Guidelines
for Laboratory Accreditation
according to ELOT EN ISO 15189

ESYD G-CLINLAB

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INTRODUCTION

This Guideline refers to procedures required for Accreditation of Medical / Clinical Laboratories that apply, either approved standardized methods (CE IVD), which require verification by the Lab, or laboratory-developed tests (LDTs or else in-house methods), modified approved methods and Research Use Only (RUO) methods, which require analytical validation and clinical verification.
The verification of the applied methods for each parameter, under accreditation, consists of the evaluation of specific analytical characteristics, with regard to the suitability of the method for fitness of purpose. It is fully applicable for the initial evaluation and extension (addition of new methods or analyzers, or change of generations of existing methods or of analyzers) of the scope of accreditation, and reference is made to the requirements during the re-evaluation of the scope of accreditation. It refers to each of the tests under accreditation that is verified for its intended use on a specific analyzer, with a specific CE IVD standard method kit, and a specific substrate.

CONTENTS

1. TESTS PERFORMED ON AUTOMATED ANALYZERS
1.1 *Quantitative tests*
1.1.1 Verification
1.1.2 Estimation of uncertainty
1.1.3 Quality Assurance
1.2 *Qualitative methods*
1.2.1 Verification
1.2.2 Estimation of uncertainty
1.2.3 Quality Assurance

2. HEMATOLOGICAL AND HEMOSTASIS TESTS PERFORMED ON AUTOMATED ANALYZERS
2.1 *Tests*
2.1.1 Verification
2.1.2 Estimation of uncertainty
2.1.3 Quality Assurance
2.2 *Peripheral Blood Smear*
2.2.1 Verification
1.2.2 Quality Assurance

3. BLOOD TRANSFUSION SERVICE TESTS PERFORMED ON AUTOMATED ANALYZERS
3.1 *Qualitative methods*
3.1.1 Verification
3.1.2 Estimation of uncertainty
3.1.3 Quality Assurance

4. MICROBIOLOGICAL TESTS
4.1 *Quantitative tests*
4.1.1 Verification
4.1.2 Estimation of uncertainty
4.1.3 Quality Assurance
4.2 *Qualitative tests*
4.2.1 Verification
4.2.2 Quality Assurance

4.3 *Microscopy tests*
4.3.1 Verification
4.3.2 Estimation of uncertainty
4.3.3 Quality Assurance

5. MOLECULAR TESTS
5.1 Verification of qualitative CE IVD tests
5.2 Verification of quantitative CE IVD tests
5.3 Validation of laboratory-developed (or in - house) tests
5.4 Estimation of uncertainty
5.5 Quality Assurance
5.6 *Cytogenetic tests - Karyotype & FISH*
5.6.1 Verification
5.6.2 Estimation of uncertainty
5.6.3 Quality Assurance

6. FLOW CYTOMETRY TESTS
6.1 *Quantitative tests*
6.1.1 Verification
6.1.2 Estimation of uncertainty
6.1.3 Quality Assurance
6.2 *Qualitative tests*
6.2.1 Verification
6.2.2 Validation
6.2.3 Quality Assurance

7. CYTOLOGICAL TESTS
7.1 Verification
7.2 Estimation of uncertainty
7.3 Quality Assurance

8. PATHOLOGY TESTS
8.1 Histopathological diagnosis
8.2 Immunohistochemical tests
8.2.1 Validation / Verification
8.2.2 Estimation of uncertainty
8.3 Quality Assurance

9. TOPICS ON THE ACCREDITATION OF MEDICAL / CLINICAL LABORATORIES
9.1 Primary Sample Collection
9.2 Multisite Accreditation
9.3 Point of Care Testing (POCT)
9.4 Umbilical Cord Blood Banks
9.5 Accreditation under a Flexible Scope

10. REFERENCES

11. USEFUL WEBSITES

1. TESTS PERFORMED ON AUTOMATED ANALYZERS

1.1 Quantitative tests

This category includes examinations such as: biochemical, immunochemical, immunological, nephelometric, haematological, serological tests, protein electrophoresis, immunofixation of immunoglobulins, etc.

1.1.1 Verification

Verification is performed, either by using commercial control samples (control samples), or by using CRM (Certified Reference Materials), or in case of unavailability of the above, mixtures obtained by mixing patient samples (pooled samples), at two concentration levels at least, within and above the reference range (normal - abnormal) or within and below the reference range (normal - sub normal), according to the critical medical decision limits of the parameter measured (see the relevant note 2):

Trueness: is calculated by recovery experiments (mixing, on one hand, a calibrator (which is preferred depending on the case) or a quality control sample, with a routine sample of known concentration, on the other hand, and measuring the mixed sample at least six (6) times, within the same day. The % recovery (R%) and the % error (bias) are calculated respectively. It is supplemented and monitored by participation in proficiency testing schemes. An alternative method for calculating Trueness (exclusively for tests where mixing cannot be applied – see note 1) is to calculate the SD and CV% for the subtraction "our result - group mean" from a competent series of external quality control results (6-10 measurements) based on the reports of the organizer of the interlaboratory scheme. The indicator "100 - average Z-score of measurements" is an independent index of Trueness % of the method.

Repeatability: At least six (6) measurements of the same control sample, divided into aliquots or a pooled sample, divided into aliquots. Use at least 2 levels of quality control samples (or more if available) or pooled samples. In case pooled samples are used, they should be at the same concentration levels as the reproducibility controls. The CV_r% of the measurements is calculated.

Intermediate precision/intra-laboratory reproducibility: At least six (6) measurements of the same control sample under different conditions, e.g. on different days and whenever possible using at least two different lots of the analysers reagents. Use at least 2 levels of quality control samples (or more if available) or pooled samples, preferably at the same concentration levels as those used in the repeatability tests, to allow direct comparison between CV_r% and CV_R%. The results of the internal quality control charts (QC) may also be used. The CV_R% of the measurements is calculated.

Limit of Detection (LOD) and Limit of Quantification (LOQ), are calculated when they are critical for drawing conclusions from the parameter examined. The characteristics are calculated by a six times measurement of a diluted sample near the LOQ stated by the manufacturer, within the same day. Alternatively, the imprecision plots (SD versus concentration diagram) and the standard error of the intercept, can be used for the calculation.

1.1.2 Estimation of Uncertainty

The components of the uncertainty budget are estimated as:

Type A: By the standard deviation of at least six (6) measurements under conditions of laboratory reproducibility, at all concentration levels tested during verification and the standard deviation of six (6) measurements of each parameter of the recovery experiments or the subtraction differences from the external quality control comparisons (see Trueness).

Type B: Appraisal of sources of uncertainty for the calibrators used by the automated analyzer. In the case of a calibrator being reconstituted, the contribution of the uncertainty of the volumetric equipment used can also be estimated. It is also possible to take into account the uncertainty that in some cases is given by the organizers of external quality control schemes, especially for those tests that are not calibrated according to the manufacturer's instructions. Generally, all sources of type B uncertainty are recorded and advice is given in order to restrict their contribution to the final result (equipment, sampling, sample transportation, pre-analytical sample preparation etc.).

The combined uncertainty is calculated by the law of uncertainty propagation. To assess the suitability of the application of the method by the laboratory, the expanded uncertainty is compared with the values provided by the manufacturer of the device and / or the values mentioned in the literature.

For the overall assessment of the suitability of the method, beyond the expanded uncertainty, it is recommended to compare with the values given by the device manufacturer and/or with the values given by the literature, for all the parameters of the verification of paragraph 1.1.1.

NOTE 1: The mixing process and the process of calculation of recovery can not be applied in the cases of free forms of parameters, such as fT3, fT4. In order to calculate the accuracy of these parameters during verification, only analysis of control samples and the results of participation in interlaboratory comparison schemes (external quality control) are used (see alternative ways of calculating Trueness).

NOTE 2: In the case of using an Immunochemical or Turbidimetric method with a CE-IVD kit (e.g. serological antibody tests, infectious disease tests, antibody detection tests against the COVID-19 virus, etc.) where the analytical systems or methodologies used are based on numerical-quantitative data (e.g. photometric units or chemiluminescence) and a numerical decision limit (cut off) for the extraction of the result, paragraph 1.1 of this guideline is applied for the laboratory to carry out the verification of the method (Analytical verification). The following paragraph 1.2 of this guideline for Qualitative methods applies ONLY in the case where numerical-quantitative data is not traceable. In any case, it is required in the tests of repeatability/reproducibility and trueness that a level close to the numerical decision limit (cut off) has been used. Assays with a level close to the numerical decision limit (cut off) are proposed to be used to replace the negative levels of quality controls of the tests mentioned in this note, which, due to the analytical performance of the sample, give unacceptable verification results.

NOTE 3: If the laboratory wishes to include in its Scope of Accreditation, additional matrices for the same test on the same analyzer, in tests where applicable, then the above verification and uncertainty calculation tests are required to be performed on all substrates (provided they are proposed for use in the insert of the manufacturer).

NOTE 4: During re-evaluation of the scope, the laboratory should at least re-estimate the uncertainty by readjusting the reproducibility with recent data, and where possible by trueness experiments with recovery testing (trueness is a component of the uncertainty). Upon uncertainty calculation the metrological traceability data should be updated, taking into account current calibrations and updated manufacturer's data for calibrators.

1.1.3 Quality Assurance

Internal Quality Control

Internal quality control is always performed for all tests and is based on the manufacturer's instructions, methodology, biological and analytical variation. The laboratory should select IQC material that is fit for its intended purpose. The internal quality control procedure should allow detection of either reagent lot to lot, or calibrator variation, or both, of the test method. Use of third-party (neutral) IQC material should be considered.

External Quality Control

Annual participation in appropriate proficiency testing schemes (PTs) is required for all tests included in the laboratory accreditation scope, with a frequency that will be defined taking into account the volume of laboratory work. The external quality control programs selected should, as far as possible, be accredited according to ISO/IEC 17043. When selecting programs, the laboratory should take into account the type of target value offered. When a program is either not available or not considered suitable, the laboratory should use alternative methodologies to monitor the performance of the test method (e.g., interlaboratory comparison schemes involving at least three laboratories where possible). In the evaluation of external quality control, the laboratories should take into account the specifications of the reagent for intended use.

For clinical tests applied to matrices apart from serum or peripheral blood (e.g. urine), at least one participation in a suitable interlaboratory proficiency scheme is required for those matrices, within the four-year or five-year accreditation cycle.

In case of calculated parameters participation in a PT scheme is not required provided that there is participation for the parameters used for the calculation, having set an acceptable z–score, ranging from -2 to 2 and within the range of concentrations that the relative equation applies. For calculated parameters, however, a declaration of suitability is required after application of the formula in all stages of the verification (repeatability, reproducibility and trueness) wherever they apply, with the results of the verification of the individual tests.

1.2 Qualitative methods

This category includes methods with a qualitative result, e.g. detectable or undetectable, positive or negative.

1.2.1 Verification

Verification of qualitative methods is performed, either by using commercial control samples, or by using Certified Reference Materials (CRM), or in case of unavailability of the above, mixtures obtained by mixing patient samples (pooled samples), at

different concentration levels, according to the critical medical decision limits of the parameter measured (see the relevant note 2, § 1.1.2).

The verification is achieved by determining the following relevant performance characteristics:

Trueness: At least six (6) negative and six (6) positive samples with a known value should be analysed (if positive and negative samples are not available, then for each category of samples that are available). Trueness is calculated from the ratio [true positive + true negative] / [total number of samples]. In case the kit contains both a negative and a positive control sample, these could be analyzed in duplicate for six (6) days. In case of semi-quantitative methods with a qualitative final expression, samples with a known value should be analysed (at different concentration levels) and the % error (bias) or the % recovery should be determined.

Repeatability: At least six (6) measurements of the same control sample (positive and negative), divided into aliquots or a pooled sample (positive and negative), divided into aliquots. Use at least 2 levels of quality control samples (or more if available) or pooled samples.

Intermediate precision/intra-laboratory reproducibility: For purely qualitative tests, analysis of positive and negative samples by different analysts during six (6) different days is required. In the case of semi-quantitative methods with an ultimate qualitative result, analysis of samples (known or unknown) between different dates is required as well as calculation of the coefficient of variation (CV%) of the measurements. Measurements have to be made at concentration levels with clinical significance. Use at least 2 levels of quality control samples (or more if available) or pooled samples, as far as possible the same as those used in the repeatability tests. Trueness experiments can also be used, only in the case of duplicate analysis for six (6) days and for at least 2 concentration levels.

Limit of detection (LOD): For purely qualitative tests, serial dilutions of a known standard sample is required, measurement of every dilution, and determination of the concentration level above which the sample is reliably classified as positive (through repeated measurements). In case of semi-quantitative methods with an ultimate qualitative result, analysis of standards or low concentration positive samples is required, as well as calculation of the standard deviation (SD) of multiple measurements (e.g. 6). The detection limit is 3.3 times the SD. It may, also, be necessary to determine the Cut Off Value (COV) and the area around it for which the outcome is uncertain (Unreliability Region, from the level with False Positive Rate to level with False Negative Rate).

1.2.2 Estimation of Uncertainty

Qualitative tests belong to the category of tests, for which strict metrological and statistical calculations of uncertainty cannot be implemented. For semi-quantitative methods which result in a qualitative output, the estimation of uncertainty is required (as in § 1.1.2).

1.2.3 Quality Assurance

Internal Quality Control

Internal quality control for the qualitative tests is performed by analyzing positive and negative control samples (controls), before analyzing patient samples. Moreover, in

accordance with the quality standards used (e.g. CLSI), these procedures should, where appropriate, be incorporated into the daily laboratory workflow. (See also requirements of §1.1.3 where applicable).

External Quality Control

The requirements of § 1.1.3 apply.

NOTE: In the case of accrediting more than one automated analyzers to perform the same tests, documentation of equivalence of results is required, preferably with analysis of real samples in a wide range of concentrations or alternatively if it is impossible to collect the above, quality control samples, and their statistical elaboration (t-test and correlation). Also, the analysis of proficiency testing scheme samples could demonstrate the equivalence of analyzers.

2. HEMATOLOGICAL AND HEMOSTASIS TESTS PERFORMED ON AUTOMATED ANALYZERS

2.1 Quantitative tests

This category includes the examinations:

Full or Complete Blood Count (FBC or CBC) (e.g. WBC, RBC, Hb, HCT, MCV, MCH, MCHC, PLTs, RDW, MPV and White Blood Cell Differential Count with # (absolute number) of Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils)

and

Hemostasis tests (in sec, % and measurement units).

2.1.1 Verification

Verification is performed, either by using commercial control samples (control samples), or by using CRM (Certified Reference Materials), or in case of unavailability of the above, mixtures obtained by mixing patient samples (pooled samples), at two concentration levels at least, within and above the reference range (normal - abnormal) or within and below the reference range (normal - sub normal), according to the critical medical decision limits of the parameter measured.

Trueness: is calculated by recovery experiments (mixing, on one hand, a calibrator (which is preferred depending on the case) or a quality control sample, with a routine sample of known concentration, on the other hand, and measuring the mixed sample at least six (6) times, within the same day. The % recovery (R%) and the % error (bias) are calculated respectively. It is supplemented and monitored by participation in proficiency testing schemes.

An alternative method for calculating Trueness (exclusively for tests where mixing cannot be applied – see note 1) is to calculate the SD and CV% for the subtraction "our result - group mean" from a competent series of external quality control results (6-10 measurements) based on the reports of the organizer of the interlaboratory scheme. The indicator "100 - average Z-score of measurements" is an independent index of Trueness % of the method.

A second alternative method for calculating Trueness is by using the control samples with six (6) measurements within the same day. The results will be checked against the target value of the control sample (%Trueness = $\frac{C}{\mu} x100$ where C= the value of the parameter for each measurement of the control sample, and μ= the target value of the control sample as defined by the manufacturer). Trueness will be assessed on at least 2 levels.

Repeatability: At least six (6) measurements of the same control sample, divided into aliquots or a pooled sample, divided into aliquots. Use at least 2 levels of quality control samples (or more if available) or pooled samples. In case pooled samples are used, they should be at the same concentration levels as the reproducibility controls. The CV_r% of the measurements is calculated.

Intermediate precision/intra-laboratory reproducibility: At least six (6) measurements of the same control sample under different conditions, e.g. on different days and whenever possible using at least two different lots of the analysers reagents. Use at least 2 levels of quality control samples (or more if available) or pooled samples, preferably at the same concentration levels as those used in the repeatability tests, to allow direct comparison between CV_r% and CV_R%. The results of the internal quality control charts (QC) may also be used. The CV_R% of the measurements is calculated.

NOTE: During re-evaluation of the scope, the laboratory should at least re-estimate the uncertainty by readjusting the reproducibility with recent data, and where possible by trueness experiments with recovery testing (trueness is a component of the uncertainty). Upon uncertainty calculation the metrological traceability data should be updated, taking into account current calibrations and updated manufacturer's data for calibrators.

2.1.2 Estimation of Uncertainty

The components of the uncertainty budget are estimated as:

Type A: By the standard deviation of at least six (6) measurements under conditions of laboratory reproducibility, at all concentration levels tested during verification and the standard deviation of six (6) measurements of each parameter of the recovery experiments or the subtraction differences from the external quality control comparisons (see Trueness).

Type B: Appraisal of sources of uncertainty for the calibrators used by the automated analyzer. In the case of a calibrator being reconstituted, the contribution of the uncertainty of the volumetric equipment used can also be estimated. It is also possible to take into account the uncertainty that in some cases is given by the organizers of external quality control schemes, especially for those tests that are not calibrated according to the manufacturer's instructions. Generally, all sources of type B uncertainty are recorded and advice is given in order to restrict their contribution to the final result (equipment, sampling, sample transportation, pre-analytical sample preparation etc.).

The combined uncertainty is calculated by the law of uncertainty propagation. To assess the suitability of the application of the method by the laboratory, the expanded uncertainty is compared with the values provided by the manufacturer of the device and / or the values mentioned in the literature.

For the overall assessment of the suitability of the method, beyond the expanded uncertainty, it is recommended to compare with the values given by the device manufacturer and/or with the values given by the literature, for all the parameters of the verification of paragraph 2.1.1.

2.1.3 Quality Assurance

Internal Quality Control

Internal quality control is always performed for all tests and is based on the manufacturer's instructions, methodology, biological and analytical variation. The laboratory should select IQC material that is fit for its intended purpose. The internal quality control procedure should allow detection of either reagent lot to lot, or calibrator variation, or both, of the test method. Use of third-party (neutral) IQC material should be considered.

External Quality Control

Annual participation in appropriate proficiency testing schemes (PTs) is required for all tests included in the laboratory accreditation scope, with a frequency that will be defined taking into account the volume of laboratory work. The external quality control programs selected should, as far as possible, be accredited according to ISO/IEC 17043. When selecting programs, the laboratory should take into account the type of target value offered. When a program is either not available or not considered suitable, the laboratory should use alternative methodologies to monitor the performance of the test method (e.g., interlaboratory comparison schemes involving at least three laboratories where possible). In the evaluation of external quality control, the laboratories should take into account the specifications of the reagent for intended use.

In case of calculated parameters (e.g. Hct, INR etc.) participation in a PT scheme is not required provided that there is participation for the parameters used for the calculation, having set an acceptable z–score, ranging from -2 to 2 and within the range of concentrations that the relative equation applies. For calculated parameters, however, a declaration of suitability is required after application of the formula in all stages of the verification (repeatability, reproducibility and trueness) wherever they apply, with the results of the verification of the individual tests.

2.2 Peripheral Blood Smear

a. White Blood Cell Manual Differential Count % percentage and absolute number: Neutrophils, Lymphocytes, Monocytes, Eosinophils, and Basophils

b. Morphology of red blood cells, white blood cells, and platelets

2.2.1 Verification

The verification of the above tests is performed by determining the diagnostic reproducibility-repeatability.

Diagnostic repeatability: Each diagnostician (microscopist) microscopizes 3 smears (1 normal and 2 pathological). Each smear is examined at least twice by each diagnostician. The White Blood Cell Differential Count is calculated and the existing

alterations of the 3 hematopoietic series (White Blood Cells, Red Blood Cells, Platelets) are noted.

Regarding the White Blood Cell Manual Differential Count (absolute number or %) calculations should be made for Mean, SD and CV%.

Morphological changes are assessed by participating in an interlaboratory scheme (external quality control).

2.2.2 Quality Assurance

Internal Quality Control

Internal quality control (2 or 3 levels) is always performed for the parameters tested on patient samples and is based on the manufacturer's instructions, methodology, biological and analytical variation.

Retrospective re-microscopy should be performed every two months (for all diagnosticians who belong to the laboratory staff: 2 smears, 1 normal and 1 pathological).

External Quality Control

Annual participation in appropriate proficiency testing schemes (PTs) is required for all tests included in the laboratory accreditation scope, with a frequency that will be defined taking into account the number of diagnosticians and the daily workload of the laboratory. The participation of all diagnosticians is required, and each diagnostician must participate at least once per year in an external quality control.

3. BLOOD TRANSFUSION SERVICE TESTS PERFORMED ON AUTOMATED ANALYZERS

3.1 Qualitative tests

Before the first use of any new analyzer / new method / new examination, the Blood Transfusion Unit proceeds with an evaluation of the performance characteristics of the method and verification that the relevant specifications, as determined by their manufacturer, are met.

In addition to the initial confirmation of the correct application of the method, a re-evaluation of the Laboratory's competence to correctly perform the specific methods is carried out at least once per year. The annual review of the Laboratory's competence is carried out through the analysis and systematic evaluation of all the data from the internal and external quality controls.

3.1.1 Verification

Verification of the qualitative methods is performed, either by using commercial control samples (control samples), or by using CRM (Certified Reference Materials), or in case of unavailability of the above, by using known samples.

The verification is achieved by determining the following relevant performance characteristics:

Trueness: Trueness is checked by using commercial control samples or known samples / reference materials and from the results of participation in external quality control schemes. It is always performed for the parameters tested on patient samples and is based on the manufacturer's instructions and the methodology.

In particular, to check the trueness of the analytical methods for determining blood groups ABO & RHESUS D, the analysis of at least four (4) samples is required for the typing of blood groups of the ABO / RhD system for all categories, reverse groups, phenotypes of the Rh system (C, c, E,e), Kell with control samples compatible with all methods (gel, microbeads, microplates). In addition, the analysis of 6 known samples for the indirect Coombs test (3 positive / 3 negative samples), and 6 known samples for the direct Coombs test (3 positive / 3 negative samples) is performed, as well as the detection and identification test for alloantibodies.

Within day repeatability: At least six (6) measurements of the same control sample for all categories of tests. Specifically, for:

- Determination of ABO and RhD blood group combined with reverse grouping
- Indirect Coombs test (positive and negative sample)
- Direct Coombs test (positive and negative sample)
- Crossmatching test with the Indirect Coombs test (IAT) and enzyme (compatible and incompatible)

A minimum of 6 replicates are performed on the same control sample, using the same reagents, the same analyzer, and the same personnel.

It is recommended that for the repeatability and reproducibility tests (see next paragraph) the participation of all personnel (by name) involved in the performance of the tests is documented as far as possible.

Intra-laboratory reproducibility: Analysis of control samples for all test categories is required (ABO/Rh blood group typing with different combinations, reverse groups, Rh system phenotypes, indirect Coombs (positive/negative sample), direct Coombs (positive/negative sample), and compatibility test, by different analysts in six (6) different days and, if possible, using at least two different lots of reagents of the analyzer.

Limit of Detection (LOD): Determination of the Limit of Detection is only performed for tests where this is critical for drawing conclusions from the parameter being tested. Assessment of the detection limit is carried out by examining a known standard sample / in house quality control to which a series of dilutions is applied in a specific ratio with the case-by-case suitable diluent as determined by the manufacturing company. The test is repeated at each dilution and the level above which the sample is classified as positive is estimated.

In the Blood Transfusion Service, antibody titration is applied mainly in the following situations:

- Pregnancy/Hemolytic disease of the fetus and newborn (HDFN)
- ABO incompatible kidney transplantation
- Stem cell transplantation

3.1.2 Estimation of Uncertainty

Qualitative tests belong to the category of tests, for which strict metrological and statistical calculations of uncertainty cannot be implemented.

3.1.3 Quality Assurance

Internal Quality Control

The internal quality control of the analyzers of the immunohematology laboratory of the Blood Transfusion Service is carried out using commercial control samples. It is always performed for parameters tested on patient samples and must be performed prior to analysis of patient samples. Specifically, for the Blood Transfusion Service laboratory analyzers, the control samples should ensure control for the typing of ABO/Rh blood groups with different combinations, reverse groups, Rh system phenotypes, indirect Coombs (positive/negative sample), direct Coombs (positive/ negative sample), detection and identification of anti-erythrocyte antibodies at low titers, and the compatibility test.

NOTE: In case of using more than one analyzer to perform the same tests, documentation of equivalence / comparability of results is required, preferably by analyzing real samples in a wide range of concentrations. Also, analysis of the interlaboratory scheme (external quality control) samples could document the equivalence of the analyzers.

External Quality Control

Annual participation in appropriate proficiency testing schemes (PTs) is required for all tests included in the laboratory accreditation scope. The organization that will carry out the external quality control must be independent, accredited to carry out external quality assessment schemes according to the requirements of the EN ISO / IEC 17043 standard. The company should offer a complete set of external quality control programs, provide scientific interpretation in case of deviation of results and the samples should be compatible with the analyzers and the methods. In case of unavailability of external quality control schemes, the laboratory must use alternative methodologies to monitor the performance of the test method (e.g. interlaboratory comparison schemes).

External Quality Control schemes for the Blood Transfusion Service include:

- ABO grouping (forward and reverse group)
- D typing and Rh (Cc,Ee) and K phenotyping
- Indirect Antiglobulin Test (IAT) / Screening Test
- Direct Antiglobulin Test (DAT)
- Compatibility Test (Crossmatching)
- Antibody identification by performing an antibody panel using phenotyped erythrocytes and enzyme treated erythrocytes
- Extended red cell phenotyping (ERP) for MN, Ss, Kk, Fya, Fyb, Jka, jkb

NOTE: In the event that any of the above methods are also performed by a manual method, in addition to the automatic analyzer, and based on the manufacturer's instructions, it is required that all verification tests mentioned above are performed separately, and that internal and external quality control is documented for the manual methods.

4. MICROBIOLOGICAL TESTS

The validation / verification of microbiological tests must be performed on one or more representative microorganisms for each group determined by the specific test and for each matrix on which the specific test is performed. In addition, it must include all stages of testing using naturally or artificially contaminated biological materials.

The methods used should be appropriate for the tests carried out in the laboratory, which are indicated by the manufacturers of analyzers and / or based on methods established by international scientific organizations, according to the requirements of clinical evaluation (community patients, patients in the level of a secondary, tertiary, quaternary healthcare setting, etc.).

Beyond the initial confirmation of the correct application of the method, re-evaluation of the efficiency of laboratories to perform specific methods correctly is performed at least once per year. The annual review of the Laboratory's efficiency is conducted through analysis and systematic evaluation of all data from internal and external quality schemes.

During re-evaluation of the scope, it is proposed to repeat all the tests required in the initial verification of the correct application of the method.

4.1 Quantitative tests

This category includes tests that are either performed or not performed on automated analyzers, such as quantitative urine culture, etc.

4.1.1 Verification

Trueness: Trueness is assessed by using CRM reference materials (Certified Reference Materials / standard strains) and by results from the participation in external quality control schemes or alternatively in a scheme of interlaboratory comparisons (such as § 2.2.2).

Repeatability: In order to check repeatability at least six (6) measurements per organism and per matrix, are required. The standard deviation and coefficient of variation (% CV_r) of measurements is estimated.

Intermediate precision / reproducibility:

First method: Use of two or three levels of inoculated samples (spiked samples). At least six (6) measurements are made for each sample if possible, at different dates, with different operators or different reagents.

Second method: From quality control charts over a period of one year.

Third method: From factorial experimental design of multiple measurements of the same sample in different experimental conditions. One - way ANOVA application may be used for calculating the SD_R.

The precision of a method is satisfactory, if the relative standard deviation (% CV) is less than 10%.

Limit of detection (LOD): The detection limit is determined by examining artificially contaminated samples, which are the product of successive decimal dilutions of the tested microorganism. The initial number of cfu should be known. The samples analyzed are at least six (6), within the same day. The lowest number of microorganisms that can be detected is the detection limit of the method.

4.1.2 Estimation of Uncertainty

The estimation of uncertainty is based on reproducibility data and on elements of systematic errors (bias), as determined by the results of proficiency testing schemes. For estimation of the uncertainty, examination of at least six (6) samples is required.

This parameter should be addressed with a precise protocol for sample size and processing (suitable / non-suitable sample): Analysis of six (6) different samples (i) (artificially contaminated) at various levels, in both conditions of reproducibility A and B (e.g. two sample manipulations, different lots of reagents, etc.), measurement of the number of microorganisms / cfu for XiA and XiB, and calculation of expanded uncertainty.

NOTE: In the case of quantitative microbiological tests performed on automatic analyzers (e.g. biochemical parameters of biological fluids, such as urine and cerebrospinal fluid) the guidelines of §1.1.1 and §1.1.2 are followed where applicable.

4.1.3 Quality Assurance

Internal Quality Control

The laboratory should select IQC material that is fit for its intended purpose. In the case of quantitative urine culture, IQC is performed using CRM reference materials (Certified Reference Materials / standard strains).

External Quality Control

Annual participation in appropriate proficiency testing schemes (PTs) is required for all tests included in the laboratory accreditation scope. The external quality control programs selected should, as far as possible, be accredited according to ISO/IEC 17043. In the absence of the above, the laboratory may use alternative methodologies to monitor test method performance (e.g. interlaboratory comparison schemes).

4.2. Qualitative tests

This category includes methods that are either performed or not performed on automatic analyzers, such as blood culture, identification of microorganisms, antimicrobial susceptibility testing for microorganisms, antibody/antigen testing for pathogenic microorganisms, with a qualitative result e.g. detectable or undetectable, positive or negative, susceptible, resistant or other category (e.g. susceptible, increased exposure to antibiotic / antifungal agent).

4.2.1 Verification

The verification of qualitative methods is performed using CRM reference materials (Certified Reference Materials / standard strains) as specified by the method. It is possible to check the repeatability / reproducibility of the annually received external quality control samples of known result. For rare microorganisms, repeatability / reproducibility testing may include strains collected over a five-year period.

Trueness: It is assessed through the Laboratory's participation in external quality control / inter-laboratory schemes.

In a multiparametric methodology, such as the identification of microorganisms, the control should extend to a sufficient number, at least 20, standard strains and/or known strains from external quality control schemes.

Repeatability / Intermediate precision / laboratory reproducibility: Analysis of at least six (6) positive and negative samples per microorganism and matrix is required from the laboratory analysers. It is required to check the repeatability by measuring control samples three (3) times, according to the requirements of the respective methodology, and the reproducibility by measuring control samples six (6) times under different conditions. The number of samples to be analyzed depends on the requirements of clinical evaluation (community patients, patients in the level of a secondary, tertiary, quaternary healthcare setting, etc.).

Limit of detection (LOD): The detection limit of the method, if documented in the validation studies of the method or the relevant literature, is determined by examining artificially contaminated samples, which are the product of successive decimal dilutions of the microorganism examined. The samples analysed by the laboratory analysers are at least six (6), under conditions of intra-laboratory reproducibility. The lowest number of microorganisms that can be detected is the detection limit of the method. The number of samples to be analysed depends on the microorganism and the matrices, according to the requirements of clinical evaluation (community patients, patients in the level of a secondary, tertiary, quaternary healthcare setting, etc.). In the case of semi-quantitative methods with a qualitative final expression, the confirmation of the Limit of Detection is required, which is performed by analyzing six (6) samples (reference material or control provided by the manufacturer) diluted in the region of the already known Limit of Detection (up to +20% LOD).

Sensitivity and Specificity: It is possible to calculate sensitivity from the ratio: correct positive results / total positive results, and specificity from the ratio: correct negative results / total negative results, if not derived from validation studies.

4.2.2 Quality Assurance

Internal Quality Control

The laboratory should select IQC material, e.g. CRM Reference Materials (Certified Reference Materials / standard strains) as determined by the method. It may include strains from external quality control samples of known result. The type and number of tested strains is determined according to the method as derived from the relevant literature. The IQC frequency is determined according to the type of examination, the frequency of test performance and the daily program of the Laboratory.

External Quality Control

Participation in an appropriate proficiency testing scheme (PT) is required at least annually or more frequently, in accordance with the program of the provider of the proficiency testing scheme and for all tests, or in the case of absence of an approved interlaboratory program, participation is required in an interlaboratory comparison scheme, whose provider complies with specific requirements (ESYD PDI) and is evaluated by participants on the basis of these requirements.

NOTE 1: Serological tests include methods for antibody testing, antigen testing, biomarker testing, etc. They may be quantitative (see § 4.1), semi-quantitative or qualitative (see § 4.2).

NOTE 2: In the case of using more than one analyzers to perform the same tests, documentation of equivalence of results is required by analyzing preferably real samples or, alternatively, if it is not possible to collect the aforementioned samples, by analyzing quality control samples, and performing statistical processing of the results (t-test and correlation). In addition, analysis of the interlaboratory scheme (external quality control) samples could document the equivalence of the analyzers. In the case of incorporating a new analyzer or a new methodology to the scope of accreditation, documentation of comparability of results is required. It is recommended to analyze a large number (≥ 100) of unselected samples.

NOTE 3: In the case of antimicrobial susceptibility testing, the study of analyzers equivalence / method comparability should include ≥ 35 samples of known resistance. Depending on the case, it is suggested to seek for precision essential agreement (PEA) or precision categorical agreement (PCA).

NOTE 4: Urinalysis includes methods and techniques, such as analysis of biochemical parameters of urine performed on an automated analyzer, dipstick urine test strips (automated or non-automated) for physical and chemical parameters, measurement of urine formed elements by an automated analyzer, urine digital microscopy, light microscopy of urine sediment. Analysis of biochemical parameters of urine are quantitative tests (see §4.1). Urine dip test strips (automated or non-automated) for physical and chemical parameters are qualitative tests (see §4.2). The measurement of urine formed elements with an automatic analyzer can give quantitative results or results with a qualitative expression (see §4.1, §4.2). Urine digital microscopy gives semi-quantitative results with a qualitative final expression (see Note 5). Light microscopy of urine sediment gives semi-quantitative results with a qualitative final expression (see § 4.1, § 4.2).

NOTE 5: Digital urine microscopy can only be checked for trueness, which is assessed via the Laboratory's participation in an external quality control scheme offering digital visual field images.

NOTE 6: Measurement of urine formed elements with an automated analyzer provides acceptable accuracy of results for negative samples. It is considered appropriate that specimens with pathological findings be tested by another method of the accreditation scope, such as light microscopy (reference method). Comparability of results between methods is suggested to be documented by analysis of real, unselected, negative and pathological samples, or alternatively by analysis of interlaboratory control samples.

NOTE 7: For the quality control of all of the parameters, if possible, included in the accreditation scope, that are examined in urinalysis, it is suggested that both internal and external quality control includes samples that can be processed according to the

laboratory techniques listed in the scope. Alternatively, for microscopy, digital visual field images from external quality control schemes can be used.

NOTE 8: In the internal quality control of urinalysis, it is suggested that for real, unselected samples, matching of the physical and chemical parameters with the presence of urine formed elements to be performed, in order to check the identity of the respective sample.

4.3 Microscopy tests

This category includes tests, such as the direct microscopic observation of matrices either or not concentrated (e.g. urine, semen) and the observation of fixed preparations, from matrices or cultures of microbes, stained with Gram stain, acid-fast stain, etc.

4.3.1 Verification

The verification of the microbiological microscopic tests is done by determining the diagnostic reproducibility - repeatability.

Diagnostic reproducibility - repeatability: It is assessed by random retesting of a representative percentage of samples, commensurate with laboratory workload, by the same or different diagnosticians (microscopists), and measurement of inter-observative and intra-observative agreement (see NOTE 1, NOTE 2).

4.3.2 Estimation of Uncertainty

Microbiological microscopic tests belong to the category of tests, for which strict metrological and statistical calculations of uncertainty cannot be implemented.

4.3.3 Quality Assurance

Internal Quality Control

Internal quality control in direct microscopic observation of matrices is performed by checking a representative percentage of samples at regular intervals by the same or different diagnosticians (microscopists).

Internal quality control in the observation of stained preparations is performed on smears from different matrices or microbial colonies, pure or mixed, recovered from the internal quality control of cultures / antibiograms. As a measure of internal quality control, it is suggested that random retesting, in which diagnosticians (microscopists) review a representative percentage of samples, commensurate with the laboratory workload (measurement of inter-observative and intra-observative agreement), as well as comparing microscopy results of stained preparations with the corresponding identification results by the automatic analyzer (calculation e.g. percent agreement), to be done.

NOTE: In the case of internal quality control of stained indirect immunofluorescence preparations, the control is performed on the same matrix on the day of performing the test, using commercial positive and negative control samples or samples with known results. In cases where a significant degree of substrate autofluorescence can be observed, wells without sample or control sample may be used for this purpose alongside the samples and controls.

External Quality Control

The participation of the Laboratory in appropriate proficiency testing schemes (PTs) should be at least annual, ensuring as far as possible the participation of all diagnosticians (microscopists).

NOTE 1: In the direct microscopic observation of matrices, the measurement of inter-observative agreement can be done by blind testing the same individual or pooled samples with pathological findings, at least two (2) at a time, by the diagnosticians (microscopists), at regular time intervals, depending on the workload of the laboratory. From time to time, it is suggested to additionally test one (1) sample without pathological findings. The samples are examined under the same light microscope each time. The measurement of intra-observative agreement can be done by blind testing at least two (2) individual or pooled samples with pathological findings by each diagnostician (microscopist). It is suggested to additionally test one (1) sample without pathological findings. Each sample is examined three (3) times under the same light microscope.

NOTE 2: In the observation of stained preparations, the measurement of inter-observative agreement is done by blind testing the same preparations, at least two (2) at a time, e.g. one (1) with Gram-positive bacteria and one (1) with Gram-negative bacteria, by the diagnosticians (microscopists), at regular time intervals, depending on the requirements and workload of the laboratory. Intra-observative agreement measurement is performed by blind testing three (3) preparations from different matrices or microbial colonies, pure or mixed, by each diagnostician (microscopist). Each preparation is examined three (3) times.

5. MOLECULAR TESTS

5.1 Verification of CE IVD qualitative tests

Trueness: Assessment of trueness is achieved by using certified reference materials (CRMs), or diagnosed samples from EQA schemes with results from interlaboratory schemes (EQA samples).

In multiparametric methodologies where more than one microorganisms / genes / mutations / polymorphisms are detected, it is not always easy to control all of them (e.g. 32 mutations in CFTR), especially if they are many. However, the laboratory must obtain a satisfactory panel of control samples with e.g. at least the most frequent mutations / polymorphisms detected in the Greek population in order to verify the trueness of the kit used.

Repeatability/Reproducibility: In qualitative molecular methods, it is required for repeatability to be assessed with ten (10) measurements of control samples (CRMs or EQA samples) at a level of positivity (with clinical value in the diagnosis) in the same experimental run, and for reproducibility with twenty (20) measurements of control samples under different conditions (different runs on different days).

In genetic sequencing tests (NGS, Sanger, MLPA, FA, WGS, WES, CES, ddPCR) it is required to check the repeatability with three (3) measurements of two (2) control samples (CRMs or EQA samples) in the same experimental run and the

reproducibility with four (4) measurements of three (3) control samples under different conditions (different runs on different days).

Appropriate statistical analysis of these experiments should follow. Alternatively, measurements for internal quality control (e.g. Levey Jennings Charts) can be used to calculate reproducibility.

Limit of Detection (LOD): In the case of determination of parameters with clinical significance, confirming the Limit of Detection at a 95% confidence level is required and is achieved by analysing at least five (5) control samples (CRMs or EQA samples) diluted close to LOD.

Compatibility of reagents: The reagents used for Real-time PCR, NGS, genetic analyzers or other analytical equipment should be compatible with the respective equipment on which the test to be accredited is performed. Otherwise, the validation procedure is followed as for in house tests (§5.3).

The above applies to all CE IVD qualitative tests performed on automated molecular point-of-care analyzers.

5.2 Verification of CE IVD quantitative tests

In addition to the requirements of § 5.1, linearity of the calibration curve should be checked three (3) times, by using at least 4 calibration points (in duplicate), if calibrators are available.

Repeatability/Reproducibility: In quantitative molecular methods, it is required for repeatability to be assessed with ten (10) measurements of control samples (CRMs or EQA samples) at a level of positivity (weakly positive near the detection limit of the method) in the same experimental run, and for reproducibility with twenty (20) measurements under different conditions (different runs on different days). Measurements should also calculate final concentrations in addition to Ct values.

Appropriate statistical analysis of these experiments should follow. Alternatively, measurements for internal quality control (e.g. Levey Jennings Charts) can be used to calculate reproducibility.

The above applies to all CE IVD quantitative tests performed on automated molecular point-of-care analyzers.

5.3 Validation of laboratory-developed internal (in-house) tests

In-house methodologies and Research Use Only (RUO) methods are validated the same way CE-IVD methodologies are validated by their manufacturers. In the case of accreditation according to ISO 15189, in addition to analytical validation, clinical validation is also required (diagnostic sensitivity and specificity, NPV, PPV, clinical utility).

Requirements regarding the validation of in-house laboratory developed methods are the following:

Trueness: Assessment of trueness is achieved by using control samples (CRMs or EQA samples).

Furthermore, trueness is validated by:

- *Method comparison*: For in-house methods, it is required to compare the method with a CE-IVD kit, or when absent, with another commercial kit or other established methodology of the laboratory itself or the designated referral laboratory, with 20 samples (e.g. 10 negative and 10 positive in qualitative parameters, 10 negative and 10 positive with a broad range of values in quantitative parameters) and appropriate statistical analysis.

In multiparametric methodologies where more than one microorganism / gene / mutation / polymorphism is detected under one technique, the laboratory must have a satisfactory panel (at least 50% of the pathogens belonging to the test's detection panel or the set of target genes which are analyzed in the cases where these constitute a small percentage of a polygenic panel) of control samples with e.g. at least the most frequent mutations/polymorphisms for each gene in the Greek area or the most frequently detected microorganisms in the human microbiome in order to validate the trueness of the method used.

Repeatability / Intermediate precision / intra-laboratory reproducibility: For Repeatability/Reproducibility it applies as defined for the corresponding CE-IVD tests. The CV% of the measurements is calculated (it can be drawn from control charts within a period of 3 or 6 months).

Limit of Detection (LOD): A series of five dilutions (with nominal concentrations at the expected or required detection limit) of a reference material (CRM) is required to be tested on 3 different days (runs) with 8-10 replicates. Determination of detection limit at a 95% confidence limit is achieved by a graphical representation of the probit analysis. In multiparametric methodologies the process is repeated for each target detected by the method.

Limit of quantification (LOQ) in quantitative methods: According to the equation LOQ=3xLOD.

Linearity and Measuring Range for quantitative methods: For an in-house Q-PCR methodology able to measure in the range of 10 log units, using at least 5 points (in triplicate) by diluting an appropriate reference material (CRM) is necessary for obtaining the standard curve (n = 5) and performing a linearity check (dilutions shall cover at least 5 log units).

Analytical Specificity: check with electrophoresis and DNA Sequencing the obtained PCR product (and Tm in Q-PCR), check for interfering substances that could inhibit or block the measurement (e.g. haemoglobin, heparin, etc.), or genetically similar organisms or organisms commonly found in the samples analyzed by the laboratory, or pseudogenes or homologous regions depending on the test to be accredited.

5.4 Estimation of Uncertainty

As in § 1.1.2

5.5 Quality Assurance

Internal Quality Control

- In conventional / Q-PCR: always use a blank (no DNA / blank), a negative and a positive control (in molecular microbiology and in somatic mutation detection, a weak

positive shall be used in place of the positive, e.g. 2-3 x LOD), in Molecular Genetics at least two control samples shall be used: blank and mutant. It is recommended that the isolation of genetic material includes a quality control (endogenous control of a housekeeping gene or exogenous control of the manufacturer).

- For high complexity testing (e.g. microarrays) where additional method or DNA isolation controls exist, the laboratory should use one positive or negative control sample either per kit of 20-40 samples or at least once a month, for small number of samples.

-The controls should be either diagnosed samples from EQA schemes or a CRM, and not the control samples provided in each kit by the manufacturer.

- It is recommended that laboratories choose CRMs from companies/bodies accredited according to ISO 17034 and should be accompanied by traceability credentials according to the guidelines of ILAC P10.

The internal quality control should be carried out at regular intervals or depending on the methodology and the manufacturer's instructions, in each run if this is possible, (with a frequency that will be defined taking into account either the workload of the laboratory e.g. at least every 50 samples, or the time intervals of execution e.g. at least every three or four months), and be analyzed in control charts (Levey-Jennings) and the evaluation should be based on Westgard criteria that the laboratory has documented in the QMS.

It is strongly recommended that, as part of internal audits and reviews of test suitability, retesting of already tested samples should be performed at a frequency based on the stability and robustness of the test method and the risk of patient harm from a false result, and analyzed in control charts (Levey-Jennings).

Equivalence of equipment: In case the test to be accredited is based on the use of alternative similar equipment units (e.g. automatic nucleic acid isolation systems, Real-time PCR thermal cyclers, genetic analyzers) then an equivalence study of all relevant units used should be carried out for each test to be accredited, using the same control samples (CRMs or EQA samples).

In genetic sequencing tests:

1) Acceptance criteria should be stated: a) of the primary sample, such as % of cancer cells, quantity, etc., b) of the concentrations and purity of the extraction products (DNA, RNA) for PCR-sequencing/FA/MLPA or, in the case of NGS, the libraries (quantification and quality control, such as size of genomic regions included in the libraries, and quantification and quality control of the final product before sequencing); c) The criteria for sensitivity (Limit of detection), appropriate read depth and coverage may vary depending on the target agents (e.g. bacteria), the method and the purpose of the sequencing test (e.g. drug resistance testing, genotyping/subtyping).

2) In widely used short-read sequencing platforms, it is recommended that the DNA segments between adapters be 100 to 500 bp long (if these are varied or not defined in the assay protocol). The Laboratory should define the acceptable concentration values of the libraries synthesized before following the next experimental steps (and justify the choice of the specific criteria).

3) To avoid failures when separating sequencing products into different samples (demultiplexing), strategies such as the use of dual indexing must be developed.

4) The sequences to be amplified during Whole exome or genome sequencing should, in addition to the regions of the corresponding exons, include at least the regions -1 to -16 and +1 to +5 of the adjacent introns.

5) The Laboratory should define quality indicators such as Phred quality score, LOD, estimate of the (total) error of the NGS process (or at least of the sequencing), the minimum read depth and the % of investigated bases sequenced with the minimum required depth, the total number of "target reads" that cover the region, in which a genetic change is detected, as well as the number of "reads" that carry this change.

6) A minimum requirement of Q score≥20 (long reads), and Q score≥30 (short reads), is generally recommended, while the required depth varies depending on the type of samples and the targets under investigation (depth>=15x).

7) Samples used for method standardization should not be the same as those used for validation. However, they can contribute to defining the acceptance / rejection criteria of samples.

8) Pathological findings should be verified with complementary methodology (e.g. Sanger sequencing, MLPA, real time PCR, etc.) or a combination of methods if the allele load is expected to be low (targeted mutation analysis).

9) When commercially available bioinformatics analysis software is used, the laboratory should have all records and validation data provided by the manufacturer. A validation process is required for software created in-house by the laboratory (with at least 10 control samples that will be analyzed and compared with other commercially available software or analyzed with another method that will confirm the result e.g. Sanger Sequencing) so as to establish their suitability for clinical use. In the case of direct sequencing (Sanger): the software for the analysis and the acceptance or not of the segments to be sequenced (base calling) should be defined and the quality indicators should be given (Phred Qscore ≥20). The criteria by which genetic changes are evaluated/categorized should be stated.

External Quality Control

For Molecular Diagnostics laboratories, participation in external quality assessments for each category of tests shall be done according to the following:

For initial assessment and at any extension of the scope of accreditation, the laboratory must have successful results of interlaboratory comparisons for all laboratory tests for which accreditation is requested. The laboratory could then make groups according to parameter and technique/equipment and rotate on a yearly basis. Within the four or five year accreditation cycle, the full scope of accreditation should be covered (matrices/tests/techniques).

In the molecular detection of pathogens or somatic mutations, external quality control shall be more demanding in order to check the sensitivity of the method (to include rare strains or very dilute samples). In the molecular detection of inherited mutations in genes where multiple mutations exist, external quality control is recommended to include the range of all mutations appearing in the Laboratory Accreditation Scope (and not just specific mutations) or combinations of them.

Following the first accreditation cycle (4 or 5 years) providing the laboratory has demonstrated excellent results in all the previous External Quality Assessments, the frequency of interlaboratory comparisons may be reduced (once every 2 years) per group of parameters and/or techniques/equipment, after evaluation of laboratory's performance by ESYD and the consent of the assessment team.

It is recommended that laboratories choose bodies that carry out EQA schemes that are accredited according to ISO 17043.

If there is no commercially available External Quality Control scheme for a parameter (pathogen or mutation) detected by the test to be accredited, or for a matrix analyzed by the test to be accredited, then the Laboratory must participate in an interlaboratory comparison of at least 15 samples for Real-time PCR and at least 8 samples for genetic sequencing with a similar diagnostic laboratory per year.

In any case, for multiparametric tests, only the parameters of the test for which the Laboratory applies External Quality Control, either by an official body or, in the absence thereof, in cooperation with similar diagnostic laboratories, will be listed in the accreditation scope.

5.6 Cytogenetic tests - Karyotype & FISH

5.6.1 Verification

The verification of this category of tests is done by determining the diagnostic accuracy and the diagnostic reproducibility-repeatability.

Diagnostic accuracy: It is determined with the help of specific statistical parameters such as specificity, sensitivity, positive and negative predictive value. These parameters are a measure of the reliability of the diagnosis and may vary per material and per laboratory, however they must range at levels accepted by the international literature.

Diagnostic reproducibility-repeatability: It is estimated by:

-Analysis of each sample by two independent analysts, at least one of which has appropriate experience in the cytogenetic analysis of this category of samples (e.g. investigation of hematological malignancies).

-Random retesting by the same or different analysts of a representative percentage of samples, commensurate with the laboratory workload (at least 1 sample per month).

Additionally, in the case of FISH (fluorescence in situ hybridization) any new labeled commercial probes (IVD) introduced into diagnosis requires verification (determination of repeatability, reproducibility, trueness and limit of detection determination) of its performance prior to diagnostic use. The verification also requires testing for target specificity in terms of analytical sensitivity and specificity (preferably in normal controls and pathological cases, which are positive for the probe being tested). Sensitivity and specificity must be high to avoid misdiagnosis. Thresholds and confidence limits must be defined for all FISH probes and probe sets. To define the cut-offs of each probe, at least 5 normal and 2 pathological samples should be analyzed by at least 2 analysts. Where applicable, each time a hybridization is performed, the respective probes should also be included in control samples.

The guidelines of authoritative international scientific societies are also taken into account.

5.6.2 Estimation of Uncertainty

Microscopy tests belong to the category of tests, for which strict metrological and statistical calculations of uncertainty cannot be implemented.

5.6.3 Quality Assurance

Internal Quality Control

As measures of internal quality control, it is required to perform proper sampling (e.g. for myeloid leukemias: bone marrow or peripheral blood when >10% blasts are present in the peripheral blood), evaluation based on recorded sample acceptance/rejection criteria (transfer to appropriate sterile containers collection and under appropriate conditions, macroscopic evaluation of the quality and quantity of the sample, etc.) and implementation of a risk minimization policy.

a) Cell cultures - Prenatal screening (amniotic fluid, chorionic villi): At least 2 independent cultures in different incubators. For chorionic villus sampling (CVS) it is required to establish and analyze long-term cultures (trophoblast cells and mesenchymal cells). Back-up cultures of prenatal samples should be maintained until the final report is prepared and, when practical and possible, viable cells should be frozen, as well as fixed cells for cases where further cytogenomic or molecular analyzes may be required.

b) Cell cultures - Postnatal control: For the peripheral blood conventional karyotype, 2 independent cultures of 48 and/or 72 hours. For hematologic-oncology samples (karyotype for acquired chromosomal alterations): at least 2 independent cultures of 24 and 48 and/or 72 or 96 hours depending on the type of neoplasm.

c) Banding using banding techniques. For the use of different banding techniques, equivalence tests should be performed. The vast majority of samples require complete banding pattern analysis for all chromosomes. ISCN (2020) defines five band levels. For hematological malignancies or solid tumors, a minimum level of banding resolution cannot be defined. Laboratories should be able to have the highest level of resolution possible (300-550 bphs) and analyze cells with different levels of chromosome banding resolution and cells of different quality in order to maximize the likelihood of detecting a neoplastic clone. Where additional tests are required to clarify chromosomal abnormalities, FISH or molecular analysis may be performed in addition. A minimum level of conventional karyotyping banding analysis (minimum G-banding quality, QAS-score) should be defined depending on the reason for referral.

d) Analysis and evaluation:

i) Karyotype or FISH by at least 2 independent analysts.

ii) Conventional karyotype: Analysis of at least 15 metaphases (of which 5 fully analyzed). To rule out mosaicism, check at least 30 metaphases.

iii) Karyotype of hematological neoplasms: In case of a normal karyotype at least 20 metaphases should be examined to rule out the presence of a chromosomally

abnormal clone. If a normal result is based on examination of fewer than 20 metaphases, the result report should state that the presence of an abnormal clone cannot be excluded. When a clonal abnormality is identified during diagnosis, analysis of 20 metaphases is recommended, where possible, to identify a possible additional minor clone. If there is a suspicion of an alteration belonging to the conventional karyotype, a conventional karyotype test must be performed.

iv) Comparison of the karyotype with the results of other auxiliary diagnostic techniques, such as FISH, molecular karyotyping, molecular analysis for mutations.

v) FISH for congenital alterations: In case of uncultured cells check ≥50 cells and in case of mosaicism ≥100 cells for each probe. In case of cultured cells additional control of at least 5 metaphases. FISH results are recommended to be accompanied by karyotyping analysis.

vi) FISH in hematological malignancies:

-Diagnostic samples: Analysis of at least 100 interphase nuclei and additional analysis of at least 5 metaphases in case of cultured cells, if possible.

-For follow-up of patients in which a clonal abnormality was found during diagnosis: analysis of at least 200 interphase nuclei and additional analysis of at least 5 metaphases in the case of cultured cells, if present.

-Where there is a suspicion of a deletion or a translocation, the adequacy of the hybridization efficiency can be confirmed either by the presence of a normal second signal in the hybridized cell e.g. in the case of a translocation, or by the presence of an internal control in the used probe e.g. in the case of a deletion.

e) Cytogenetic Result Reports: The karyotype is described according to the most recent version of the International System of Human Cytogenetic Nomenclature (ISCN). Gene names should be written according to HUGO nomenclature. Abnormal FISH results should always be reported using the most recent ISCN.

f) FISH in paraffin sections: In case of diagnostic samples the selection of the section and the hybridization area should be indicated by a Pathologist:

- the paraffin section must be 3.5-5μm thick
- the analysis must be done on at least 60 cells. In case that 60 cells cannot be analyzed, this is written as a comment in the results.

The guidelines of authoritative international scientific societies are also taken into account.

External Quality Control

Participation of the Laboratory in appropriate proficiency testing schemes (at least annual for karyotype and FISH), ensuring the participation of all members of the Laboratory.

6. FLOW CYTOMETRY TESTS

6.1 Quantitative Tests

Two standardized flow cytometry methods exist:

1. Calculation of lymphocyte subpopulations
2. Calculation of CD34+ hematopoietic progenitor cells

both belonging to the calculation of immunologically identified cell populations by flow cytometry (CLSI Former NCCLS H42- A2 (vol 27 No 16)). The following suggestions are also based on Validation of assays performed by flow cytometry (CLSI H62).

6.1.1 Verification

All the applied methods must be verified on existing equipment in the laboratory premises. The verification is performed by using commercial control samples of fixed whole blood, at least at two concentration levels, wherever available. The verification of the methods implemented for each parameter under accreditation, consists of the calculation of the following analytical characteristics:

Trueness: It is evaluated by participation in interlaboratory programs. For trueness, the results of at least three years of participation in the external quality control scheme of EEK can also be used.

Repeatability: At least six (6) measurements of the same control sample. The % CV_r of the measurements is calculated. Alternatively, three (3) samples may be analysed, two (2) times each, at respective time intervals.

Intermediate precision / reproducibility: At least six (6) measurements of the same control sample on different days. The % CV_R of the measurements is calculated (it can be drawn from the results of control charts within 6 months).

Limit of Detection (LOD) and Limit of Quantification (LOQ): wherever it is crucial to draw conclusions from the parameter examined. The parameters are calculated by a six times measurement of a diluted sample at a concentration similar to the limit of detection stated by the manufacturer, within the same day.

Determination of reference values : Initially, those given by the literature with a large number of samples in a Caucasian population (especially for pediatric samples) are accepted. If possible, confirmation is made with 20 normal samples.

6.1.2 Estimation of Uncertainty

As §1.1.2

6.1.3 Quality Assurance

Internal Quality Control

It is required to use one sample of internal quality control weekly (fixed blood sample for method 1, an appropriate sample supplied by the manufacturers for method 2). In method 1 all major lymphocyte subpopulations shall be determined (CD3+, CD3+CD4+, CD3+CD8+, CD19+, CD3-CD16/56+). In method 2 mandatory use of a control sample on the day of sample measurement is recommended (when the method is not used continuously – then weekly use is not required).

Internal Quality control includes control of equipment and control of methods. More specifically, it is required:

A. Daily use (or at each opening of the instrument) of alignment beads (type I) according to the manufacturer's instructions. The allowable limits have been set by the technician during the initial installation of the instrument, usually for an alignment with CV <2.5%. Storage of corresponding Levey Jennings diagrams in the flow cytometer is required.

B. Weekly use of beads for the confirmation of the repeatability of measurement for each fluorescence (type 2) according to the manufacturer's instructions. Storage of corresponding Levey Jennings diagrams in the flow cytometer is required.

C. Annually (or after each major repair) use of validation beads for instrument alignment and calculation of the response of the instrument to the fluorescence signals with special beads with different fluorescence intensities (output of a linear calibration curve for each fluorescence detector). Carryover estimation is also recommended: measure 3 replicate samples (A1, A2, A3) with a high number of a population followed by 3 replicates without the population (B1, B2, B3) and calculate the carryover from the equation carryover%= [(B1-B3)/(A3-B3)]*100.

D. Control of fluorescence compensation with suitable beads or appropriate cells. It is suggested that the testing is done in the case of a significant change of PMTs and in general in the case of a significant change of the instrument’s settings.

E. At least weekly or on the day of sample measurement (when testing is infrequent) use a commercial fixed blood sample or an equivalent commercial control sample to measure CD34+ cells as an internal quality control sample.

External Quality Control

Participation in appropriate Proficiency Testing Schemes, with at least 4 samples per year for both the aforementioned tests, is required.

6.2 Qualitative tests

The tests concerning acute leukemia, Paroxysmal Nocturnal Hemoglobinuria (PNH) and lymphoproliferative syndromes typing panels can be considered as qualitative or semi-quantitative, since each marker is required to be classified as positive or negative. The requirements of §1.2 apply.

6.2.1 Verification

The method verification concerns the specific material for which it is applied (e.g. peripheral blood). In case it is applied to another biological material, the verification (possibly requiring method validation) should be carried out with the specific biological sample (e.g. CSF, Bone Marrow, BAL etc.).

Otherwise, the verification is the same as for the quantitative tests (par. 4.1.1).
Limit of Detection (LOD) and Limit of Quantification (LOQ): wherever it is crucial to draw conclusions from the parameter examined. The parameters are calculated by a six times measurement of a diluted sample at a concentration similar to the limit of detection stated by the manufacturer and generally at some measurable low level, within the same day. The limit of detection is 3.3 times the standard deviation (SD).

Especially for measurement of minimal residual disease, the calculation guidelines given by scientific societies should also be taken into account: a minimum limit for LOD of 20 events and a minimum limit for LOQ of 30-50 events. They should be reported together with the final result accompanied by the total number of cells (events) analyzed and the number of the cells under investigation that were recovered.

LOB (Limit of Blank): defined as the sample analysis without the population under detection (e.g. normal marrow) up to 10 events (cells) in the area where the pathological population is expected to be detected. It can also be determined by cut off determination experiments on FMOs samples. LLOD can be calculated as LOB+1.645SD, or mean of the blank +3SD.

It is necessary to determine the limit of events (cells)/run that will be analyzed in each technique.

6.2.2 Validation

Those tests for which there are no kits, and in-house protocols - panels are used, e.g. combinations of reagents, mainly, but not only CE-IVD, validation is required in addition to verification.

The use of FMO (fluorescence minus one) for at least one protocol (the main protocol of a panel) is suggested as the validation of the method. This gives us on one hand an indication of the LOB for the missing marker, and on the other the repeatability for the other markers.

"Empty spaces" can also be determined using normal or disease-free samples or even samples with regenerative bone marrow in the case of MRD.

For sample stability, what is recommended in international literature guidelines applies: 72 hours for whole blood and 48 hours for bone marrow, unless stability experiments have been performed that allow or not to change the times.

The stability of a processed sample at the respective time points could be assessed, because it does not significantly set a workload to the laboratory.

6.2.3 Quality Assurance

Internal Quality Control

The use of internal quality controls is important in flow cytometry.

For tests such as leukemias and lymphomas, populations within the sample that are known not to express the specific antigen can be used as negative controls and to determine the positive-negative threshold as well as the degree of autofluorescence. In immunophenotyping leukemia/lymphoma samples internal controls can be used to identify remaining normal cells within the sample, aid in the interpretation of results, identify normal versus pathological samples, and ensure reagent efficiency. It would be good to use the same cell type as the target cell for the internal controls, because some cell types have high autofluorescence (monocytes, granulocytes). An example of a negative population is B lymphocytes for CD3 and T lymphocytes for CD19. A relevant SOP can document the expected use of internal controls.

External Quality Control

Participation in appropriate Proficiency Testing Schemes, with at least 2 samples per year, is required, depending on availability.

7. CYTOLOGICAL TESTS

Cytology laboratory equipment does not require calibration. All pre-analytical procedures related to slide staining must be verified at regular intervals using control samples.

The Cytologist should correlate the clinical and the microscopic findings, when issuing the report, and follow a nationally accepted classification system for the cytological results (diagnostic terminology). The diagnostic terminology used must be in accordance with the current international literature, the guidelines of the Hellenic Society of Clinical and Molecular Cytology, the Quality Committee of the Ministry of Health and International Scientific Societies (e.g. College of American Pathologists).

The laboratory must observe and apply sample acceptance criteria, in accordance with the current international literature, the guidelines of the Hellenic Society of Clinical and Molecular Cytology, the Quality Committee of the Ministry of Health and International Scientific Societies (e.g. College of American Pathologists).

The percentage of unacceptable or non-diagnostic samples must be monitored with the objective of providing periodic feedback to the personnel - external partners who are authorized to perform the sampling.

Previously examined cytology specimens from patients with abnormal findings should be reviewed. In case of discrepancy-disagreement with previous cytological diagnosis from the laboratory, the previously generated cytological and histological results should be reviewed.

The laboratory should define criteria for the review of cytology cases (which can be ancillary quality indicators of the diagnostic work produced). The criteria must be in accordance with the current Greek and international literature and the guidelines of the Hellenic Society of Clinical and Molecular Cytology, the Quality Committee of the Ministry of Health and International Scientific Societies (e.g. College of American Pathologists).

The volume of diagnostic work carried out by each Cytologist must be recorded. The laboratory must impose limits on the diagnostic work to be carried out by each diagnostician. The limits of diagnostic work vary by type of examination and must agree with those proposed by the current international literature, the guidelines of the Hellenic Society of Clinical and Molecular Cytology, the Quality Committee of the Ministry of Health and International Scientific Societies (e.g. College of American Pathologists).

All cytological reports must be issued within a reasonable period of time, that will not risk the progress of the patient's health under any circumstances. The laboratory must document and implement procedures for monitoring the time between examination of samples and issue of the results (turnaround times).

The cytological report should be written in accordance with the requirements arising from the guidelines of the Hellenic Society of Clinical and Molecular Cytology, the Quality Committee of the Ministry of Health and International Scientific Societies (e.g. College of American Pathologists) and should definitely include the following:
a) Description-Report of cytological findings and acceptance of sample
b) Conclusion
c) Comments-Recommendations (when necessary)

All requests must be retained in paper or electronic form for a reasonable period of time, commensurate with the clinical significance of the examination, and in any case not less than 1 year. In addition, the reports given to the interested parties should be retained. This can be done in paper or electronic form. This file is never destroyed. All slides should be kept in special archives for a reasonable period of time, commensurate with the clinical importance of the examination and in any case not less than 3 years.

Special archival specifications may apply for special types of specimens. Overall, the archiving must be in accordance with the current National and European legislation and the guidelines of the Hellenic Society of Clinical and Molecular Cytology, the Quality Committee of the Ministry of Health and International Scientific Societies (e.g. College of American Pathologists).

7.1 Verification

The diagnostic verification of cytological test results is achieved by determining special statistical indicators.

Diagnostic accuracy: is determined by specific statistical parameters such as specificity, sensitivity, positive and negative predictive value. These parameters are a measure of the reliability of the diagnosis and may vary per material and per laboratory, but should fall within levels acceptable by the international literature.

The calculation of sensitivity, specificity and the ratio of false-negative cytological results are performed by using the histological examination as the reference method (the gold standard). The calculation of the positive predictive value (PPV), and the negative predictive value (NPV) of cytology results is performed by using the histological examination as the reference method (the gold standard).

Diagnostic Reproducibility-repeatability: It is estimated with random retesting a representative number of samples commensurate with the workload of the laboratory, by the same or different diagnosticians (measurement of inter-observative and intra-observative agreement - κ statistical parameter).

The guidelines of authoritative international scientific societies are also taken into account.

7.2 Estimation of Uncertainty

Cytological tests belong to a category of tests, for which strict metrological and statistical calculations of uncertainty cannot be implemented. Cytological tests are qualitative tests, which can be converted into semi-quantitative in many cases, for the purpose of statistical exploitation of the extracted results.

7.3 Quality Assurance

Internal Quality Control

Laboratories must monitor their performance, as a whole, but also that of each Cytologist separately. The implementation of internal quality control measures is essential and the use of one or more of the following is recommended:

a) Implementation of regular Clinical Pathological Conferences (C.P. Conference). Periodic meetings of clinicians with personnel from the Laboratories Division are recommended, where all clinical, imaging, cytological, pathological, microscopic and other relevant data are discussed, critically analysed and correlated.

b) Random retesting: The laboratory staff reviews a representative percentage of samples, commensurate with laboratory workload (measurement of inter-observative and intra-observative agreement - κ statistical parameter).

c) Mechanical retesting of cytological samples by special scanners (imagers). (measurement of inter-observative and intra-observative agreement - κ statistical parameter).

d) Targeted retesting of cytological samples with pathological diagnosis or with diagnosis of atypia: (measurement of inter-observative and intra-observative agreement - κ statistical parameter).

e) Comparison between biopsy and cytological results (calculation of sensitivity, specificity, positive and negative predictive value using ROC curve)

f) Comparison of cytological diagnosis with the results of other ancillary diagnostic techniques such as electron microscopy, immunocytochemistry, molecular cytology, image analysis, flow cytometry, cytogenetic tests.

g) Use of special statistical indicators in order to monitor the general performance of the laboratory. Examples include the percentage of non-diagnostic samples, the periodic review of the quality of the stains used, the recording of the TAT time, the percentage of positive samples, the percentage of ASCUS, ASC-H and HSIL samples, the ASC/SIL index. The guidelines of authoritative international scientific societies are also taken into account.

External Quality Control

External quality control of cytology tests is achieved through comparative proficiency testing. Proficiency testing programs should, as far as possible, control all pre-analytical, analytical and post-analytical procedures and not be limited to the confirmation of the cytological diagnosis. Samples used for external quality control should, as far as possible, mimic routine samples as far as receipt, examination and diagnosis by the laboratory is concerned.

The participation of the Laboratory in proficiency testing programs should be at least annual, ensuring the participation of all members of the Laboratory. For initial assessment and at any extension of the scope of accreditation, participation in proficiency testing programs is required for all the tests for which accreditation is requested. Within the four or five year accreditation cycle, the full scope of accreditation should be covered.

Laboratories must monitor their performance, as a whole, but also that of each Cytologist individually. The implementation of external quality control measures is essential and the use of one or more of the following is recommended:

a) Participation in a program of interlaboratory tests (measurement of inter-observative and intra-observative agreement - κ statistical parameter). The interlaboratory testing program should be accredited according to EN ISO 17043 where possible.

b) Participation in a periodic program of exchange of slides with other cytological laboratories (measurement of inter-observative and intra-observative agreement - κ statistical parameter).

c) Participation in a program of periodic examination of standard samples from the archives of Reference Laboratories or Scientific Societies (measurement of inter-observative and intra-observative agreement - κ statistical parameter).

The guidelines of authoritative international scientific societies are also taken into account.

8. PATHOLOGY TESTS

Pathology tests are divided, for the purpose of this document, into three categories:

1. Histopathological (morphological) diagnosis
2. Ancillary diagnostic tests (immunohistochemical and histochemical tests)
3. Tests with Predictive Significance (immunohistochemical and molecular tests)

8.1 Histopathological diagnosis

The histopathological diagnosis is a qualitative test and is based on the assessment of the morphological changes of tissues and cells at the light microscopy level, by utilizing the training and experience of the pathologist.

The diagnostic material on which the test is applied are histological sections mounted on slides and stained with the basic hematoxylin-eosin (H&E) stain. These sections are produced during the sectioning of paraffin blocks containing embedded tissue derived from the sampling carried out during the grossing of the histological samples (usually biopsies and surgical resections). The above procedures are preceded by the pre-analytical handling and processing of tissues in order to achieve their stabilization / fixation and to stop the autolytic processes that begin immediately after the interruption of blood circulation.

The method of tissue processing is a critical process that affects, to varying degrees, all categories of pathology and molecular testing performed on histological specimens.

Labelling of histology preparations (paraffin blocks and histology slides): The standardized, optimized, and secure labelling of paraffin blocks and histology slides, promotes safety, by ensuring that all derived products from patient tissues, are uniquely identified and linked to a specific patient. The College of American Pathologists has published a relevant guideline which should be used as a guide for this specific procedure.

Pre-Analytical parameters

The College of American Pathologists has published relevant recommendations regarding appropriate practices to ensure the molecular integrity of cancer samples to be subjected to molecular testing for the purpose of implementing personalized therapies.
Adherence to the implementation of techniques and recording of deviations regarding the pre-analytical parameters, for tissues, as described in relevant guidelines, is a prerequisite for ensuring the quality of the entire range of pathology tests.

8.2 Immunohistochemical tests

8.2.1 Validation / Verification

The choice between validation or verification is determined by the categorization of the immunohistochemical test (Type I Class I or Type II Class III according to IVD) as determined by the instructions for use (IFU) that separate the tests into:

1. Type I = CE-IVD with strict application of IFU's = Verification
2. Type II = CE-IVD with deviation from instructions for use or non-CE-IVD test (LDT / RUO) = Validation

The process of validation / verification is required for each test mentioned in the accreditation scope of the laboratory before introducing the tests into clinical practice. It is recommended to create a list of accredited tests, with at least a description for each test at least of: 1. The antibody and the clone, 2. The reference material, 3. The intended use(s).

Instructions for use of immunohistochemical tests should include the following information:
- Identification of sample type(s) and intended use of the test
- Clear instructions for:
    - Deparaffinization
    - Antigen pretreatment
    - Dilution of primary antibody
    - Antibody incubation times
    - Detection system
    - Enhancing step (linker)
    - Application platform (device).
    - Preprogrammed (locked) protocol or method with a full description of the application protocol

Objective criteria: Must be demonstrated and recorded. Data from the comparative assessment of the validation/verification samples with the reference samples, should be traceable to the staining, the predefined performance characteristics and the acceptance criteria.

Performance characteristics: In order to demonstrate or confirm the performance characteristics it is necessary to compare the new test with a comparator or a reference / standard.

In immunohistochemistry there is no access to standard calibration materials. Access is available to tissues. Therefore, concerning the choice of a reference standard we should decide, for each test (antibody), what type of tissue we will use, which cells

should be positive, at what staining intensity and in what count, based on the international literature and/or standard lists of reference materials available on the websites of the external quality control schemes. Exemplary reference materials could be:

- Specimen types with known morphology and antigenic expression (e.g. iCAPS)
- Confirmed tissue samples or controls (controls) stained with an already validated or verified method (in-laboratory or in another laboratory)
- Tissues with references in the instructions for use of the test, concerning staining patterns, performance, etc.
- Cell lines with pre-standardized staining expression
- External quality control (EQA) samples

A. Accuracy = Trueness and Overall Precision

Trueness:
- Comparison with known results of validated test (reference samples, samples from validated tests (in-laboratory or at another laboratory)
- Comparison with another validated test (e.g. in situ hybridization, PCR), other validated platform or third-party reagents
- Tertiary comparison - External quality control (EQA) or Inter-laboratory samples
- Population study

Overall Precision

- Repeatability: within a group of tests (intra run).
- Intermediate precision: between a test group.

If more than one platform (device) is used the overall precision should be determined for each one separately.

B. Sensitivity: It is divided into:
- Analytical (the ability of the test to detect small amounts of antigen (it also determines the dilution range for the limit of detection)).
- Diagnostic: Evaluation of true positive (TP) staining versus false negative (FN) staining. Defined as Sensitivity: TP/(TP+FN).

C. Specificity:
- Analytical (the ability of the test to detect the antigen without being affected by cross-reactions).
- Diagnostic: Assessment of true negative (TN) staining versus false positive (FP) staining. Defined as Specificity: TN/(TN+FP).

D. Overall concordance:
- Analytical (The degree of agreement of the new test and the reference test ± = Trueness).
- Diagnostic: Evaluation of true positive and negative staining against the total of true and false positive and negative stains. Defined as Concordance: TP+TN/(TP+TN+FP+FN).

E. Reproducibility

F. Robustness: Parameters that affect the analytical result:
- Pre-analytical: (Cold ischemia time – Fixation time Section thickness).
- Post-analytical: (Antigen stability - time and method of storage of sections - retention time of diluted antibody / Others: (Desalting)).

G. Evaluation of result (Readout) by a pathologist (initial validation/verification):
- Training: of a Pathologist in the use of a grading system (by test application experts of the manufacturer/supplier, by professional/educational organizations)
- Evaluation of a new test by more than one pathologist and in relation to expected results and known cases or controls that were:
- Validated by application experts, team of experts
- Determination of diagnostic sensitivity and specificity for each pathologist
- Comparison of the results of the pathologists and evaluation against defined acceptance criteria (e.g. 90% agreement – percentage dependent on the number of validation / verification samples)
- Optimizing the agreement between observers in the context of: Internal Quality Control (IQC) and External Quality Control (EQC).

H. Quality of staining: It is necessary to develop or adopt a scoring system in terms of, for example, intensity of staining, uniformity, specificity, absence of underlying staining, counterstaining. Particularly for tests with predictive value, where specific staining features are associated with a positivity threshold this should be determined and scored accordingly (*This procedure assesses 'basic' analytical specificity and sensitivity).

Table 1. Performance characteristics evaluated during verification or validation

	Verification	**Validation**			
Performance characteristics	**CE-IVD test**	**LDT - "Off label with reference to instructions"**	**LDT - "Off label without reference to instructions "**	**LDT -RUO with references to instructions**	**LDT - laboratory developed**
Precision / Trueness	x	x	x	x	x
Overall Precision	x	x	x	x	x
Sensitivity			x	x	x
Specificity			x	x	x
Robustness	x*	x*	x*	x*	x*
Quality of staining	x	x	x	x	x
Concordance between pathologists	(x)	(x)	(x)	(x)	(x)

Types of samples used during validation / verification:

- Materials related to intended use of the test (instructions for use)
- Controls (those prepared in the laboratory are preferred)
- Sections from already evaluated samples
- Sections from samples with different expression levels (high, low) and different tissue types
- All samples should be prepared the same way as for routine laboratory samples

Table 2. Number of samples used during validation / verification

Test Type	Non-Predictive	Predictive	Criteria
CE/IVD with strict adherence to instructions for use (for each antibody)	10 (+) & 10 (-) -or - Selection of number with a justified responsibility of the scientific Director	>20 (+) & 20 (-) Include different levels of expression Guidelines for ER/PR/HER-2 or other predictive biomarkers	Accuracy Precision Reportable range Overall concordance >90%
CE/IVD with modifications of the instructions for use, Non CE/IVD (LDT, RUO)	10 (+) & 10 (-) -or - Selection of number with a justified responsibility of the scientific Director	ER/PR/HER-2 : >40 (+) & 40 (-) Others : 20 (+) & 20 (-)	Accuracy Precision Analytic sensitivity Analytic specificity

Re-validation / re-verification due to test modifications:

1. Modifications associated with a specific antibody. Scope of verification / validation:

- New Antibody Lot: Verification of results is achieved by "running" a known positive and a known negative sample.
- Changes in: Antibody Dilution, New Supplier (Same Antibody Clone), Changes in Antibody Incubation Time or Antigen Retrieval Time (Same Antigen Retrieval Method): Verification of results is achieved by "running" two known positive and two known negative samples. In the above two cases it is recommended to run an additional sample to indicate the lower sensitivity range.
- New antibody clone: New complete verification / validation process.

2. Modifications concerning the totality of antibodies:

- Type of fixative
- Antigen retrieval method: Changes in pH, Changes in buffer type, Changes in heating type
- Changes in antigen detection system
- Changes to tissue preparation devices (Histokinettes)
- Changes in environmental conditions: Translocation of the laboratory, Change of water supply.

Selection of a representative number of tests performed in the laboratory and comparison between samples 'run' after the modifications with samples 'run' under the original conditions.

The verification of the above tests is done by determining the diagnostic accuracy and the diagnostic reproducibility-repeatability.

8.2.2 Estimation of uncertainty

Pathology tests belong to a category of tests, for which strict metrological and statistical calculations of uncertainty cannot be implemented.

8.3 Quality Assurance

Internal Quality Control
Histopathological examination

Pre-analytical stage, Check for:

- Status of samples before accessioning (e.g. unsuitable or non-compliant samples).
- Adherence to pre-analytical tissue processing parameters. Recording of cold ischemia time (if the information is available), type of fixative, total fixation time, section thickness, storage conditions of blocks.
- Completeness of accompanying documents (e.g. request form, adequacy of identification information).
- Correctness of sample accessioning (ISO 27001 certified electronic LIS system, preferred) and assignment of a unique identifier for each sample.

Risk management is applied to the above and actions are recorded in case of deviation from the prescribed procedures.

Analytical Stage

Internal quality controls of histopathological testing are performed in accordance with job descriptions and departmental operating manuals to ensure that testing is compliant. The number and frequency of reviewed tests per control category is determined as a percentage of the total number of tests performed in the laboratory. Test reviews are indicatively divided into the following categories:

A. Intraoperative Consultation Review (Frozen Biopsy): Frozen biopsy cases are reviewed at pre-determined intervals by the Laboratory Director and evaluated for concordance with the final diagnosis (on permanent sections) and against literature data.

B. Randomized Review of Histological Cases: Histopathological cases are reviewed at predetermined intervals according to the following criteria:

- Clinical history
- Gross description
- Gross photos (if available)
- Adequacy of Sampling
- Quality of histological slides (labelling, H&E staining, coverslipping)
- Correctness of histopathological diagnosis
- Notes (if any)
- Critical data (for neoplasms based on CAP or equivalent protocols)
- Special processing (if any)
- Processing time

C. Review Based on Clinical Indicators: The designee determines the type of specimen or disease to be studied. This choice can for example be about:

- Type of histological sample regardless of diagnosis (e.g. Breast)
- Specific disease entity (e.g. Colon carcinoma)

During the process, the reports from all consecutive cases corresponding to the type of sample / disease entity under study are retrieved from the archive at predetermined intervals. Reports are evaluated for completeness, accuracy, and consistency against a predetermined list of required data (e.g. College of American Pathologists CAP cancer protocols).

D. Measurement of inter-observative and intra-observative agreement: Review of a representative percentage of samples (calculation of κ statistical parameter).

E. Implementation of regular clinical-pathological discussions either in the context of a predetermined periodic meeting of the multidisciplinary team (MDT) or in the context of the Tumor Boards where the clinical and imaging findings are presented, discussed, critically analysed and correlated with the corresponding pathological findings and other microscopy data. Conclusions are recorded as internal quality evidence.

The guidelines of authoritative international scientific societies are also taken into account.

Immunohistochemical tests

In the context of internal quality control and to detect failures in immunohistochemistry tests, controls are used. These controls can be tissues or cell lines and their use is intended to investigate specific performance characteristics of individual tests. The selection of controls for each specific test is derived from the published literature. Controls are applied, depending on the quality requirements, either to each individual patient slide or to a slide "run" in the context of grouped similar tests for different patients. Controls are run for each test on a daily basis. They are divided into positive and negative.

The positive control (section of selected tissue) gives the following information:

A) As long as the external (separate from the patient sample) control is true positive it is an indication that the method works by checking for:
- The specific reactivity of the primary antibody.
- The range of staining expression, which levels helps define sensitivity, and by
- Controlling the detection system.

B) If it is negative, it is an indication that part of the method failed:
- It doesn't detect the failed part though.

C) Internal (included within the patient's section) control:
- It is an indication of true positive staining.
- It is an indication of the quality of the tissue in terms of compliance with the pre-analytical and storage parameters of the samples.

The negative control (tissue section without application of primary antibody) gives the following information:

A) It is an indication of the specificity of the primary antibody (cross-reactivity).

B) It is an indication of failure of the detection system (background staining).

Failures identified in the internal quality control results of immunohistochemistry are recorded, failure trends are tracked, and on their basis corrective actions are implemented.

External Quality Control

The participation of the Laboratory in proficiency testing schemes in terms of histopathological diagnosis should be performed at a frequency determined by the respective program, ensuring at the same time the participation of all pathologists who carry out diagnostic work in the laboratory.

The Laboratory's participation in external quality control programs for immunohistochemical tests should be done at a frequency determined by the respective program ensuring that all tests in the scope of accreditation are covered (in their entirety in the case of initial assessment or extension and subsequently for all tests of the scope in depth of 4 years/5 years depending on the validity period of the accreditation certificate). For immunohistochemical tests, participation by grouping similar tests (modules) is determined by the available EQA schemes.

Participation in external quality control schemes accredited according to ISO:17043 is recommended.

9. TOPICS ON THE ACCREDITATION OF MEDICAL / CLINICAL LABORATORIES

9.1 Primary Sample Collection

Legal Entity: According to the requirements of the ESYD KAD regulation and the standard 15189, in terms of legal entity, the point of collection of a primary sample must fulfill the legal requirements. The evaluation team notes the fulfillment of these without evaluating them. According to Law 4461/2017, it is not allowed to operate blood sampling sites outside the establishment of private diagnostic laboratories of biological materials.

Quality Management System: Maintaining the integrity of the sample at all stages of collection, handling, transport to the main laboratory and processing, plays a vital role in ensuring the reliability of test results. Therefore, it is important to ensure quality at the primary sample collection sites.

The primary sample collection sites are defined as:

- Collection sites owned by the Laboratory, or its parent organization and staff are employees of the Laboratory (see Multi-Site Accreditation).
- Collection sites that do not belong to the Laboratory but there is a contract/agreement for the collection of the sample and its analysis in the Laboratory.

In addition to the above, the laboratory will state details of all other sources of sample collection, e.g. home blood draws. The laboratory will ensure the integrity of samples from these sources.

Instructions for the proper collection and handling of samples at the site of primary sampling and their transport to the Laboratory shall be documented in a primary sample collection manual, which shall form part of the Laboratory's quality system and be readily available at the site of primary sampling.

The Laboratory will have policies and procedures in place to ensure that proper hygiene, environmental conditions, and patient comfort and privacy are maintained at primary sampling sites, which should have adequate space to avoid contamination.

The Laboratory will document that the integrity of the samples is not affected during collection, storage, and transport. During the transport of samples, the stability of the required temperature will be ensured (it is recommended to monitor it and record the transport times), and the safety of the transporter. The importance of the integrity of temperature sensitive parameters during sample transport is emphasized. It is recommended to use suitable containers / bags for transporting biological material. The guidelines of valid international scientific societies should be followed for the transport of Microbiology, Pathology and Cytology specimens.

The Laboratory will ensure that primary sampling sites dispose infectious waste in accordance with national legislation.

For samples requiring separation, the Laboratory will ensure that personnel are adequately and appropriately trained, and that the required equipment is suitable, maintained and/or calibrated. Assessment of training provided to staff at primary sampling sites will be ensured and relevant records will be maintained.

The Laboratory will schedule and conduct internal audits of primary sampling sites, which will be included in the annual management review.

If the primary sample is taken at multiple locations belonging to a single legal entity, those specific locations, and the activity performed at each location, will be listed in the scope of accreditation.

9.2 Multisite Accreditation

Multi-site accreditation is the accreditation of a laboratory under a single legal entity for activities carried out at more than one sites (see ESYD DDEPT Procedure). The ESYD DDEPT procedure must have been included in the file of external documents.

A prerequisite for multi-site accreditation is that all parts of the laboratory are subject to a single quality management system, which must comply in all its aspects with the requirements of the accreditation standard.

Laboratories that have departments in different locations must fully document the relationships between their departments located in different locations as well as the degree of overlap between them (e.g., division of labor, transfer of samples between different locations, movement of personnel and/or equipment, evaluation, and release of results).

Laboratories must ensure and document that their clients and prospective clients are informed of their accreditation status.

9.3 Point of Care Testing (POCT)

A provider is defined as the one who performs the outpatient diagnostic tests.

Legal Entity: It must be clearly defined who has the legal responsibility (responsible legal entity) for the activities and results of the POCT, including responsibility for compliance with all requirements of the EN ISO 15189 standard.

EN ISO 15189 applies to POCT testing when performed in a hospital, clinic and by a healthcare organization providing ambulatory care. It is not intended for patient self-monitoring in a home or community setting. Only medical laboratories can be accredited as POCT providers.

Roles and Responsibilities:

Quality management system (according to the requirements of the ISO 15189 Standard): The POCT provider must have a quality management system that meets the requirements of the EN ISO 15189 Standard.

Document control, registration control (according to the requirements of ISO Standard 15189): All documents listed and required at the point of testing must be included and considered in the accredited entity's QMS to provide adequate control.

Staff competence (according to ISO 15189 requirements)

Equipment (in accordance with the requirements of ISO 15189): The provider must implement policies and procedures to ensure that all equipment is commissioned prior to use and adequately maintained through the implementation of a preventive maintenance program. Equipment used by POCT operators must be properly calibrated by authorized personnel. Equipment should not be moved regularly between areas to avoid any accidental cross-contamination. Correlation/equivalence studies with the POCT provider's existing laboratory analyzer are mandatory for new equipment. Electronic connection of the POCT equipment to the laboratory information system (e.g., within a hospital network) is desirable, where applicable.

Facility and environmental conditions (according to the requirements of ISO Standard 15189): The POCT provider determines and manages the working environment necessary to achieve good working conditions, privacy, and patient safety, and to comply with the POCT requirements and device manufacturers' recommendations. Depending on the type of POCT being performed, access should be limited to authorized personnel.

Space must be sufficient to allow work areas to be kept clean and tidy. The space required should be commensurate with the volume of analyses performed and the overall internal organization of the premises (including staff, patient, and storage facilities). The space allocated should be as required by national legislation and regulations.

An appropriate program for monitoring environmental conditions should be developed, and special attention should be paid to sterility, dust, electromagnetic interference, radiation, humidity, power supply, temperature, sound, and vibration, as appropriate to the technical activities concerned. This monitoring program will be designed by the POCT provider and followed by the POCT sites/entities.

Testing methods (according to the requirements of the ISO 15189 Standard): Validation of POCT methods (devices/equipment) is performed and provided by their manufacturers, as the POCT methods are mainly closed systems.

Verification must be provided by the laboratory. The verification is done in an accredited medical laboratory with an established QMS according to ISO 15189. The choice of verification parameters depends on the specific POCT method.

Quality assurance (as per the requirements of ISO Standard 15189): Periodically the POCT provider should evaluate the POCT results against those of the laboratory. Each site will participate in an EQA program at an appropriate frequency as determined by the provider. The ISO 15189 Standard specifies clear requirements for IQC and EQA. For IQC, the recommendations of the POCT device/equipment manufacturers, if any (according to the IVD EC directive for medical devices), will be followed. Otherwise, the IQC/EQA assurance plan is based on medical societies, international literature, etc.

Pre-analytical procedures (according to ISO Standard 15189): The provider must ensure that pre-analytical procedures are appropriate for POCT. The responsibility for establishing and implementing pre-analytical procedures belongs to the laboratory staff. For POCT tests there are usually no special sample storage and transport requirements (primary samples are usually tested immediately by POCT devices).

Post-analytical procedures, reporting of results (according to ISO Standard 15189): The POCT provider assumes legal responsibility for the results produced. When required, the provider must have the competence and the appropriate staff for the interpretation of POCT results. The POCT provider is responsible for the validity of the results, considering quality control, proper operation of the equipment and the training status of the operators.

9.4 Umbilical Cord Blood Banks (UCBB)

Legal requirements: Licensing by the Ministry of Health and the National Transplant Organization (No. Α3γ/οικ 18092 FEK 1005 /B March 24, 2017). The license must have been published in the Government Gazette (FEK). There is an official list in the Ministry of Health of the licensed UCBBs (Public or Private).

It is a strong recommendation that banks obtain accreditation from the FACT-NETCORD Organization within four (4) years from the approval of their operation.

Quality Management System: Covers all policies and standard procedures of all critical aspects of the operation and management of the umbilical cord blood bank, including: personnel, security, validation/verification of critical methods, internal and external quality control, agreements with the interested parties, consent of the donor (parent in this case), confidentiality, identification and tracking of the Umbilical cord blood (UCB) and stem cell units, documentation of the handling, processing of the UCB, control and storage of the stem cells, electronic monitoring systems, errors, discrepancies and adverse events, equipment, critical supplies and reagents, storage stability control, clinical outcome, data collection and management, regular quality review and internal audits.

Personnel:

- The Director of the Bank is a Hematologist or a Biopathologist, that must have proven professional and scientific experience in the field of Hematopoietic Cell Transplantation for at least five (5) years.
- The Medical Director is a Hematologist with at least five years of experience in hematopoietic cell transplantation. If the Director of the UCBB is a Hematologist, he may also exercise the duties of the Medical Director.
- The Biologist (at least one (1)) has at least two years of experience in control, processing, and cryopreservation of hematopoietic grafts.

- Medical laboratory technologist (at least one (1)).
- The Clerk/Secretary handles the receipt of transplants and the delivery of the collection materials and the IT with the information program of the UCBB.

Position descriptions and authorizations of personnel should be clear, documented and up to date. It is clearly described which members of staff inform parents, handle the consent process for the collection of UCB and implement this in collaboration with maternity hospitals. Staff training is ongoing and documented. Staff evaluation is clearly described in the QMS and implemented periodically.

Content of Consent or Contract with the family for cryopreservation:

The required elements of the parental consent or contract should include:

- Declaration of consent (i.e., consent for use in transplantation, research, commercialization, or quality control)
- Who can sign the consent or the contract
- The tests that must be done on the blood of the umbilical cord and the blood of the mother
- Storage and disposal
- Potential benefits
- Possible risks and inconveniences
- Alternative solutions other than cryopreservation
- Storage of personal information of the donor
- Future contact details (for test results and monitoring of the baby's health status)
- Costs and claims
- Criteria for withdrawal of consent or termination of contract
- Consent for collection only (otherwise permission for use in research or commercialization should be included if identity is anonymous or information on how to approach the family for additional consent for other uses of the collected umbilical cord blood material).

It should be ensured that information to parents and health care providers is fair, balanced and documented. Prohibition of false statements, omissions, unsubstantiated claims, and emotional appeals likely to mislead parents or health care providers.

Screenings and information on the origin of UCB: The required pre-analytical stage screenings should include information about the mother and the child, such as personal information and lifestyle (e.g., substance use), medical history, including family history, pregnancy and delivery history and baby's health, travel history as it could be related to communicable diseases (e.g., malaria).

Laboratory testing of maternal blood should be done for the following infectious agents: HBV (HBsAg and HBV NAT), HCV (anti-HCV and HCV NAT), CMV (anti-CMV (IgG and IgM)), HIV (anti-HIV 1 & 2 and HIV NAT), Syphilis (non-specific and/or specific tests for Treponema pallidum). HTLV-I/II antibody detection tests are performed when the donor's parents reside in or come from areas with a high incidence of infection. It must have been done 7 days before or after the UCB unit is collected.

Collection of UCB: Collection of UCB is done according to the laboratory's written instructions and is carried out by a trained, qualified healthcare professional in an approved or validated container containing anticoagulant.

The information transferred to the laboratory refers to the method of umbilical cord blood collection (e.g., in utero versus ex utero), the type of delivery (natural delivery or caesarean section), whether it is a singleton or twin pregnancy, the time between umbilical cord blood collection and cryopreservation.

UCB and/or maternal blood must be sent in an isothermal box (special transport box) with temperature monitoring, and it must be confirmed that the container can maintain the intended temperature during the transport of the blood bags to the laboratory, which must not exceed 48 hours. Each shipping box is labeled Umbilical Cord Blood, Fragile, Do Not Irradiate.

Facilities: Separate reception, control, processing areas (minimum 50 m^2 each).

In the processing areas of the UCBBs, recorded control procedures are followed for the room temperature, humidity, air quality (particle measurement), and the sterility of the laboratory area.

Equipment and Reagents: Regular and preventive maintenance of the equipment is done according to the manufacturer's instructions.

Monitoring records, warnings, alarm systems are kept for the critical equipment (processing, storage) to ensure uninterrupted operation, in order to avoid problems in the maintenance of the Units.

Storage is done in temperature and level-controlled containers of liquid nitrogen (strong recommendation for storage in the liquid phase rather than the gas phase of nitrogen). There is continuous recording and control of the nitrogen level with 24-hour notification of level and temperature failure inside the storage containers.

Supplies, reagents, and other consumables that come into contact with UCB must be approved for clinical use and recorded in registers for each lot number.

Analytical Procedures: The laboratory has documented Analytical Processing and Cryopreservation Procedures that describe unit volume (above 60 ml), total count of nucleated cells, type and concentration of cryoprotectant, method and rate of cryo-freezing, final storage temperature.

Laboratory tests required before and after treatment include total nucleated cell count (TNCC), CD34+ cell count, CD34+ cell viability measurement, microbiological testing to check for aerobic, anaerobic bacterial and fungal growth, determination of ABO and Rhesus groups, HLA-A and HLA-B testing, hemoglobinopathies testing (recommended depending on family history for autologous preservation and required for allogeneic preservation).

During processing, it is necessary to store accompanying vials (3 microsamples of 100μl for HLA, 2 cryovials of plasma at temp. < -70°C for laboratory tests).

It is recommended that for the storage of the final product (stem cells) that attached vials are available for additional ancillary tests (e.g., to check the viability of the cells at regular intervals, as will be defined in the written laboratory procedures).

Verification, internal and external quality control follow all the aforementioned guidelines for hematology, flow cytometry and microbiological testing.

Post-Analytical Procedures: The laboratory has documented Analytical Procedures that describe access control to the storage area, continuous electronic recording of temperature, humidity and liquid nitrogen level, and 24-hour electronic notification of failures.

For potentially infectious samples, storage in special quarantine containers is required.

The laboratory has documented procedures for the case of thawing the cells and using them for transplantation. Detailed reference should be made to all tests that must be performed to ensure that the product stored for transplantation is viable, adequate, and suitable for transplantation.

The laboratory should have documented procedures for investigating and dealing with adverse events related to stem cell administration and for investigating complaints about stem cell storage.

9.5 Accreditation under a Flexible Scope

The requirements for the flexible scope of accreditation in medical laboratories and for all categories of tests are divided into 2 categories:

A) the horizontal requirements described in the Guidelines of ESYD for the assessment of conformity of accredited bodies under a flexible scope of accreditation ESYD KO EYEL.

B) the specific requirements for the description of the scopes of accreditation of medical laboratories, as stated in the document EA-4/17 M Description of scopes of accreditation for medical laboratories.

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11. USEFUL WEBSITES

ELOT: http://www.elot.gr/

EPTIS: http://www.eptis.bam.de/

ISO: http://www.iso.org/iso/home.html

Eurogentest: http://www.eurogentest.org/

IFCC: http://www.ifcc.org/

CLSI: http://www.clsi.org/

CAP: http://www.cap.org/apps/cap.portal