory should request a second sample with a suitable volume; if this is not possible, it is recommended that the analyte value be replaced by a comment coded in the report "Analysis cannot be performed due to insufficient sample" or "Sample with inappropriate blood-to-anticoagulant volume ratio".

Clotted samples
Blood samples with anticoagulant with evidence of visible micro or macroclots in samples that should not contain them, therefore mainly intended for coagulation and hemocytometry tests (the suspicion may be generated by an incongruous value of platelets, associated with instrumental alarms and characteristic cytograms).
Detection of clots in samples for blood count and blood coagulation may be possible:
• by visual inspection of the sample (macroclots);
• using combinations of automatic clot detection techniques (e.g. presence sensors)
• with instrumental alarms (PLT Clumps or NRBC/PLT Clumps) that signal increased background noise and two-dimensional cytograms suggestive of the presence of platelet aggregates in the presence or absence of particularly low platelet count

The presence of clots in primary blood samples affects the result of blood counts and blood coagulation tests following the consumption of platelets, coagulation factors and incorporation of corpuscle elements of blood into clots.
With a few exceptions, most of the results of analyses performed on inappropriately coagulated serum or plasma samples are not clinically modified

In the presence of coagulated samples intended for blood coagulation and blood counts, the tests must not be performed and the laboratory operator must request a second sample.
If this is not possible, it is recommended to replace the analyte value with a comment coded in the report "Analysis not possible for coagulated sample".

Contamination
• Contaminated samples
Presence of unintended substances and/or materials such as infusion liquid (e.g.: saline or glucose), drugs and/or drugs, anticoagulants (EDTA, citrate, heparin), parenteral nutrition fluids.

Biohazard samples for operators
• Broken container/tube/slide;
• Container/tube visibly externally contaminated with blood or biological fluids;
• Accompanying documents contaminated with biological material;

Unsuitable samples due to the presence of interferents (hemolysis, lipemia, jaundice)

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In the laboratory, objective and standardized systems are adopted for the detection of NCs relating to unsuitable samples through the use of equipment and analytical modules capable of quantifying

automatically the indices of hemolysis, lipemia, hyperbilirubinemia, and the sample volume.

Hemolyzed sample
Visible hemolysis in the sample after centrifugation is defined by the presence of free hemoglobin concentrations in serum or plasma >0.30 g/L (18.8 mmol/L).
The Lab Operator assesses the nature of the interference and its influence on the analyses. Tests for which no interference is described can normally be performed and reported, while those for which interference is described should not be performed
In the report, the result of the analyte will be replaced by a coded comment due to the nature of the interferer.
(Determination not performed hemolyzed sample)
In the case of samples with an anomaly such as "mild hemolysis" such as not to block the execution of the analytical determination, but certainly interfere with the result of the same, the patient's final signed report will be described in the note of the N.C

Lipemic Champion
Samples with evident turbidity. Lipoemia in samples is defined in terms of turbidity caused by a high concentration of lipoproteins visible to the naked eye or quantifiable at 660/700 nm and usually corresponding to a concentration of triglycerides >1000 mg/dL (11.3 mmol/L) in whole blood samples and >300 mg/dL (>3.4 mmol/L) in centrifuged samples.
Presence of turbidity caused by a high concentration of lipoproteins visible to the naked eye or quantifiable at 660/700 nm and usually corresponding to a triglyceride concentration >1000 mg/dL (11.3 mmol/L) in whole blood samples and >300 mg/dL (>3.4 mmol/L) in centrifuged samples.
In the report, the result of the analyte will be replaced by a coded comment due to the nature of the interferer
(Determination not performed lipemic sample..)
In the case of samples with an anomaly such as "slightly opalescent" such as not to block the execution of the analytical determination, but certainly interfere with the result of the same, the patient's final signed report will be described in the note of the N.C.

Jaundiced sample
Bilirubin samples >2.5 mg/dL (42.7 µmol/L)
The definition of jaundice on visual inspection of the centrifuged sample alone may not be reliable; it is therefore recommended to use a photometric survey.
The Lab Operator assesses the nature of the interference and its influence on the analyses. Tests for which no interference is described can normally be performed and reported, while those for which interference is described should not be performed
In this case, timely communication of the non-compliance to the patient and/or attending physician is necessary.

Other considerations.
A considerable source of non-compliance of biological samples derives from biological variability and the treatment/transport of the sample to the laboratory that performs the determinations.
In vitro diagnostics must therefore take into account the variable of nutrition.
The introduction of food and its digestion determines the introduction of metabolic substrates in various forms (especially lipids) that can become interfering in clinical chemistry, haematology, coagulation and immunochemistry.
The definition of the fasting state before the analysis is carried out must therefore be clearly included in the laboratory's Quality Manual. The definition of food status is a good indicator of quality, as well as being an important requirement from a medico-legal point of view.

Due to the gradual decentralization of sampling points, the methods of storing and transporting biological samples to the laboratory represent a critical issue that personnel must be aware of.
A possible preliminary suggestion for users is that each laboratory, in relation to the analytical instrumentation, establishes for all parameters (especially haematological ones), the maximum time that can elapse from sample collection to analysis, also in relation to the storage temperature, and consequently reports only the parameters for which there is a guarantee of reliability

Management of non-compliant samples

All generic barcode or aliquot errors identified by the machine are taken care of individually by the operators in the pre-analytical sector and resolved on the day of arrival.
If the errors are unsolvable, an NC will be generated.

When preanalytical and laboratory operators find abnormalities in biological samples (NC relative to storage temperature, unsuitable material...), they do not perform the required analytical determination. They report the type of anomaly by sending a request for a new withdrawal to the secretariat email of the Withdrawal Point so that the customer can be contacted by telephone as soon as possible to agree on the execution of a new withdrawal.
In the case of N.C. with a request to send a new biological sample, it will be closed upon receipt of the new material for analysis even on the same day.

If the patient does not undergo a new sample within 7 days of the recall, the report will be closed, thanks to the verification of the "suspended" examinations, by the sector operator, with the missing examination reporting as a result a codified comment due to the nature of the interfering the wording "Determination not carried out cause..."
The final report of the examination will bear the note of the NC in writing.

Patient recall

The Operators of the operational sectors of the laboratory operate according to IdL 11 dedicated Rev.0 of 01/09/25 They communicate to the secretariat of the Sampling Point, via dedicated internal email, the need for a new sampling, indicating the patient's personal and identification data, the date of the sampling, the origin, the reason for the NC, and the type of sample needed.
The subsequent sampling carried out following the recall of the patient by the secretariat of the Sampling Point must be managed with a new dedicated acceptance ("recalls" agreement), and the relevant report will be attached to that of the first sample.
Biological samples must reach the Laboratory within 7 calendar days, from the time of the request, in order to be processed.
Missing analyzes or those that need to be re-performed will not be charged to the patient.